5. Tracer experiments with ^{75}Se

The effects of the selenium status on the distribution of ^{75}Se among the body compartments were analyzed in rats fed for several generations with a selenium-deficient diet or a selenium-sufficient diet. The composition of the diet and the treatment of the animals are described in section 2.1.4. Six male animals (three deficient and three sufficiently supplied) with a body weight between 340 and 490 g were labeled *in vivo* with 18 MBq ^{75}Se with a specific activity of 9 MBq/µg Se (2 µg of selenium per animal), as described in section 2.1.2.

In the second part of the experiments the effects of the selenium status on the expression of the selenium-containing proteins was investigated in the lung and trachea obtained from the labeled selenium-sufficient and selenium-deficient animals. In this experiment ^{75}Se with a high specific activity was used. The specific activity was 17.6 MBq/µg Se.1 µg of selenium was added per animal.

In order to obtain some information on sites of action of the selenium-containing proteins and thus on their possible functions, their subcellular distribution was investigated. For this the labeled tissues of lung and trachea were homogenized and than partitioned by differential centrifugation into a nuclear, mitochondrial, microsomal and cytosolic fraction. For each sample the protein concentration and the activity of the tracer were determined. Samples with equal amounts of the proteins were separated by means of SDS-PAGE or 2D electrophoresis. After staining and drying of the gels, the selenium compounds were identified autoradiographically via the radiation of ^{75}Se. The selenoproteins present in the subcellular compartments of lung and trachea were then analyzed via their molecular masses and pI value. Some of them could be identified in this way. Identification immunoassays were also applied for their. The data obtained in these experiments are shown below.

5.1 Effect of the selenium status on the distribution of ^{75}Se in different tissues

The Figure 5-1 shows the distribution of ^{75}Se in the different tissues of selenium-deficient rats after labeling periods of 3 hours, 24 hours and 8 days (three animals in each group). The activity was related to the wet mass of the tissues. The concentration of the tracer was calculated as mean ± SD. After 3 hours most of the labeled selenium was present in the liver and kidney. After 24 hours the tracer concentration increased in all tissues investigated but mainly in the thyroid, followed by the spleen, kidney, lung and trachea. After 8 days the selenium concentration had decreased again in all tissues except the spleen.

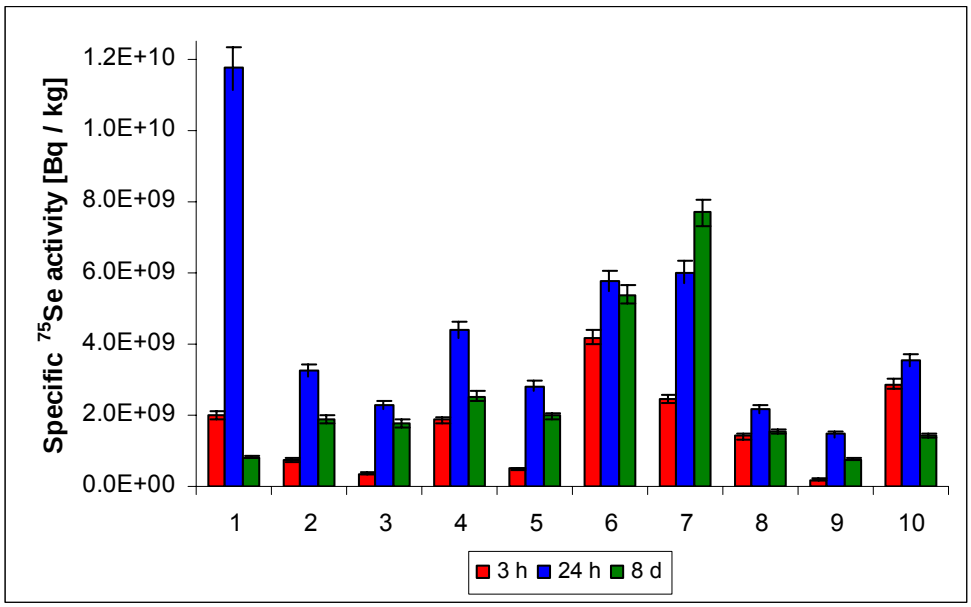

Figure 5-1 Distribution of ^{75}Se among the tissues (Bq/kg wet mass) of rats (n = 3) fed a selenium-deficient diet after different labeling periods; 1- thyroid, 2- trachea, 3- gullet, 4- lung, 5- prostate, 6- kidney, 7- spleen, 8- pancreas, 9- skin, 10- liver

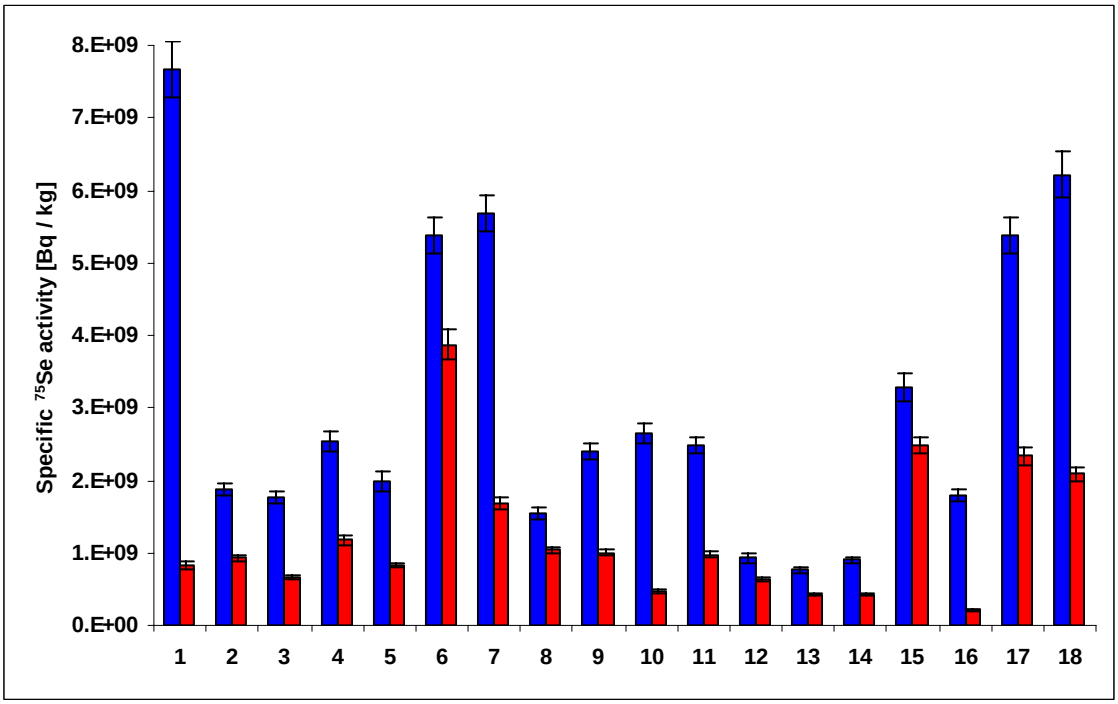

Figure 5-2 Distribution of ^{75}Se among the tissues (wet mass) of rats fed either a selenium-deficient (blue bar) or a selenium-sufficient (red bar) diet after a labeling period of 8 days; 1- thyroid, 2- trachea, 3- gullet, 4- lung, 5- prostate, 6- kidney, 7- spleen, 8- pancreas, 9- duodenum, 10- colon, 11- small intestine, 12- stomach, 13- skin, 14- epididymis, 15- testis, 16- spinal marrow, 17- liver, 18- plasma

The Figure 5-2 shows the distribution of ^{75}Se among different tissues of 2 animals fed a selenium-deficient diet and 2 fed a selenium-sufficient diet. The animals were labeled with a small amount of selenium (18.5 MBq per animal containing 2 µg of selenium) and killed after 8 days. The activity of the tracer in the tissues was measured by means of a scintillation detector. Remarkable differences in the ^{75}Se distribution were observed between the two groups, which are caused by the tissue-specific hierarchy. Considerably more ^{75}Se was found in the selenium-deficient tissues. The content of the tracer in the plasma, liver, kidney, spleen, testis and thyroid was high in the selenium-deficient and selenium sufficient rats.

5.2 Distribution of ^{75}Se and of the selenium-containing proteins in the lung and trachea

5.2.1 Effect of the selenium status on the subcellular distribution of selenoproteins in the tissues of the respiratory tract

In the homogenates and in the subcellular fractions of the lung and trachea separated by means of differential centrifugation, the ^{75}Se activity was determined and related to the protein content of each of the samples. The results for the specific tracer activity obtained in this way for the selenium-deficient and selenium-sufficient animals are shown in the Figure 5-3.

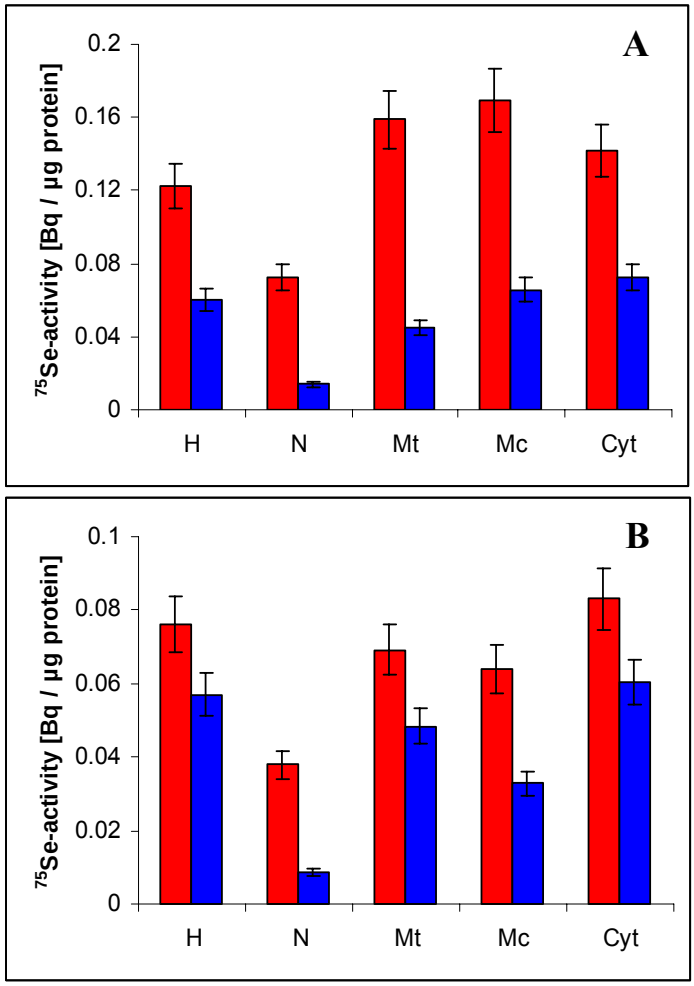

Figure 5-3 Distribution of ^{75}Se in the homogenates and subcellular fractions of the lung (A) and trachea (B), of the selenium-deficient (red bar) and the selenium-sufficient (blue bar) rats

In the homogenates and subcellular fractions of the lung remarkable differences in the ^{75}Se distribution were observed between the two groups. In the lung samples of the selenium-sufficient animals the highest specific activity was found in the microsomal fraction and the lowest in the nuclei. In the samples of the deficient animals the levels were increased in all fractions.
In the homogenates of the trachea the retention was similar, but considerable differences in the tracer distribution between the groups were found among the cell compartments. In both groups the cytosols had the highest specific activity and the nuclei the lowest. Retention factors were calculated by relating the specific activity of the samples from the deficient rats to the corresponding values in the sufficiently supplied rats. They show that after administration of a small amount of selenium to the deficient animals, the element in the lung is especially retained in the nuclei and mitochondria and in the trachea in the microsomes and nuclei.

The proteins of the homogenates and subcellular fractions of the two tissues from selenium-sufficient and selenium-deficient animals were separated by means of SDS PAGE and the selenium present in the proteins was identified by autoradiography. The autoradiograms are shown in the Figure 5-4. Eleven selenoproteins have been found in this way. Their relative molecular masses were: 10 kDa, 12 kDa, 15 kDa, 16 kDa, 18 kDa, 20 kDa, 23 kDa, 25 kDa, 53 kDa – 54 kDa, 57 kDa – 60 kDa, and 74 kDa. Information on the occurrence of these proteins in the homogenates and cellular compartments is given in the Tables 5-1 and 5-2. The autoradiograms in the Figures 5-4 and 5-5 show that there were differences between the fractions, but the distribution of these bands was similar in the two tissues. In the lung and trachea of the selenium - sufficient rats, the selenoproteins at 15 kDa and 25 kDa were most strongly labeled. The predominantly labeled selenium-containing proteins in the lung and trachea of the selenium-deficient rats were at 15 kDa and in the range between 20 kDa – 25 kDa and 57 kDa – 60 kDa.
The samples were also taken for quantitative determination of the ^{75}Se activity.

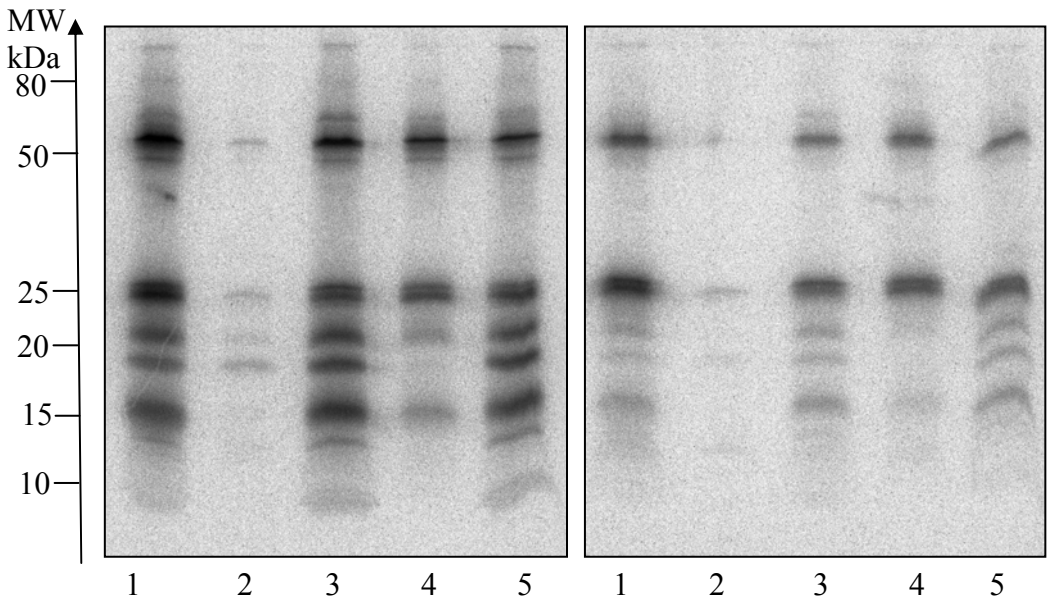

Figure 5-4 Autoradiogram of the ^{75}Se-labeled proteins, separated by SDS-PAGE, in the homogenate (1) and the nuclear (2), mitochondrial (3), cytosolic (4) and microsomal (5) fraction of the lung of rats fed a selenium-deficient diet (left) or a selenium-sufficient diet (right)

Table 5-1 Selenoproteins present in the homogenate and the subcellular compartments of the lung of selenium-deficient (-) and selenium-sufficient (+) rats; √- strongly labeled

Molecular masses [kDa]	Homogenate		Nuclei		Mitochondria		Microsomes		Cytosol	
	-	+	-	+	-	+	-	+	-	+
74	√				√	√	√	√	√	√
60 - 57	√	√	√	√	√	√	√	√	√	√
54 – 53	√	√	√		√	√	√	√	√	√
25	√	√	√	√	√	√	√	√	√	√
23	√	√			√	√	√	√	√	√
20	√	√	√	√	√	√	√	√	√	√
18	√	√	√	√	√	√	√	√		
16 – 15	√	√	√	√	√	√	√	√	√	√
12	√				√	√	√	√		
<10	√				√		√			

The proteins with molecular masses of 60 – 57 kDa, 25 kDa, 20 kDa and 16- 15 kDa were present in the homogenate and all subcellular fractions of the lung in both groups. The 18 kDa band was not seen in the cytosolic fraction, as were the 74 kDa and 23 kDa bands in the nuclei. The 10 kDa and 12 kDa bands were only found in the mitochondria, microsomes and in the homogenates of the selenium-deficient animals.

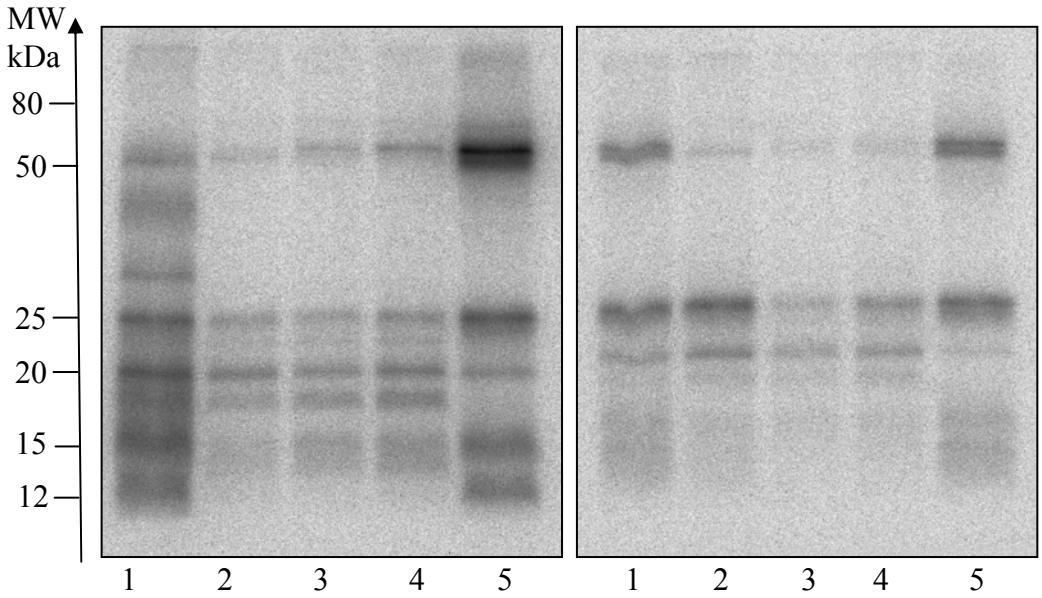

Figure 5-5 Autoradiogram of the ^{75}Se-labeled proteins separated by SDS-PAGE in the homogenate (1), and the nuclear (2), mitochondrial (3), microsomal (4) and cytosolic (5) fraction of the trachea of rats fed a selenium-deficient diet (left) or a selenium-sufficient diet (right)

In the case of the trachea the selenoproteins with molecular masses of 54 kDa -53 kDa, 25 kDa, 20 kDa and 15 kDa were present in all fractions in both groups. The band at 60-57 kDa was not found in the nuclei, as was the 18 kDa band in the cytosol and nuclei of the selenium-sufficient rats. In the nuclei, mitochondria and cytosol in this group the 16 kDa protein was not detected. The 74 kDa protein was detected in the homogenates, mitochondrial microsomal and cytosolic fraction of the selenium-deficient trachea. The 12 kDa band was present in the homogenates and the cytosolic fraction.

Table 5-2 Selenoproteins present in the homogenate and the subcellular compartments of the trachea of selenium-deficient (-) and selenium-sufficient (+) animals; √- strongly labeled

Molecular mass [kDa]	Homogenate		Nuclei		Mitochondria		Microsomes		Cytosol	
	-	+	-	+	-	+	-	+	-	+
74	√				√		√		√	
60 - 57	√	√			√	√	√	√	√	√
54 – 53	√	√	√	√	√	√	√	√	√	√
25	√	√	√	√	√	√	√	√	√	√
23	√	√	√		√	√	√	√	√	√
20	√	√	√	√	√	√	√	√	√	√
18	√	√	√		√	√	√	√		
16	√	√	√		√		√	√	√	
15	√	√	√	√	√	√	√	√	√	√
12	√	√							√	√

5.2.1.1 Quantitative analysis of ^{75}Se-labeled proteins in the subcellular compartments of the respiratory tract

The program Aida 2.43 (Straubenhardt, Germany) was used for the quantitative analysis of the tracer distribution between the proteins present in the homogenates and subcellular fractions of the lung and trachea. After autoradiography the proteins containing ^{75}Se were present as black bands and their intensity was related to the amount of the tracer. The intensity of the band blackening was determined as photostimulable luminescence units – PSL units. The ratio of the band blackening intensity to the whole blackening was calculated for each sample. For the analysis the following ^{75}Se-labeled bands were taken: 74 kDa, 60 – 50 kDa, 54 -57 kDa, 25 kDa, 23 kDa, 20 kDa, 18 kDa, 16 – 15 kDa, 12 kDa, 10 kDa. The values obtained in this way are shown in Figures 5-6, 5-7, 5-8, and 5-9.

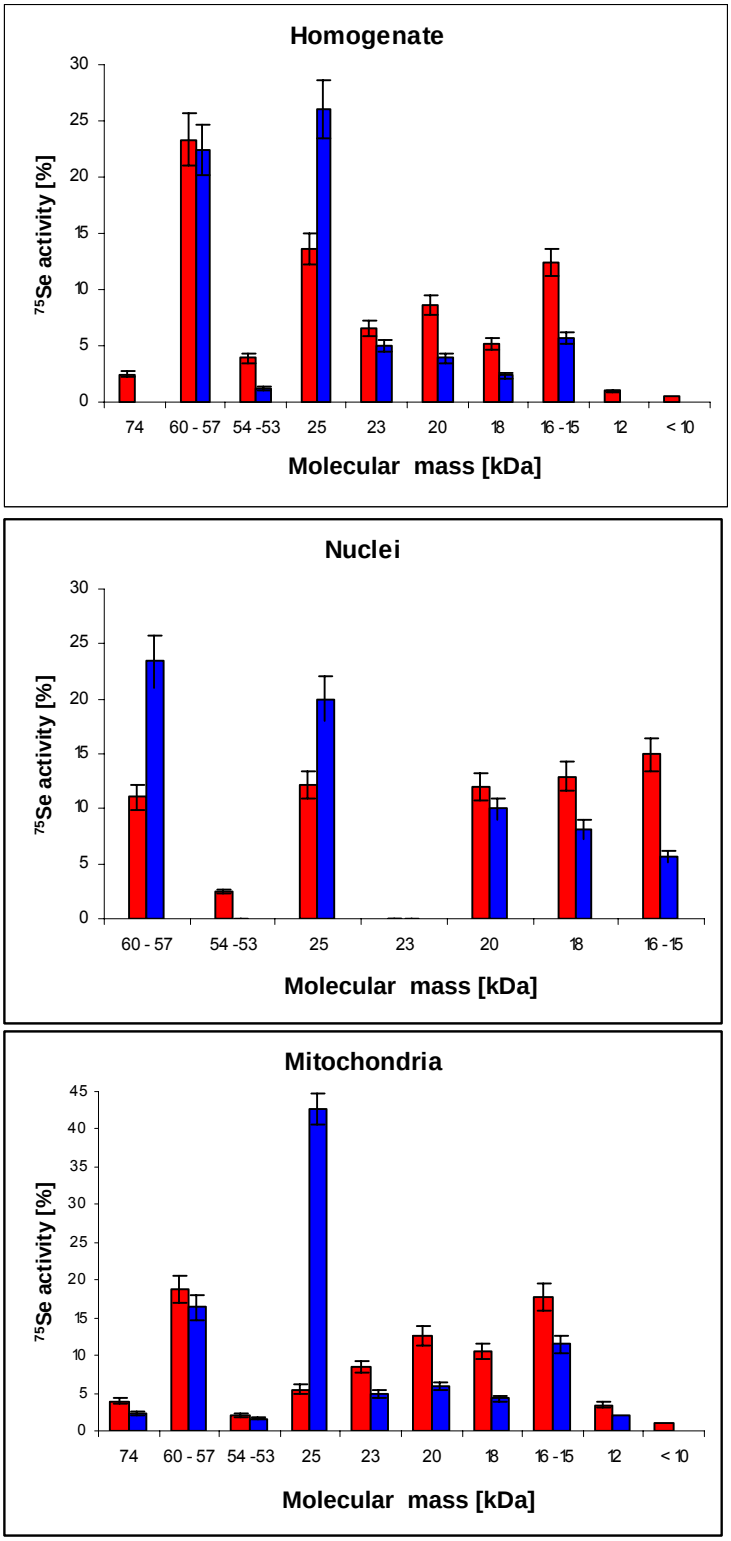

Figure 5-6 Distribution of the ^{75}Se-labeled selenoproteins among the homogenate, the nuclear and the mitochondrial fraction of the lung of selenium-deficient rats (red bar) and selenium-sufficient (blue bar) rats. The data are expressed as a percentage of the total protein-bound ^{75}Se activity of the whole blackening in the electrophoretically separated proteins (n=2).

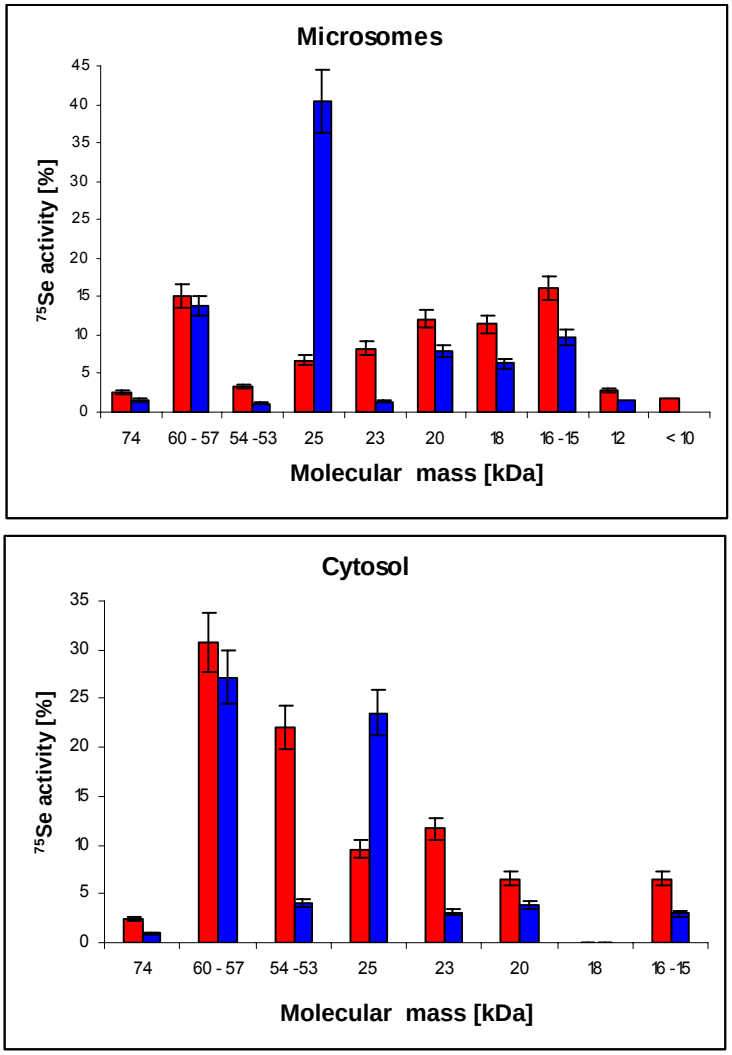

Figure 5-7 Distribution of the ^{75}Se-labeled selenoproteins among the microsomal and cytosolic fraction of the lung of selenium-deficient rats (red bar) and selenium-sufficient (blue bar) rats. The data are expressed as a percentage of the total protein-bound ^{75}Se activity of the whole blackening in the electrophoretically separated proteins (n=2).

The results show that the blackening of the ^{75}Se-labeled band found at the 60 – 57 kDa was very strong in the homogenate (27%) and the cytosol (ca. 30%). In the other fractions it was lower than 20%. There were no significant differences in the distribution of those selenoproteins between selenium-deficient and selenium-sufficient animals. In the case of other proteins, marked differences between the two groups have been found. The 25 kDa protein was mainly labeled in all samples of the selenium-sufficient animals. About 25% was in the homogenate and the cytosol, 42 % in the mitochondria and microsomes and less than 20% in the nuclei. The blackening of the 20 kDa, 23 kDa and 18 kDa bands was below 10%

in all subcellular fractions (in the cytosol and nuclei the band at 18 kDa and in the nuclei the band at 23 kDa were absent). The blackening of the 16 – 15 kDa band was mainly found in the nuclear, mitochondrial and microsomal fraction, in each below 20%. The blackening of the band between 54 -53 kDa was high in the cytosolic fraction (above 20%). 12 kDa and 10 kDa proteins were mostly present in the mitochondrial and microsomal fraction, the blackening was below 5 %. The 10 kDa protein was detected only in the homogenate, the mitochondria and the microsomes of the selenium-deficient animals. The blackening of the 74 kDa protein in all fractions (beside nuclear) was below 5%. The results are summarized in the Table 5-1.

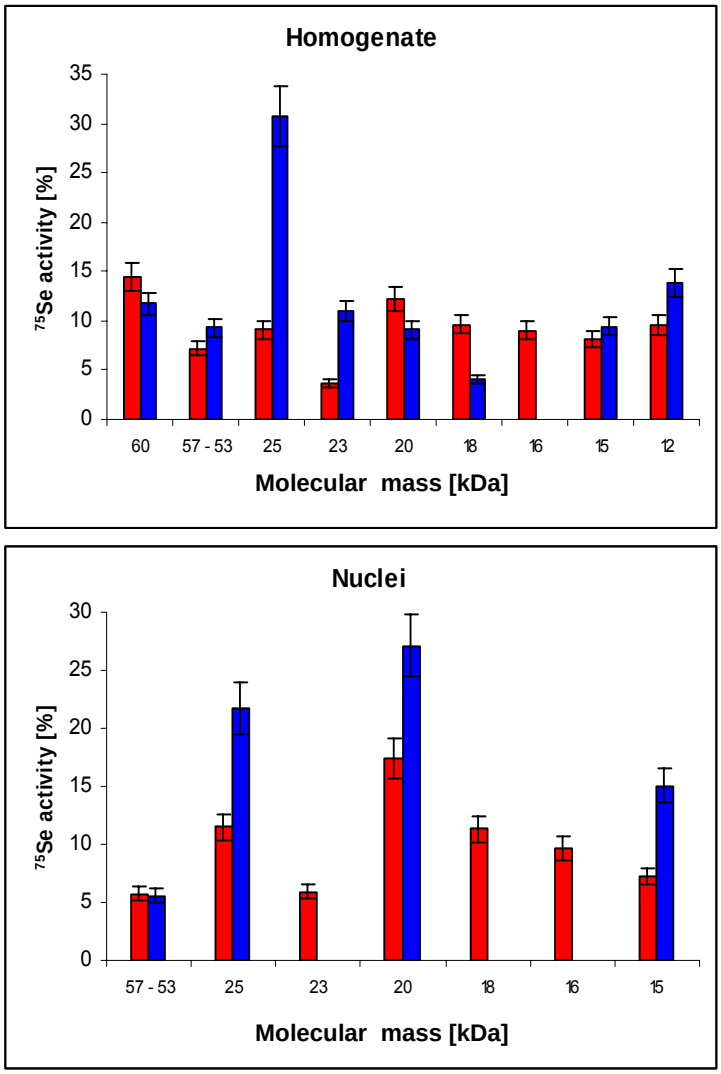

Figure 5-8 Distribution of the ^{75}Se-labeled selenoproteins among the homogenate and nuclear fraction of the trachea of selenium-deficient rats (red bar) and selenium-sufficient (blue bar) rats (n=2). The data are expressed as a percentage of the total protein-bound ^{75}Se activity of the whole blackening in the electrophoretically separated proteins.

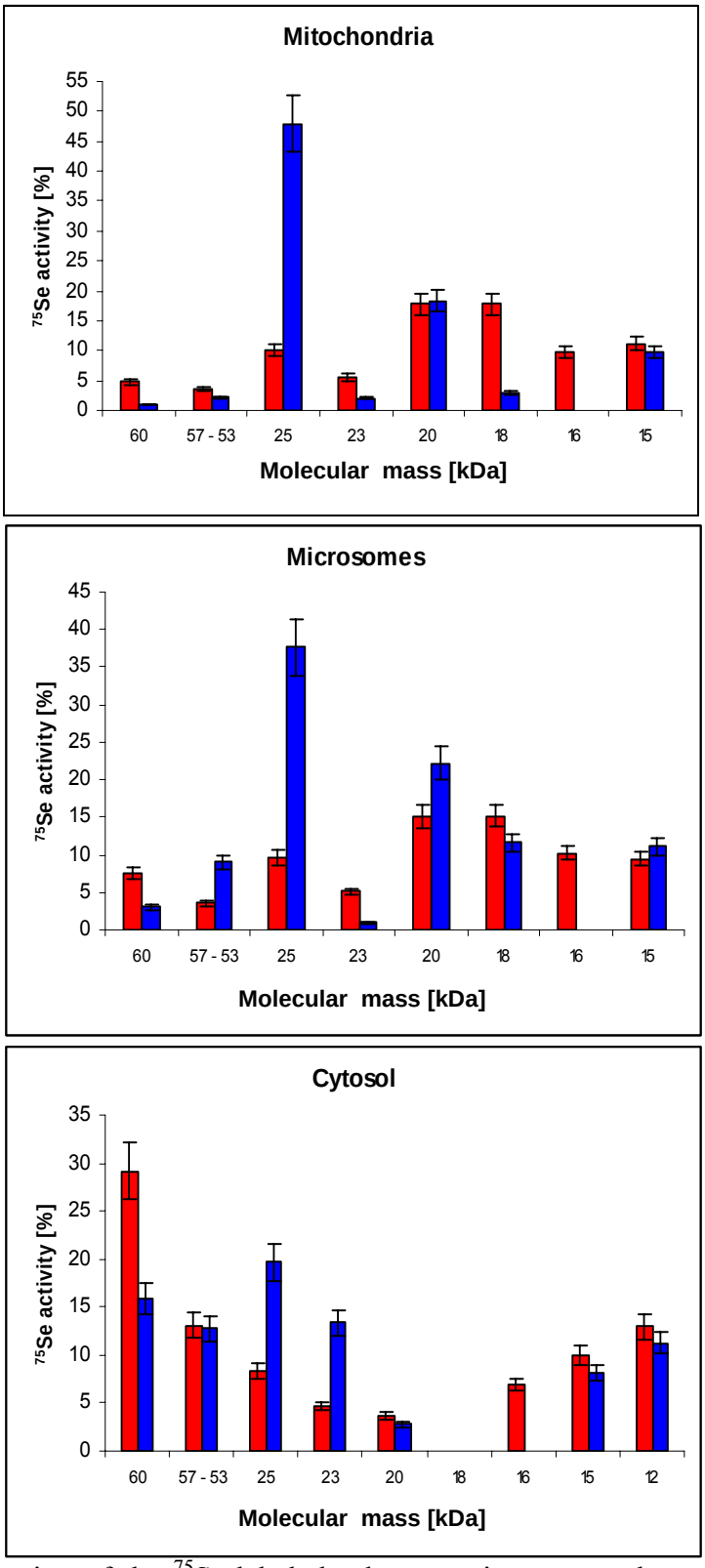

Figure 5-9 Distribution of the ^{75}Se-labeled selenoproteins among the mitochondrial, the microsomal and cytosolic fraction of the trachea of selenium-deficient rats (red bar) and selenium-sufficient (blue bar) rats (n=2). The data are expressed as a percentage of the total protein-bound ^{75}Se activity of the whole blackening in the electrophoretic separated proteins.

The results obtained from the subcellular fractions of the trachea show that also in this case the blackening at the 25 kDa band was considerably higher in all subcellular fractions of the selenium-sufficient trachea. The blackening of the band at 25 kDa was strongest in the mitochondrial and microsomal fractions with more than 40%. The bands at 23 kDa were also most strongly labeled in the homogenate (10% in the Se(+) and below 5% in the Se(-)) and cytosolic fraction (14% in the Se(+) and below 5% in the Se(-)) of the selenium-sufficient trachea. Interestingly, also the band found at 15 kDa in the nuclei of the selenium-sufficient trachea was more pronounced (15% in the Se(+) and 7% in the Se(-)). The protein with a molecular mass at 60 kDa was mainly present in the homogenate and the cytosolic fraction. The ratio of the blackening of the band at 57 – 53 kDa was the highest in the homogenates and the cytosols in both groups (15%). Interestingly, the intensity of this band was stronger (below 10%) in the microsomal fraction of the sufficient sample. The 18 kDa band was present in the homogenates (10% in the selenium-deficient sample and 5% in the selenium-sufficient sample), mitochondria (deficient 15% and sufficient below 5%), microsomes (in both samples below 15%), and nuclei (above 10% but only in the deficient sample). This band was not detected in the cytosolic fraction. The 16 kDa protein was found in all fractions of the selenium deficient trachea with a blackening intensity below 10%. The 12 kDa band was present in the homogenate and cytosol, with the intensity of the blackening below 15%. The results corresponded to those listed in the Table 5-2.

5.2.2 Comparison of the selenoproteins found after two-dimensional electrophoresis in the selenium-deficient and selenium-sufficient lung and trachea

For further characterization of these selenoproteins two-dimensional electrophoresis (IEF/SDS-PAGE) was applied. This electrophoresis technique has a better resolution, as the proteins are first separated via their pI and then via their molecular masses. The autoradiograms of the lung and trachea homogenates and their subcellular fractions are shown below.

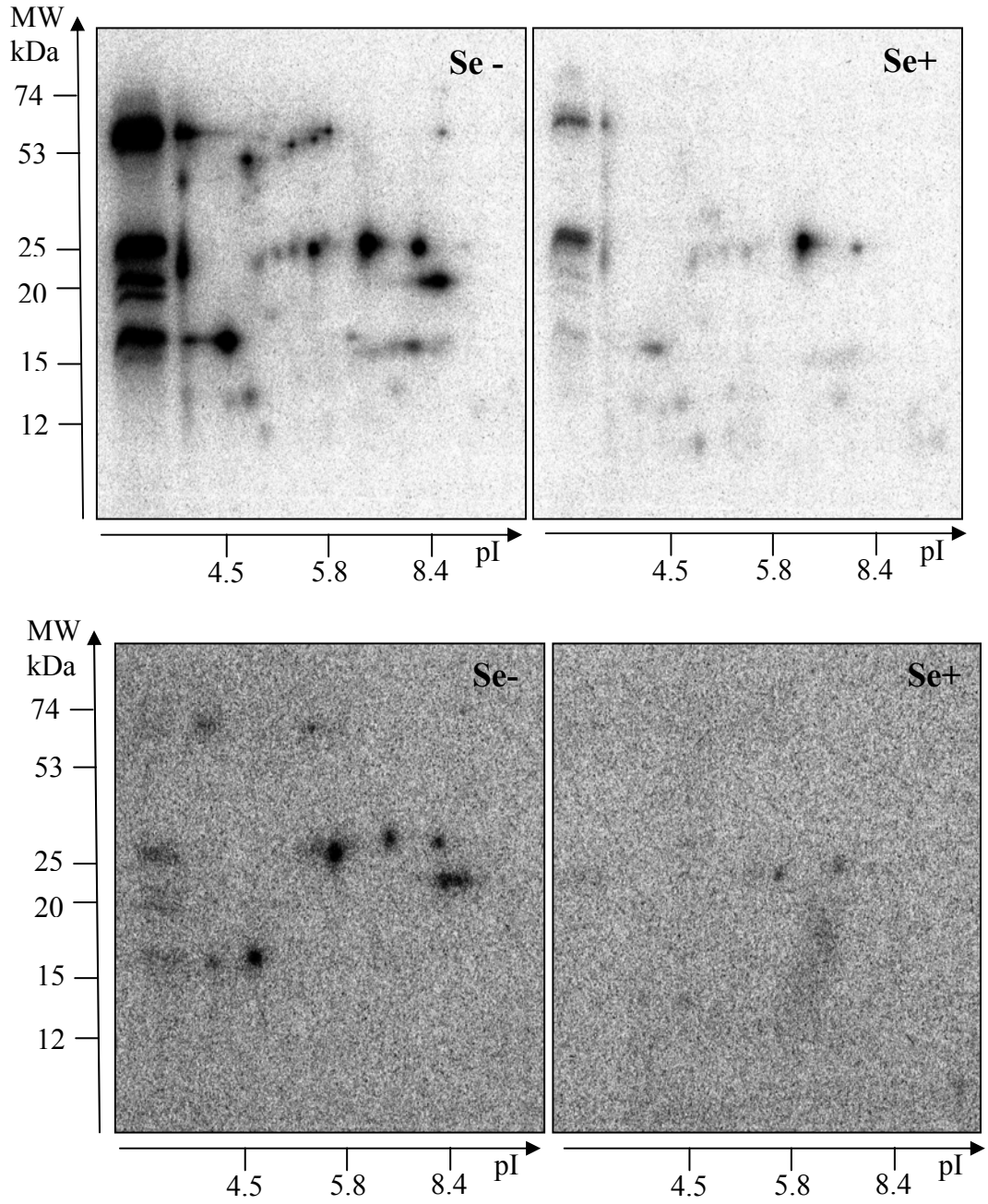

Figure 5-10 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the homogenate (upper figure) and in the nuclear fraction (lower figure) of the lung of rats fed a selenium-deficient (Se-) or a selenium-sufficient diet (Se+)

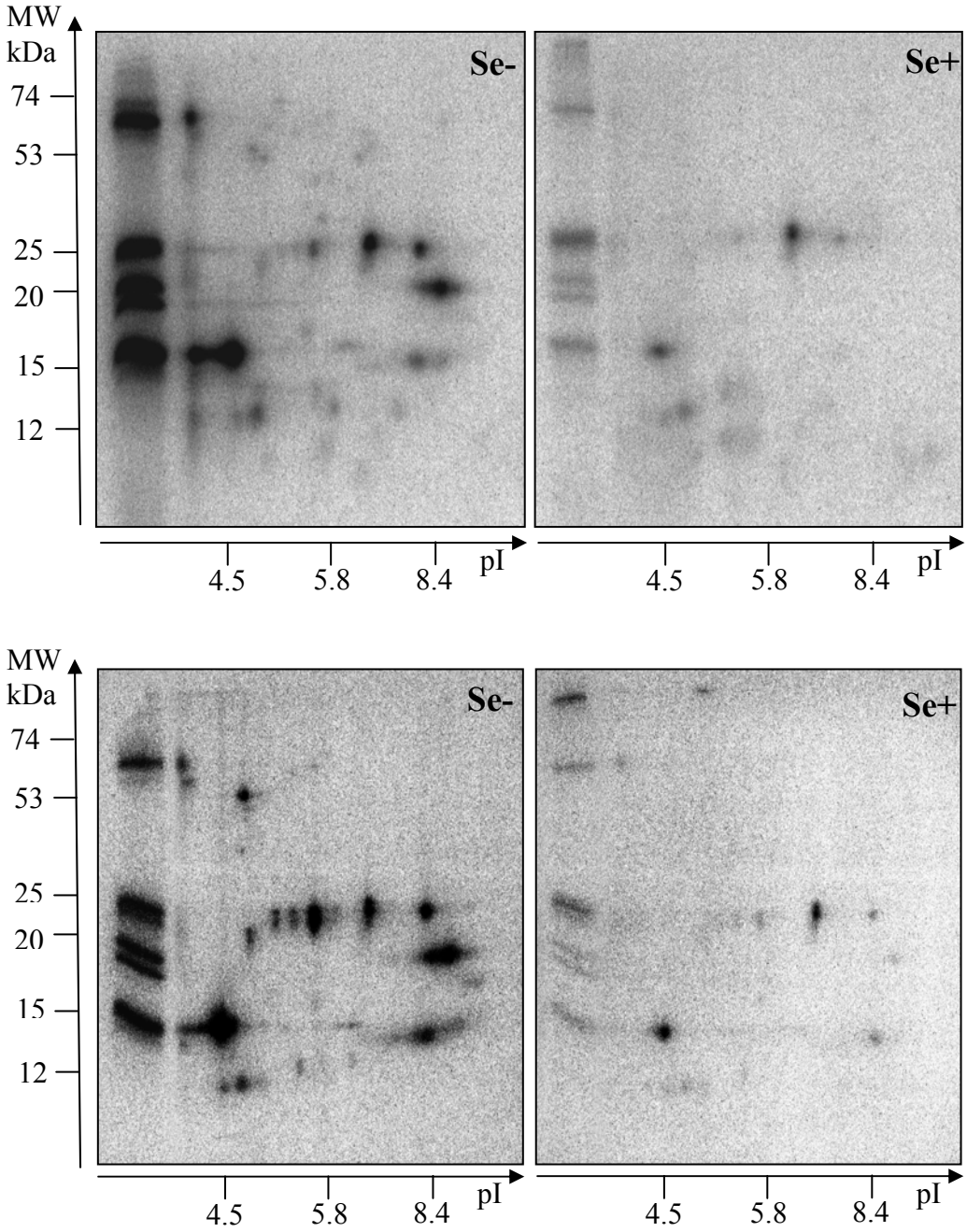

Figure 5-11 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the mitochondrial (upper figure) and in the microsomal fraction (lower figure) of the lung of rats fed a selenium-deficient (Se-) or a selenium-sufficient diet (Se+)

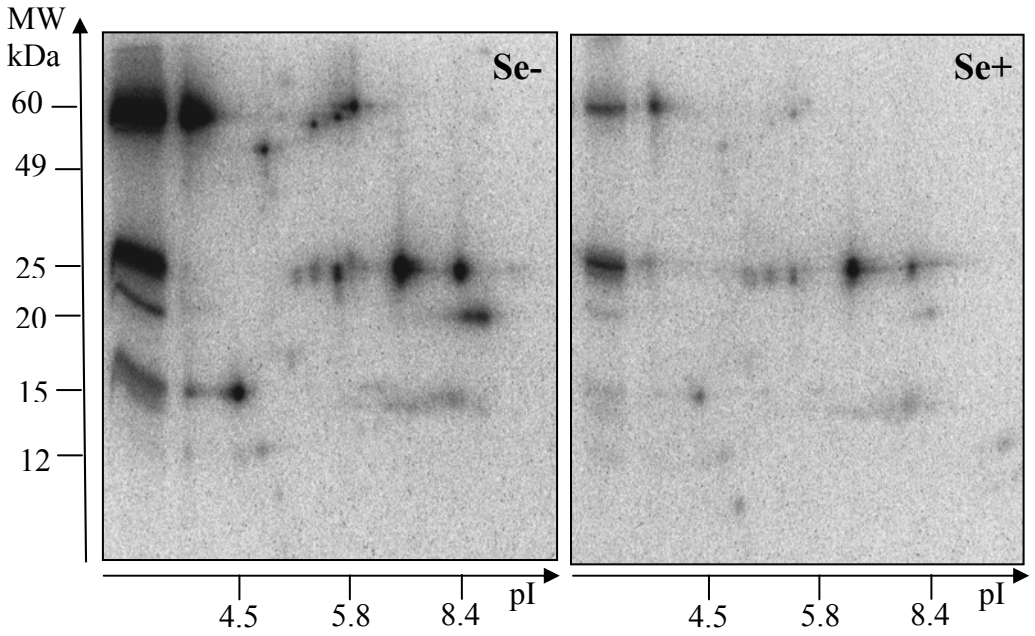

Figure 5-12 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the cytosolic fraction of the lung of rats fed a selenium-deficient (Se-) or a selenium-sufficient diet (Se+)

The distribution of the selenium-containing proteins in the homogenates of the lungs of the rats from the two groups after separation by 2D electrophoresis can be seen in the upper two autoradiograms in the Figure 5-10. The proteins in the homogenates of the Se (-) samples were resolved into several ^{75}Se-containing spots, whereas in the homogenate of the Se (+) samples distinctly fewer spots had been sufficiently labeled. In the Se (+) homogenates the most intensive spots were at 25 and 15 kDa, and in the Se (-) at 60 – 57 kDa, 25 – 20 kDa and 15 kDa.

The lower parts of the Figure 5-10 and the Figure 5-11 show the autoradiograms of the selenium-containing proteins in the subcellular compartments of the lung. In the nuclear, mitochondrial and microsomal fractions of the selenium-deficient animals the spots at 20 – 25 kDa and 15 kDa were only weakly labeled. In the selenium-sufficient animals a 25 kDa spots had the highest ^{75}Se activity in these fractions. In the nuclei, in addition to this spot only one further labeled spot could be seen which likewise was in the 25 kDa range.

The cytosolic selenium-containing proteins (Figure 5-12) were resolved into many spots in the Se (-) sample (highest tracer content in spots at 60- 55 kDa and 25 -20 kDa) and clearly fewer in the Se (+) samples (highest tracer content at 25 kDa).

The following autoradiograms show the selenium-containing proteins detected in the homogenate and in the subcellular fractions of the trachea. The nuclear fraction could not be analyzed in this way due to the small amount of the sample and the relatively low ^{75}Se activity. In the homogenates of the Se (-) trachea most of the tracer was found in the spots with molecular masses at 60 -57 kDa, 25 -20 kDa, and 15 kDa and in the Se (+) homogenates at 60 – 57 and 25 kDa (Figure 5-13). The Figure 5-14 presents autoradiograms of ^{75}Se-labeled proteins in the Se (-) and Se (+) mitochondrial fraction of the trachea. Here the strongest activity of the tracer was found at 25 - 20 kDa and 15 kDa in both groups. The main labeled spots in the selenium-deficient microsomes were at 60 -57 kDa, 25 -20 kDa, and 15 kDa, and in the selenium-sufficient samples at 25-15 kDa (see Figure 5-14 the two lower autoradiograms). The autoradiograms of selenium-containing proteins in the cytosolic fraction of the trachea are shown in the Figure 5-15. In this case the main labeled spots were at 60 -57 kDa, 25 - 20 kDa, 15 kDa and 12 kDa in the Se (-) cytosol and 60 -57 kDa, 25 -20 kDa, and 15 kDa in the Se (+) cytosol.

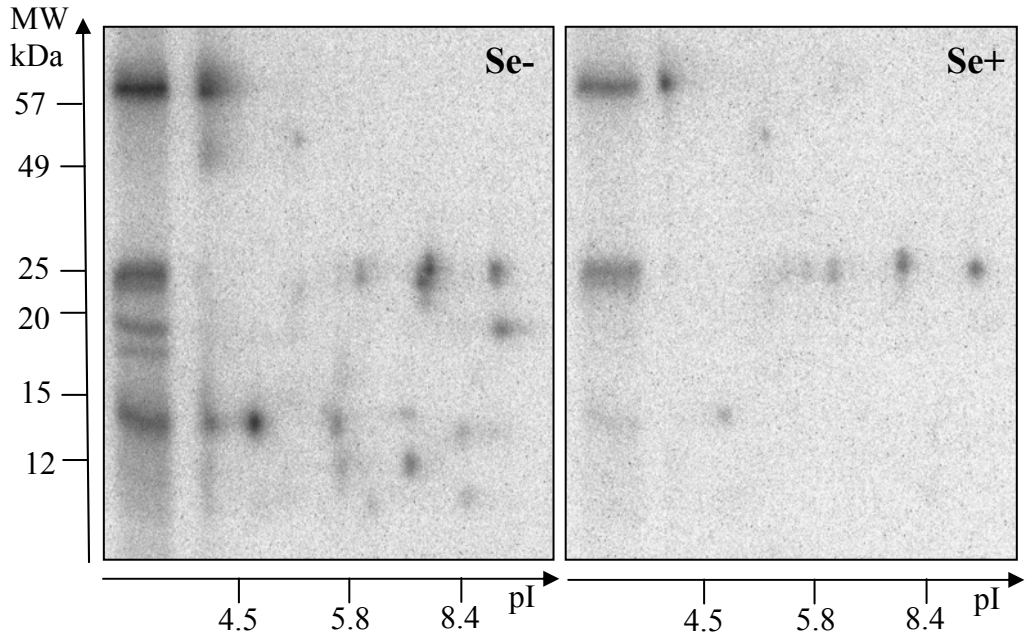

Figure 5-13 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the homogenate of the trachea of rats fed a selenium-deficient (Se-) or a selenium-sufficient diet (Se+)

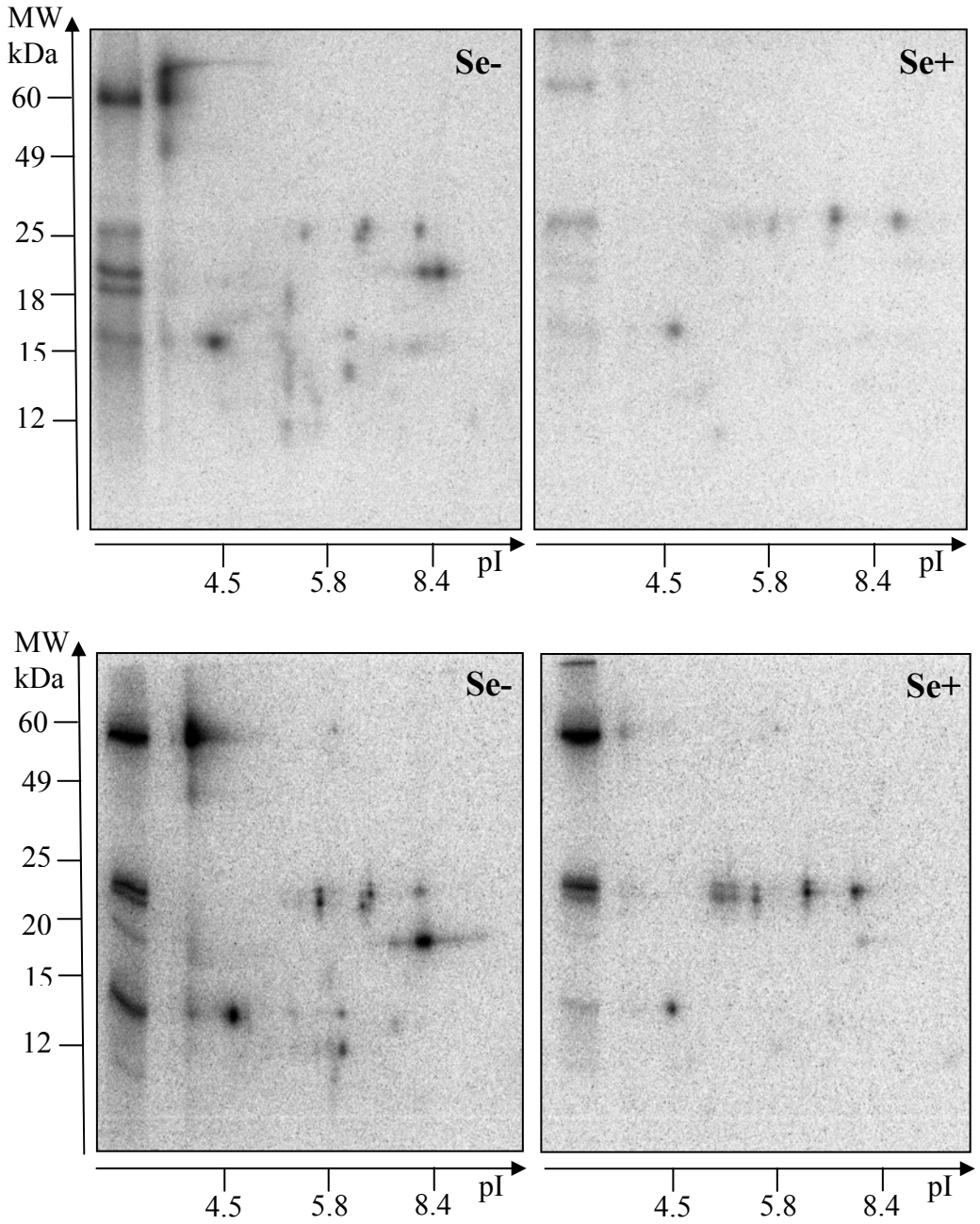

Figure 5-14 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the mitochondrial (upper part) and in the microsomal (lower part) fraction of the trachea of rats fed a selenium-deficient (Se-) or a selenium-sufficient diet (Se+)

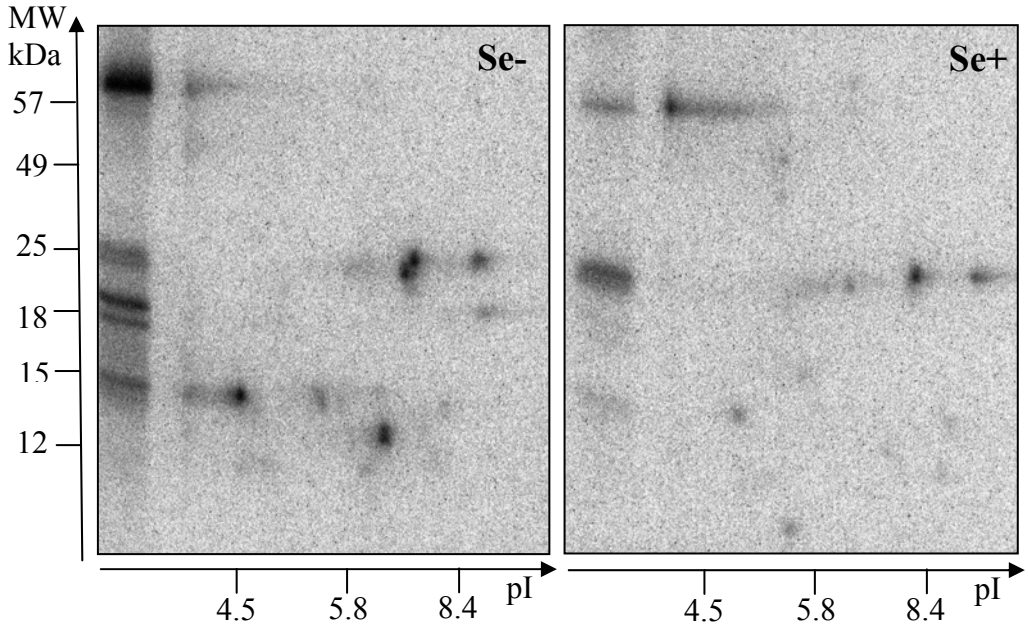

Figure 5-15 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the cytosolic fraction of the trachea of rats fed a selenium-deficient (Se-) or a selenium-sufficient diet (Se+)

The expression of the proteins was strongly dependent on the selenium status. There were similarities between the lung and trachea with regard to the pattern of the selenium-containing proteins in the homogenates and subcellular fractions. In the selenium-sufficient tissues the main labeled selenium-containing proteins were at 25 kDa and 15 kDa (mitochondria and microsomes) and in the cytosol also at 60 - 57 kDa (cytosol) while in the selenium-deficient tissues the tracer was also incorporated into other proteins.

5.2.3 Characterization and identification of selenium-containing proteins in the lung and trachea

In further experiments the selenium-containing proteins in the lung and trachea were more closely investigated. For these experiments only selenium-deficient animals were taken, because of the much stronger retention of the tracer. In this way it was also possible to compare the selenium-containing protein distribution among the tissues of the respiratory tract.

The two autoradiograms in the Figure 5-16 show the ^{75}Se-containing proteins in the homogenates and cellular compartments of the lung and trachea. The results of the evaluation of these data are listed in the Table 5-3.

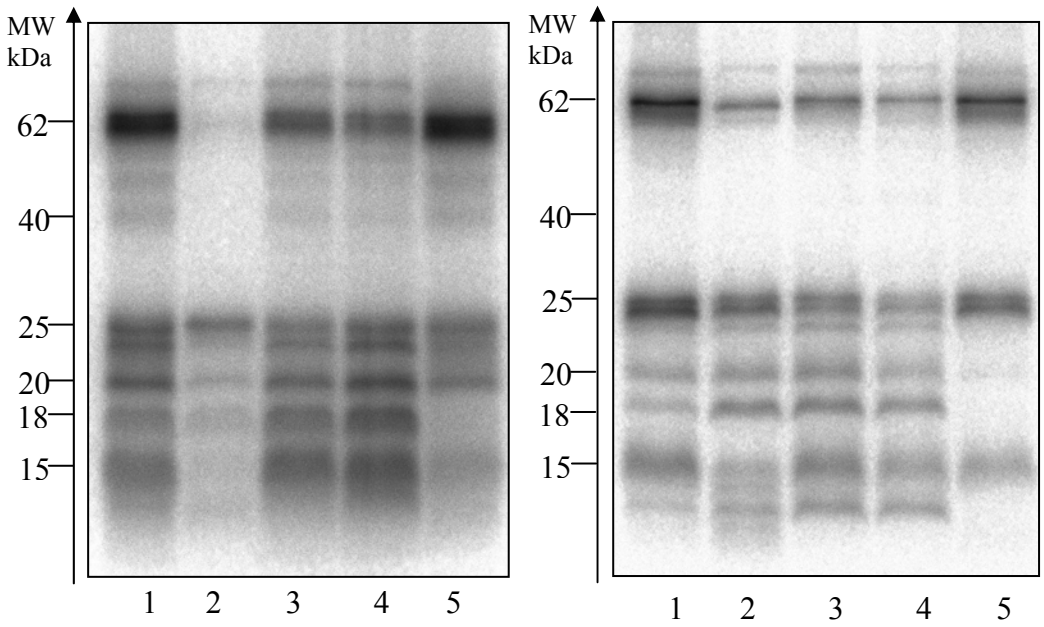

Figure 5-16 Autoradiogram of the ^{75}Se-labeled proteins in the homogenate (1) and the nuclear (2), mitochondrial (3), microsomal (4) and cytosolic (5) fractions of the rat lung (A) and rat trachea (B) after Laemmli-SDS-PAGE.

Table 5-3 ^{75}Se-labeled bands found in lung and trachea samples; ■ – strongly labeled, ▪ - medium-labeled , □ - weakly labeled, ▫ - very weakly labeled

^{75}Se-labeled bands[kDa]	Lung					Trachea				
	Hom	N	Mt	Mc	Cyt	Hom	N	Mt	Mc	Cyt
74	□	□	□	□	▫	□	□	□	□	□
67	■	▫	▪	▪	■	■	▪	▪	▪	■
57-53	■	▫	▪	▪	■	▪	□	▫	▫	▪
49	□	▫	▫	▫	□	▫	▫	▫	▫	▫
40	□	▫	□	□	□	□	▫	▫	▫	□
25	■	■	■	■	■	■	■	■	■	■
23	■	□	▪	■	▪	■	▪	▪	▪	■
20	■	□	■	■	▪	■	■	■	■	▪
18	▪	□	■	■		▪	□	■	■	
16-15	■	□	■	■	▪	■	□	■	■	▪
12-10	▪	▫	▪	▪	▫	▪	▫	▪	▪	▫

The distribution of the labeled selenium-containing proteins after SDS-PAGE differed within the cellular compounds, but there were no remarkable differences between lung and trachea.

The homogenates of the lung and trachea showed almost the same selenoprotein distribution pattern. Bands were detected at 74 kDa, 67 kDa, 57 -53 kDa, 25 kDa, 23 kDa, 18 kDa, 16 – 15 kDa and 12 kDa. In the homogenate, mitochondria, microsomes and cytosols of the tissues three bands between 49 and 40 kDa were observed. The cytosolic fraction did not contain the band at 18 kDa. The bands found at 67 kDa and 57 – 53 kDa were more distinct in the homogenate and subcellular fractions of the lung. In both tissues the band at 57 – 53 kDa was more pronounced in the homogenates and cytosols.

5.2.3.1 Identification of the selenoproteins in the respiratory tract

5.2.3.1.1 Identification of a 15 kDa protein

The protein at 15 kDa was detected in all subcellular fractions of the lung and trachea, but had the highest ^{75}Se activity in the mitochondrial and cytosolic fractions. Its pI value was comparable to that of the 15 kDa protein found in the prostate, thyroid, large intestine and brain. By co-electrophoresis of the 15 kDa selenium-containing band of the lung and trachea and the 15 kDa selenium-containing bands of the prostate and large intestine it was shown that the labeled bands migrated in the same way (Figure 5-17). The 15 kDa bands from these tissues (lung, prostate, large intestine, thyroid and brain) were subjected to trypsin proteolysis and the peptides were separated by tricine-SDS-PAGE. In each sample the same number of peptides with the same molecular masses were found. The results indicated that the selenoprotein found at 15 kDa in the respiratory tract is identical to the 15 kDa protein present in the other tissues.

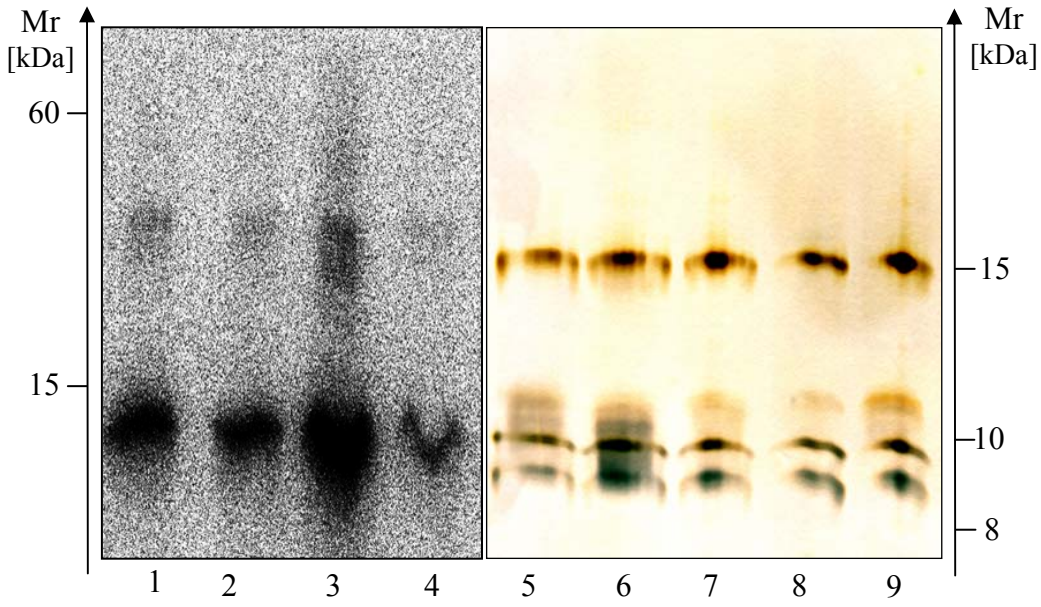

Figure 5-17 Left: Autoradiogram of the ^{75}Se-labeled 15 kDa protein of the rat lung (1), large intestine (2), prostate (3), trachea (4) after SDS-PAGE. Right: Silver-stained gel of the fragments of the 15 kDa protein of the rat lung (5), prostate (6), large intestine (7), thyroid (8), brain (9) after trypsin proteolysis separated by SDS-PAGE (17 % gel)

5.2.3.1.2 Immunoassay for the identification of selenoproteins

The immunoassay is the fastest and most specific method for the identification of proteins, if a suitable antibody is available. As most of the selenoproteins are not completely identified and characterized it was only possible to obtain antibodies for the recognition of the TrxR1, SelP, GPx1, SelT. For these selenoproteins the immunoassays were carried out. The immunoblots are shown below.

Selenoprotein P

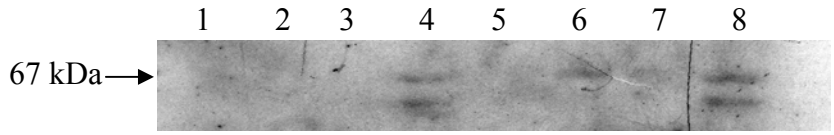

Figure 5-18 Immunological detection of Selenoprotein P in human and rat epithelial cells of the lung; 1- culture medium from rat epithelia, 2 - culture medium from human epithelia, 3 - cytosolic fraction of rat epithelia, 4 - cytosolic fraction of human epithelia, 5- mitochondrial fraction of rat epithelia, 6 - mitochondrial fraction of human epithelia, 7- homogenate of rat epithelia, 8 - homogenate of human epithelia

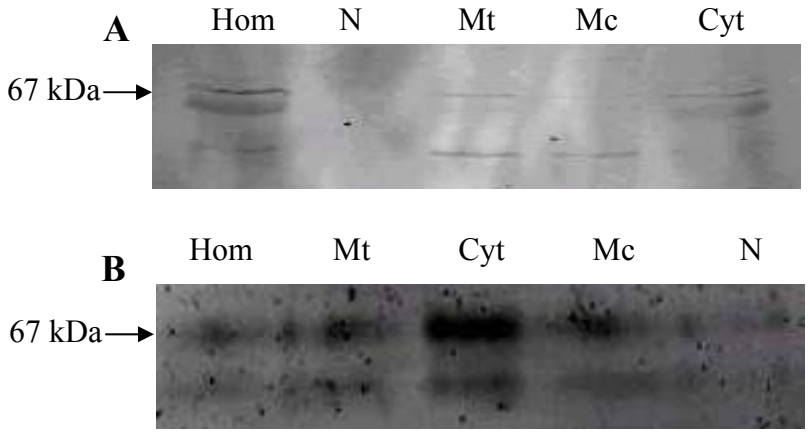

Figure 5-19 Immunological detection of Selenoprotein P in the subcellular fractions of the rat lung (A) and trachea (B); Hom- homogenate, N- nuclear, Mt- mitochondrial, Mc- microsomal, Cyt- cytosolic fraction

Trx1

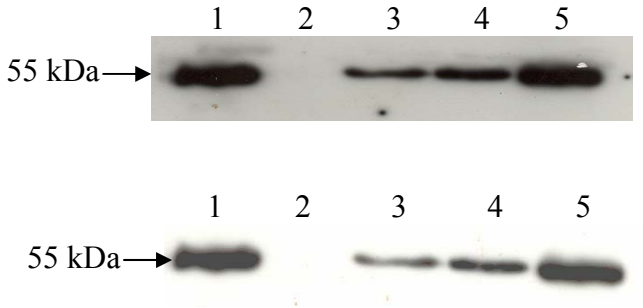

Figure 5-20 Immunological detection of TrxR1 1 in the subcellular fractions of the lung (upper part) and trachea (lower part); lines: 1- homogenate, 2 - nuclear, 3 - mitochondrial, 4 - microsomal, 5 - cytosolic fraction

Gpx1

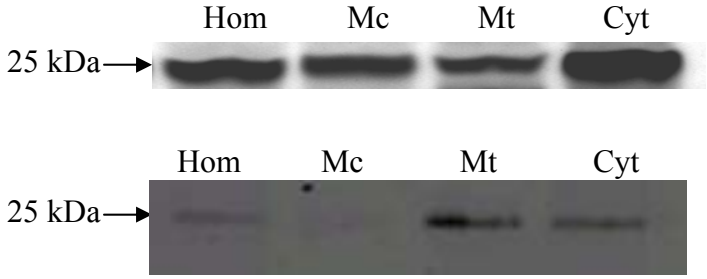

Figure 5-21 Immunological detection of glutathione peroxidase Gpx1 in the subcellular fractions of the rat lung (upper part) and trachea (lower part); Hom- homogenate, Mc- microsomal, Mt- mitochondrial, Cyt- cytosolic fraction

SelT

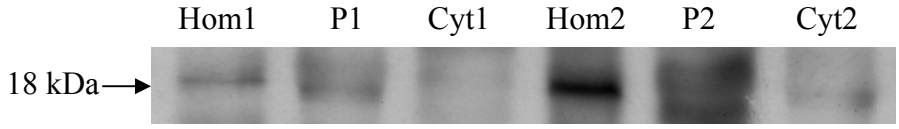

Figure 5-22 Immunological detection of the 18 kDa selenoprotein (SelT) in the homogenate (Hom), pellet at 100000×g (P) and cytosol (Cyt) of the rat trachea (1) and lung (2)

In the homogenate and subcellular fractions of the lung and trachea the selenoprotein P (SelP), thioredoxin reductase (TrxR1), glutathione peroxidase (Gpx1), 15 kDa selenoprotein

(Sel15) and 18 kDa selenoprotein (SelT) were identified. The immunoblot of SelP was also positive in the homogenate of the human epithelial cells.

5.2.3.2 Study on the selenium-containing proteins in the lung and trachea by two-dimensional gel electrophoresis

For further characterization of the selenoproteins present in the homogenates and subcellular fractions of the lung and trachea two-dimensional electrophoresis (IEF/SDS-PAGE) was applied. The following autoradiograms show the distribution of the selenium-containing proteins in the homogenates, cytosols, mitochondria, microsomes, and nuclei of the two tissues after separation by this method. In this way the molecular mass and the pI value of the selenium-containing proteins were obtained after autoradiographical localization of these proteins.

In the homogenates of the lung and trachea one spot with a molecular masse at 74 kDa and a pI value between 4.8 and 5.0 was found. It could be identified as the selenoprotein O (SelO). At 60 – 53 kDa and a pI value of 5.2 – 6.0 three spots were observed. These molecular masses and pI values are characteristic for TrxR1 and TrxR2. However the TrxR2 is a selenoprotein found so far only in the brain. In the homogenate of the lung one spot at 60 kDa and a pI value of 8.4 was observed. In both homogenates ten spots with molecular masses between 40 - 29 and pI values of about 4.5 - 8.0 were found. In the pI range between 6.4 – 7.0 seven spots with molecular masses at 25 - 23 kDa were observed. They were identified as subunits of the glutathione peroxidase Gpx1 and Gpx3. Two further spots were found at 20 kDa (pI values 7.0 - 7.8) and 18 kDa (pI value 7.2 -7.8) They stemmed from Gpx4 and the 18 kDa selenoprotein. At the molecular masses between 18 and 16 kDa one spot with a pI value of about 4.8 to 5.0 and two spots with pI values of 5.0 and 6.2 were found. Between 15 and 14 kDa , too, several spots were observed with the pI values 4.5 - 4.8, 5.8 – 6.6, 6.8 – 7.5. The spot with the pI values of 4.5 - 4.8 was previously identified as 15 kDa selenoprotein (Sel15) [57, 58] and the spot with a pI value of about 6.8 – 7.5 as the neutral 15 kDa selenoprotein. In the trachea homogenate one very strongly labeled spot with a pI value of about 5.8 – 6.0 was observed at 12 kDa. In the molecular mass range between 12 and 10 kDa and pI values of 4.5 - 4.8 three spots were found in both tissues. There were also several spots in the molecular mass range below 10 kDa with pI values of 5.2 - 6.0. In the lung homogenate there was one spot at 10 kDa and a pI value of 8.0 – 8.5, which probably stemmed from selenoprotein W (SelW).

Table 5-4 Number of ^{75}Se-labeled spots found in the lung and trachea samples after 2D electrophoresis

	Number of ^{75}Se labeled spots				
	Homogenate	Nuclei	Mitochondria	Microsome	Cytosol
Lung	34	22	34	27	37
Trachea	37	20	32	21	36

In the autoradiograms of the 2D gels a large number of ^{75}Se-labeled proteins were detected as can be seen in the Table 5-4.

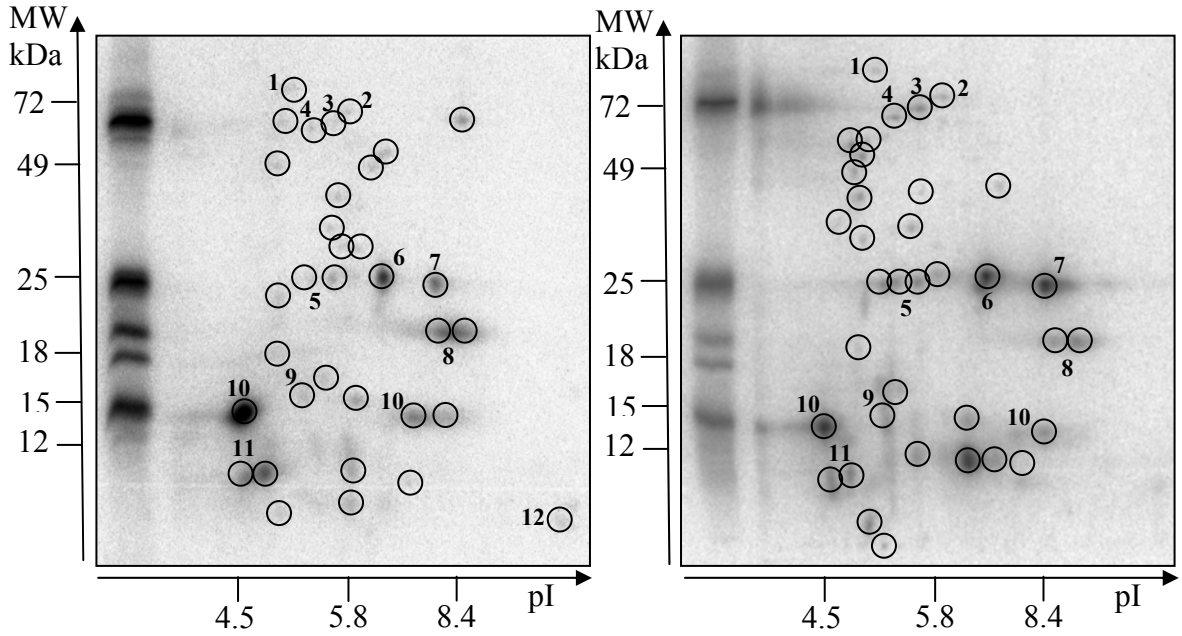

Figure 5-23 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the homogenates of the lung and trachea of rats fed a selenium-deficient (Se-) diet

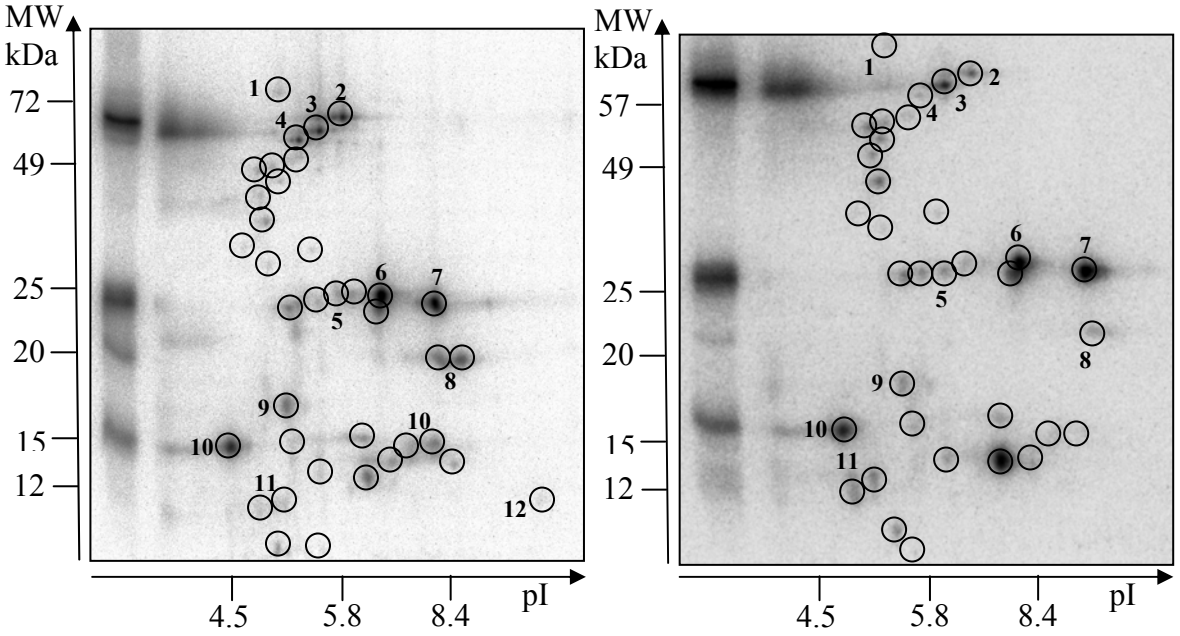

Figure 5-24 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the cytosolic fraction of the lung and trachea of rats fed a selenium-deficient (Se-) diet

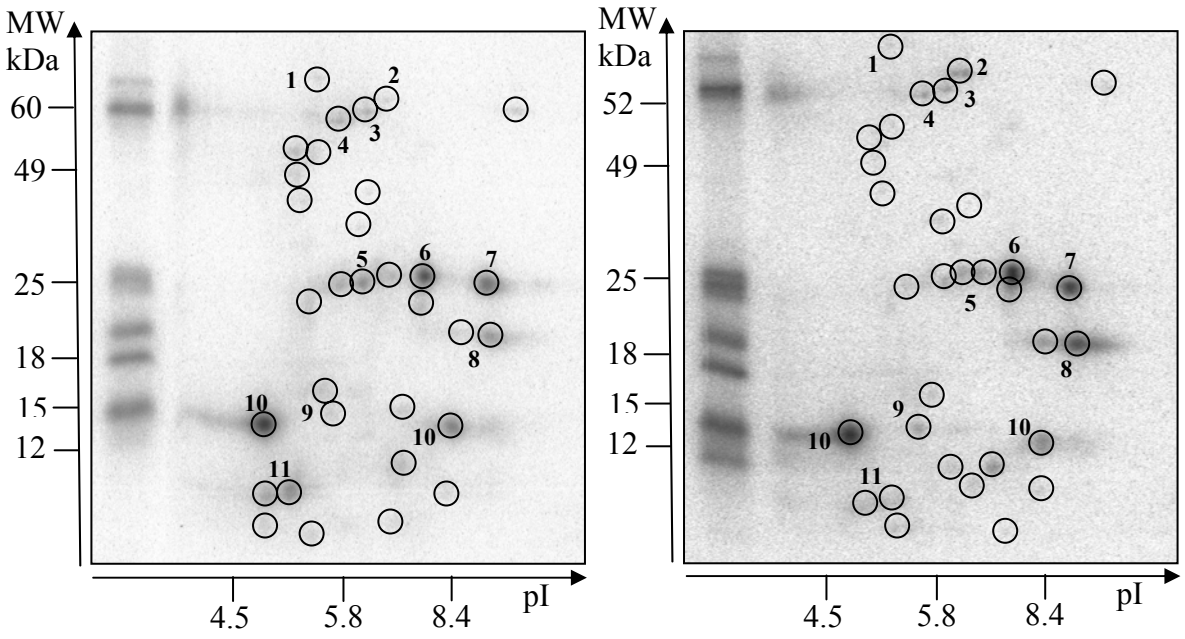

Figure 5-25 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the mitochondrial fraction of the lung and trachea of rats fed a selenium deficient (Se-) diet

In the cytosolic fraction of the both tissues the spot at 16 kDa and the three spots at 12 -10 kDa with pI values of 4.5 - 4.8 were not observed. In the lung homogenate a spot at 60 kDa

with a pI value of 8.4 could likewise not be seen. In the lung and trachea samples at the molecular masses of 12 kDa and with a pI of about 5.8 – 6.0 one strongly labeled spot was detected. The spot with a pI of 4.8 and the masse of 16 kDa, which was not seen in the homogenates, was detected in the cytosolic fractions. With the other selenium-containing subunits no differences were observed. Some of them such as two spots at 25 – 23 kDa and one at 15 kDa were more strongly labeled than in the homogenates.

The mitochondrial fractions showed almost the same pattern of selenium-containing proteins as the homogenates (Figure 5-25). Some differences, however, were observed, for example more distinct spots at 60 kDa with a pI value of 8.4, at 18 kDa with pI values of about 4.8 and 5.0, and at 15 kDa with pI values of about 6.8 – 7.5. This could mean that these selenium-containing proteins are membrane-bound. Not all of the spots with masses between 40 – 29 kDa and pI values of about 4.5 - 8.0 detected in the homogenates were found in the mitochondrial fraction.

In the microsomal fractions of the lung and trachea (Figure 5-26) the spots with masses of 40 – 29 kDa and pI values of about 4.5 - 8.0 were missing. There were seven strongly labeled spots at 25 – 23 kDa and pI values of 6.4 -7.0, two at 20 kDa with pI values of 7.0- 7.8 and five at 15 -12 kDa with pI values of 4.5 - 4.8, 5.8 – 6.6, 6.8 – 7.5 and 4.5 - 4.8, 5.8 – 6.0. In this range several additional, very weakly labeled spots could be distinguished.

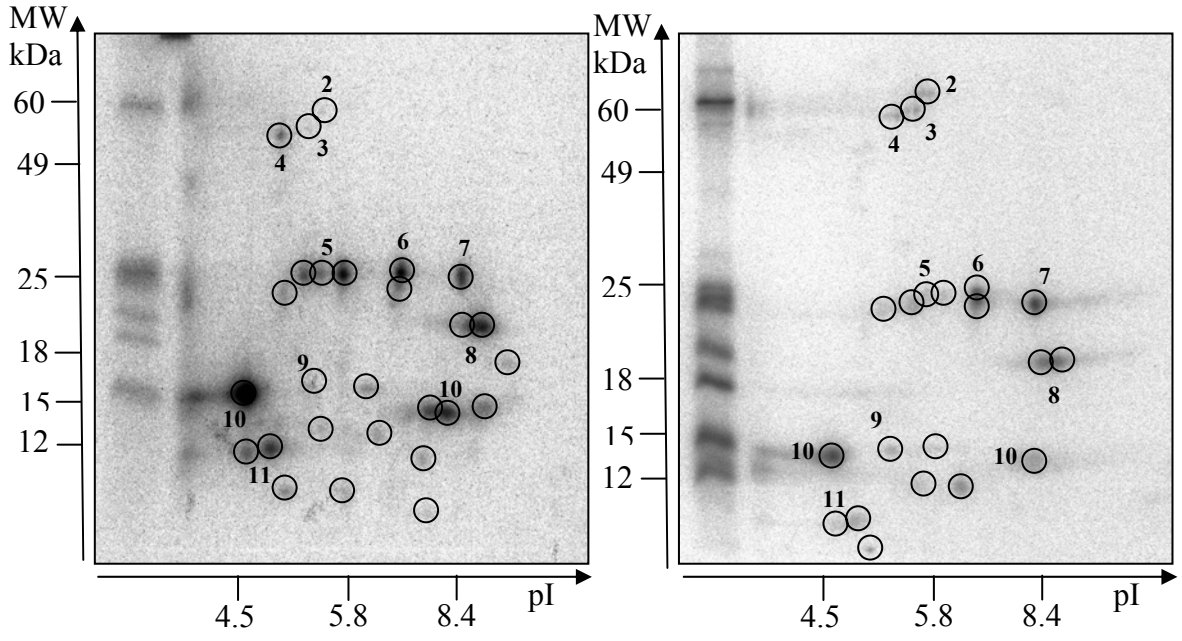

Figure 5-26 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the microsomal fraction of the lung and trachea of rats fed a selenium deficient (Se-) diet

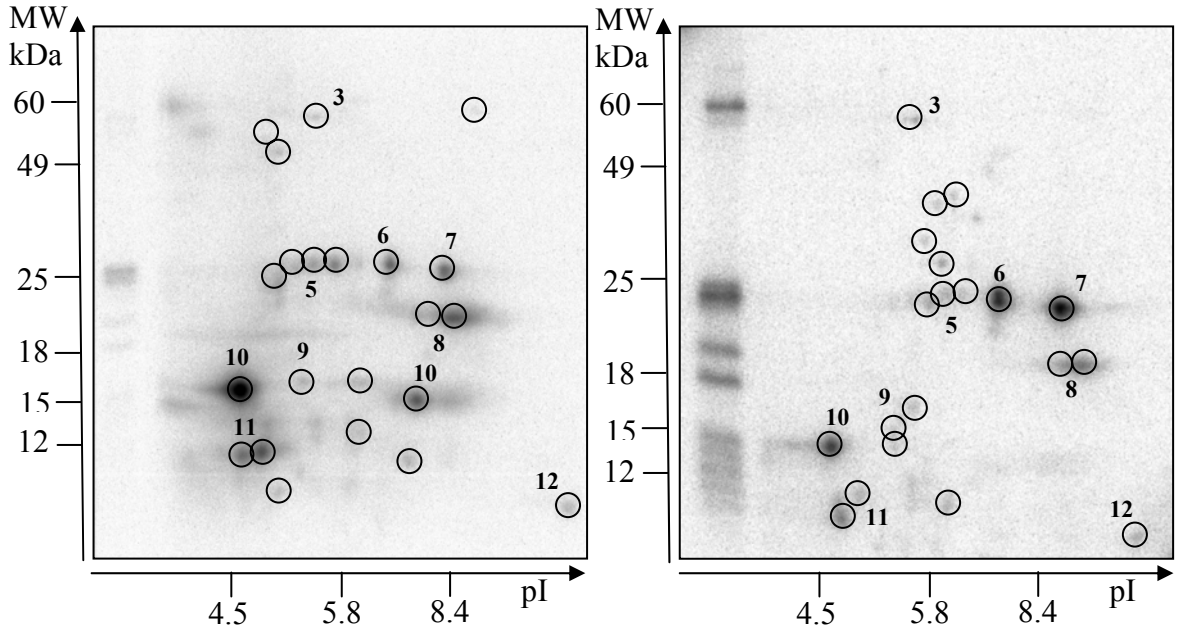

Figure 5-27 Autoradiograms of ^{75}Se-labeled proteins found after IEF/SDS-PAGE in the nuclear fraction of the lung and trachea of rats fed a selenium deficient (Se-) diet

Table 5-5 Classification of the ^{75}Se containing-proteins found in the lung and trachea samples

Nr.	selenoprotein		Nr.	selenoprotein
1	SelO		7	Gpx?
2	SelP		8	Gpx4
3	TRx1- TRx2		9	16 kDa
4	TRx1- TRx2		10	15 kDa
5	Gpx1		11	12 kDa
6	Gpx3		12	SelW

Most of the selenium-containing proteins found in the nuclear fractions of the lung and trachea had molecular masses below 25 kDa. They were identical to those detected in the homogenates (Figure 5-27). At 60 – 57 kDa only two spots with pI values of 5.2 – 6.0 were found. In both fractions one spot at 10 kDa and pI values of 8.0 – 8.5 were observed. The selenium-containing proteins found in the nuclei of the lung are described in the section 5-5.

In the analysis of the autoradiograms no differences in the distribution of the selenium-containing proteins in both tissues and their subcellular compartments were observed. Some of the selenium-containing proteins found in the lung and trachea could be identified as is shown in the Table 5-5.

5.2.4 Gpx1 activity in the homogenates and cytosols of the lung and trachea

Glutathione peroxidase (Gpx, EC 1.11.1.9) catalyzes the reduction of hydroperoxides, including hydrogen peroxide, by glutathione and in this way protects the cell from oxidative damage. This enzyme is a tetramer with four identical subunits. Each subunit contains a selenocysteine in the active site which participates directly in the two-electron reduction of the peroxide substrate. The enzyme uses glutathione as the ultimate electron donor to regenerate the reduced form of the selenocysteine [128, 129].

The 25 kDa protein was identified as Gpx1 (see section5.2.3.1.2). In the homogenates and cytosols of the lung and trachea of rats fed either a selenium-deficient or selenium-sufficient diet the activity of the glutathione peroxidase - Gpx1 (EC 1.11.1.9) was measured. The results are presented in the Figure 5-28.

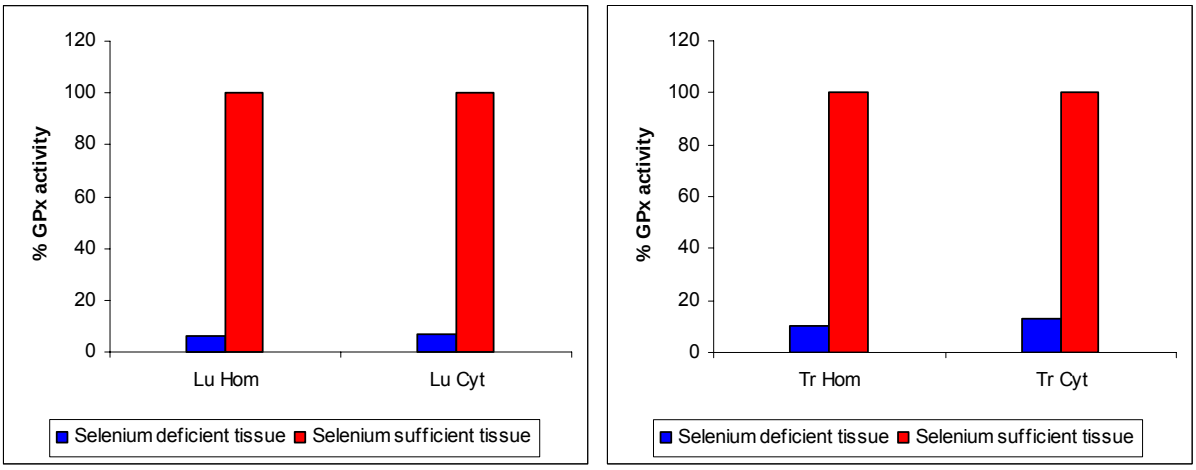

Figure 5-28 Gpx1 activity (% of control) in the homogenate and cytosolic fraction of the lung (left) and trachea (right) of selenium deficient and selenium-sufficient animals

There were distinct decreases in Gpx1 activity found in the cytosols and homogenates of the lung and trachea in the deficient rats as compared with selenium-sufficient animals. The activity of the enzyme was ten - fold lower in both lung and trachea fractions of the deficient animals.

5.2.5 Discussion

The results confirm the previous hypothesis that the distribution of selenium among the different binding forms is strongly dependent on the selenium status. After administration of labeled selenite to the selenium-deficient rats only a small percentage of the amount retained in the lung and trachea was found in the 25 kDa protein, identified as glutathione peroxidase

(Gpx1), and instead other selenoproteins such as the 20 kDa (PHGpx), the 15 kDa (Sel15) and the 12 kDa were preferentially supplied. In the lung and trachea of selenium-sufficient animals, however, the majority of the tracer was contained in the glutathione peroxidase. The phospholipid hydroperoxide glutathione peroxidase (PHGpx) is a monomer with a molecular mass of 19.7 kDa, found in many tissues in both cytosolic and membrane-associated forms. It can directly reduce phospholipid hydroperoxides. The results of several studies suggested that the enzyme may have important functions in the redox regulation of variety of processes such as inflammation [49]. A 15 kDa protein has been found in several mammalian tissues [57, 58]. Its function and its biological significance are not yet known but its further investigation is of special interest with regard to the decreased incidence of lung and prostate cancer with selenium supplementation. Kryukov et al. in their studies showed that a 12 kDa selenoprotein called also SelR or SelX is a zinc-containing protein [64]. The presence of selenium and zinc, known for their antioxidant actions, may suggest a role of the 12 kDa selenoprotein in the protection against oxidative stress and redox regulation of cellular processes. However, the function of this protein is not yet known.

Selenium protects mammals from oxidative stress. The nutritional level of this element has been correlated with the prevention and reduction of lung cancer, but the possible involvement of the selenoproteins in those mechanisms have not been elucidated [94]. The dietary selenium intake can strongly influence the selenoprotein levels. Most of the attention in this field has been focused on glutathione peroxidase. However, the role of selenium in the form of this enzyme could explain several, but not all the effects of selenium deficiency. About two thirds of the selenium present in the organism is not bound to this enzyme but is present in other compounds. This suggested that there are further biologically active forms of selenium [140]. The fact that with insufficient selenium intake the element is mainly used for restoring the level of other selenoproteins, is an indication of their importance. This is supported by the previous finding of Behne et al, which shows that the level of the 25 kDa protein is mainly built up during the later phases of repletion, after the pools of the other selenoproteins have been refilled [130]. The chemopreventive effect of selenium is probably mediated through its role in more than one component. Information on the presence of more than 30 selenium-containing proteins in the lung and trachea was obtained after labeling of rats *in vivo* with ^{75}Se and identification of the selenium containing proteins from the tracer distribution after electrophoretic separation. Those compounds differ in their distribution among subcellular compartments, which suggest that they may be involved in different cellular processes.

Further studies are needed to explain the role of selenium-containing proteins in the lung and trachea and especially the biological significance of the 15 kDa and 12 kDa selenoproteins.

5.3 Selenium-containing proteins in the cultured cells of the lung and trachea

This part of the experiments was concerned with the identification and characterization of the selenium-containing proteins in cultured cells of the lung and trachea. For this purpose the lung epithelial cell line A549 (human), the lung epithelial cell line CCL (rat) and the tracheal fibroblast cell line CRL7422 (rat) were chosen. The cells were cultured as described in the section 2.1.3.

The lung epithelium is constantly exposed to high concentrations of oxygen and oxidants and is thus a primary target for reactive oxygen species (ROS) [131]. Therefore, lung epithelial cells are fortified with high intracellular and extra-cellular levels of antioxidants [132]. However, massive amounts of ROS are generated in conditions such as inflammation or exposure to cigarette smoke, air pollutants, and drugs [131, 132]. Consequently, the oxidation -reduction state of the cell is altered, an oxidant-antioxidant imbalance results and overall tissue integrity is threatened [133]. These cells have developed sensitive and effective antioxidant defenses, in which selenium may play an important role. Another function of the lung epithelium is a formation of a restrictive barrier between the lumen and the underlying sub mucosa. In asthma and chronic obstructive pulmonary disease, this epithelial barrier is disturbed, possibly contributing to the initiation and exacerbation of the inflammatory process that occurs in these diseases [134]. Many of the inflammatory diseases can initiate a repair process, characterized by accumulation of the fibroblasts and myofibroblasts and extra cellular connective tissue [131].

The cells were labeled *in vitro* with 40 kBq ^{75}Se (0.1µg Se in 1l medium), applied to the medium 24 hours after cell plating. After 48 or 72 hours the cells were harvested and homogenized (see section 2.2.5.1). The homogenates were then separated by differential centrifugation into a nuclear, mitochondrial, microsomal and cytosolic fraction. For each sample the protein concentration and the activity of the tracer were determined. Aliquots of the fractions with equal amounts of the proteins were separated by means of SDS-PAGE or 2D-SDS-PAGE. After staining and drying of the gels, the selenium compounds were identified autoradiographically via the ^{75}Se activity. The selenoproteins present in the

subcellular compartments of lung and trachea were then characterized via their molecular mass and pI value.

5.3.1 Selenium-containing proteins in the subcellular fractions of the cell lines A549, CCL and CRL7422

The selenium-containing proteins found in the homogenates and subcellular fractions of the lung epithelial cells A549, CCL and tracheal fibroblasts CRL are shown in the Figure 5-29 and 5-30.

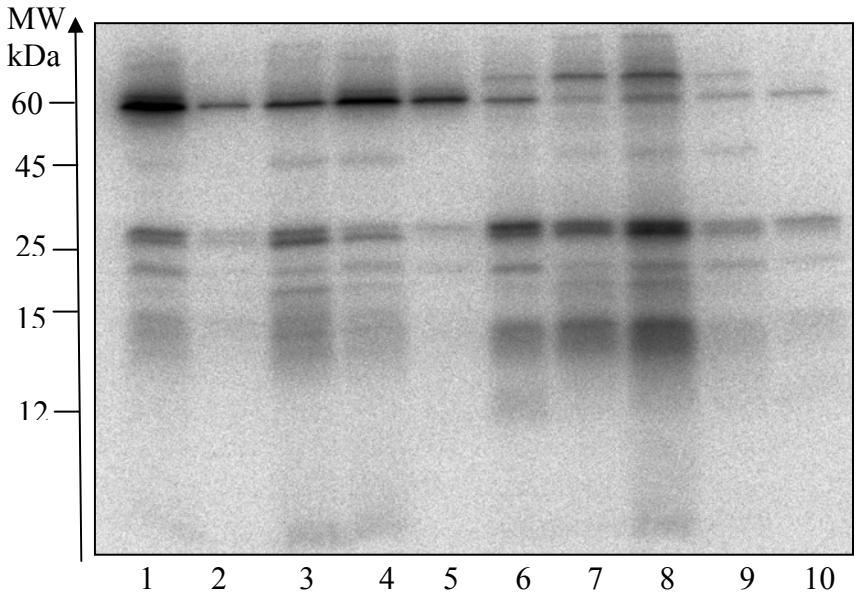

Figure 5-29 Autoradiogram of the ^{75}Se-labeled proteins in the homogenate (1) and the nuclear (2), mitochondrial (3), microsomal (4) and cytosolic (5) fractions of the A549 and in the homogenate (6) and the nuclear (7), mitochondrial (8), microsomal (9) and cytosolic (10) fraction of the CCL cells after SDS-PAGE

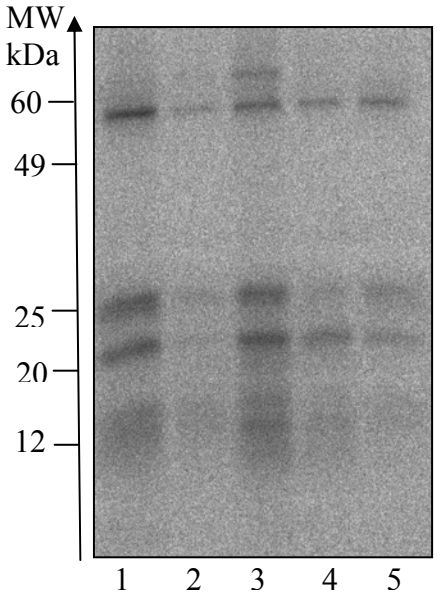

Figure 5-30 Autoradiogram of the ^{75}Se-labeled proteins in the homogenate (1) and the nuclear (2), mitochondrial (3), microsomal (4) and cytosolic (5) fraction of the CRL7422 cells after Laemmli-SDS-PAGE

As observed in the tissues, the distribution patterns of the labeled selenium-containing proteins differed also among the cellular compartments. However, there were no remarkable differences with regard to the selenoprotein characteristics between these three cell lines after SDS PAGE. In all fractions the same selenoproteins were found. ^{75}Se-containing bands at 74 kDa, 65 kDa, 57 – 53 kDa, 25 kDa, 23 kDa, 20 kDa, 18 kDa, 16 kDa, 15 kDa, 12 kDa were detected. In the epithelial cells A549 and CCL a band at about 40 kDa was observed in the homogenate, mitochondria, microsomes, and cytosols, but not in the nuclei. This band was likewise not found in the CRL cells. The band at 74 kDa was not present in the A549 cells and it was very weakly labeled in the CRL7422 cells. In the cytosolic fraction of all cell types the band at 18 kDa was missing. The bands found at 57 – 53 kDa, 25 – 23 kDa and 15 kDa were most strongly labeled.

5.3.1.1 Comparison of the selenoproteins in human lung and trachea cells

The lung epithelial cells and tracheal fibroblasts are morphologically different cell types of the respiratory tract. It was interesting to investigate if they differ in the expression pattern of the selenium-containing proteins.

The samples obtained from those cells were separated by SDS-PAGE and the selenium-containing proteins were made visible by autoradiography. The autoradiograms of the homogenate, pellet (fraction obtained after centrifugation of the cell homogenate at 100000×g) and cytosol of those cells are shown below (Figure 5-31).

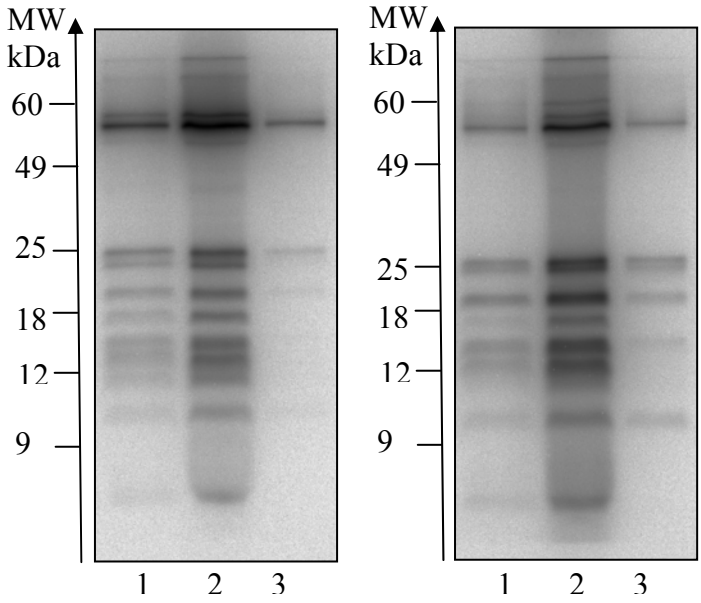

Figure 5-31 Autoradiogram of the ^{75}Se-labeled proteins in the homogenate (1), pellet (2) and cytosol (3) of the human epithelial cells (left) and tracheal fibroblast cells after Laemmli-SDS-PAGE

In the homogenates of the human lung epithelial cells twelve selenium-containing proteins with molecular masses of 57 kDa, 55 kDa, 53 kDa, 45 kDa, 25 kDa, 23 kDa, 20 kDa, 16 kDa, 15 kDa, 12 kDa and 10 kDa were detected. The same proteins were found in the pellet of the lung epithelia. The homogenate and pellet of the tracheal fibroblasts contained one additional band at 74 kDa. The cytosolic fraction of both cell types contained the selenoproteins with molecular masses of 65 kDa, 25 kDa, 23 kDa, 20 kDa, 15 kDa and 12 kDa. The proteins present in both cell types differed in the intensity of labeling. In the lung epithelia the bands at the 57 kDa was most strongly labeled, while in the tracheal fibroblasts the band at 57 kDa, 20 kDa, 16 kDa and 15 kDa had the highest tracer content.

5.3.1.2 Characterization of the selenium-containing proteins in the cultured cells by means of 2D-SDS-PAGE

The following autoradiograms show the distribution of the selenium-containing proteins in the homogenates and cytosols of the A549, CCL and CRL7422 cells after separation by 2D electrophoresis.

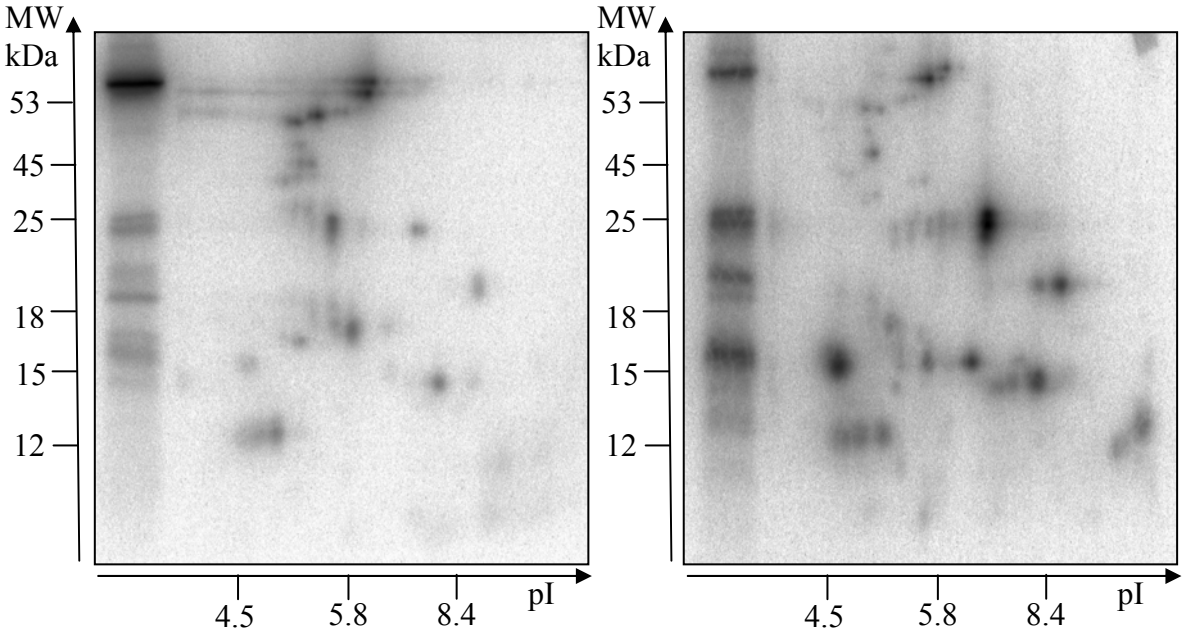

Figure 5-32 Autoradiogram of the ^{75}Se-labeled proteins in the homogenate of the human epithelial cells (left) and rat epithelial cells after 2D electrophoresis

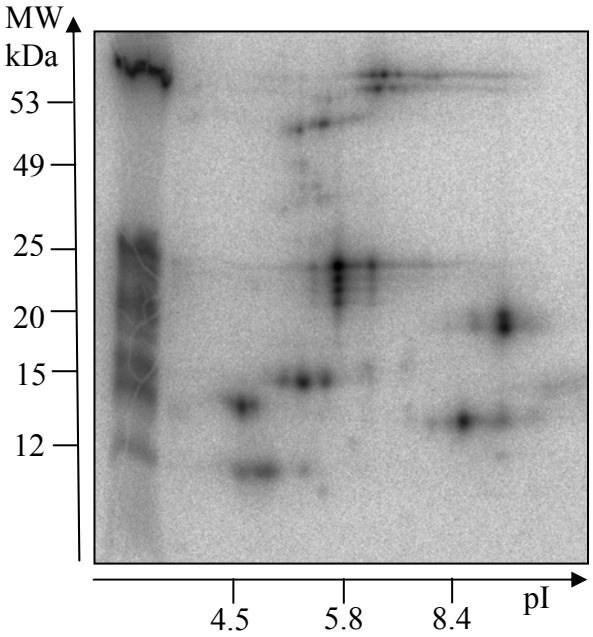

Figure 5-33 Autoradiogram of the ^{75}Se-labeled proteins in the homogenate of the human tracheal fibroblast after 2D electrophoresis

In the homogenates of all cell types one spot with a molecular mass at 74 kDa and pI a value between 4.8 and 5.0 was found. At 60 – 57 kDa and pI values of 5.2 – 6.0 two spots were observed. The molecular masses and pI values are characteristic for TrxR1 and TrxR2. Two spots with molecular masses at 55 - 53 kDa and pI values of 4.8 – 5.0 were detected. In all homogenates several spots of molecular masses between 40 - 29 kDa and pI values of about 4.5 - 8.0 were found. However, they were weakly labeled. In the A549 and CRL7422 samples two spots with molecular masses at 25 - 23 kDa and pI values of 6.4 – 7.0 were observed, whereas in the CCL homogenate six additional spots were found. Two further spots were found at 20 kDa and pI values of 7 - 7.8, and one at 18 kDa with a pI value of 4.8 – 5.0. At the molecular masses of 16 kDa one spot with a pI value of about 4.8 - 5.0 and two spots with pI values of 5.0 - 6.2 were detected. In the homogenates of CRL7422 three spots with molecular masses of 16 kDa were present. Two spots at molecular masses in the range between 15 and 14 kDa were observed. The spots had pI values of 4.5 - 4.8 and 6.8 – 7.5. In the homogenates of all cell types three spots at molecular masses between 12 and 10 kDa and pI values of 4.5 - 4.8 were found. There were also several additional spots in the molecular range below 10 kDa with pI values of 5.2 - 6.0. In the CCL homogenate there was one spot at 10 kDa and a pI value of 8.0 – 8.5.

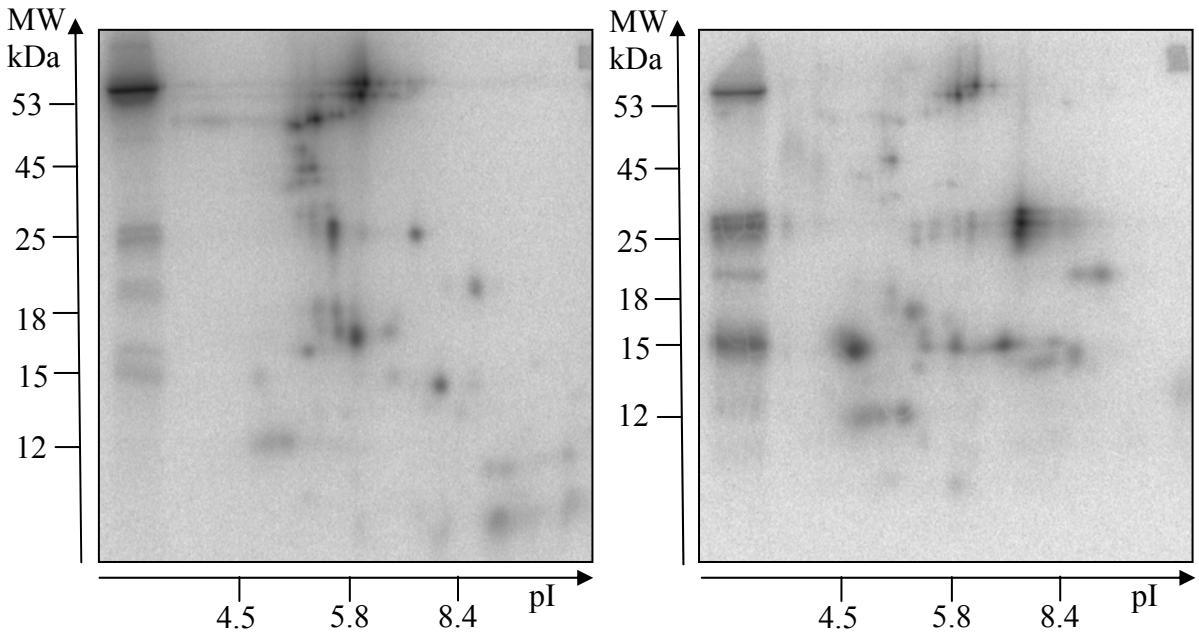

Figure 5-34 Autoradiogram of the ^{75}Se-labeled proteins in the cytosol of the human epithelial cells (left) and rat epithelial cells after 2D electrophoresis

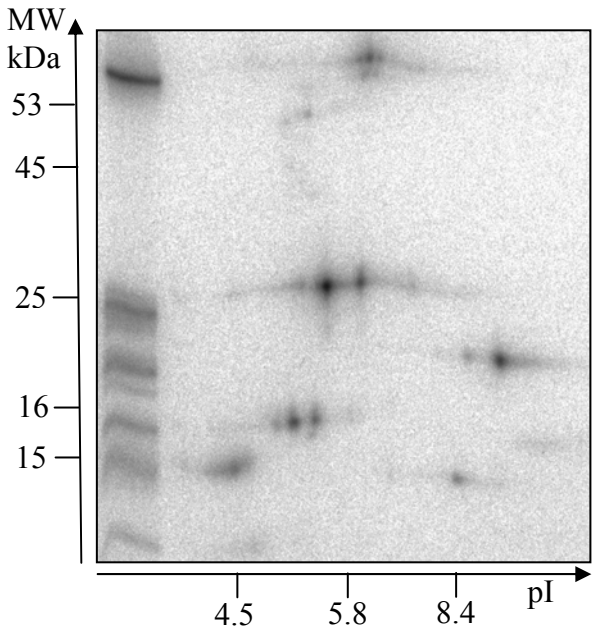

Figure 5-35 Autoradiogram of the ^{75}Se-labeled proteins in the cytosol of the human tracheal fibroblasts after 2D electrophoresis

In the cytosols of all cell types the spot at 18 kDa was not present. In the CRL7422 samples at the molecular masses of 12 kDa and a pI value of about 7.8 – 8.0 one strongly labeled spot was detected. There were also spots in the CCL homogenate in the molecular range below 10 kDa with pI values of 8.5 - 9.0. With the other selenium-containing subunits detected in the cytosolic fraction no differences were observed were compared with those in the homogenates.

5.4 Localization of selenoproteins in the cell compartments of the rat lung after special purification procedures

The subcellular fractions obtained by differential centrifugation are in most cases contaminated with other cellular compartments. The nuclear fraction may contain large mitochondria, the mitochondrial fraction endoplasmic reticulum or Golgi membranes. For better characterization of the subcellular fraction specific protocols were applied in the purification of nuclei, endoplasmic reticulum, lysosomes and ribosomes of the rat lung.

Nuclei

The nuclei were purified using a method of Windell and Tata (see section 2.2.4.2.3). The purity of the prepared nuclei was tested using a light microscope after coloration with Mayer's hemalum solution. The figure 5-36 shows the picture of the stained nuclei. The nuclear proteins were then separated by SDS-PAGE and 2D electrophoresis and the selenoproteins present in this fraction were visualized by autoradiography.

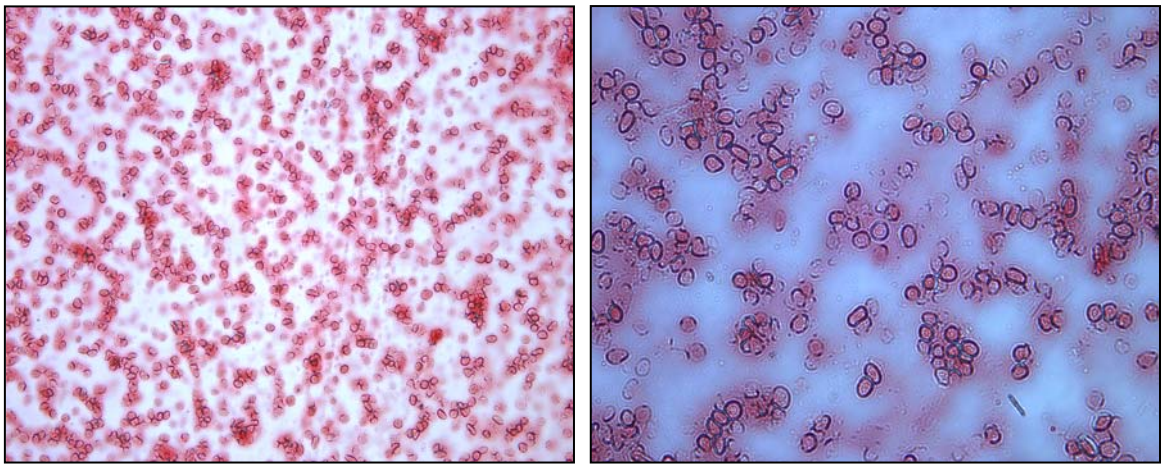

Figure 5-36 Nuclei from rat lung after coloration with Mayer's hemalum solution; magnification 200-fold (left) and 400-fold (right)

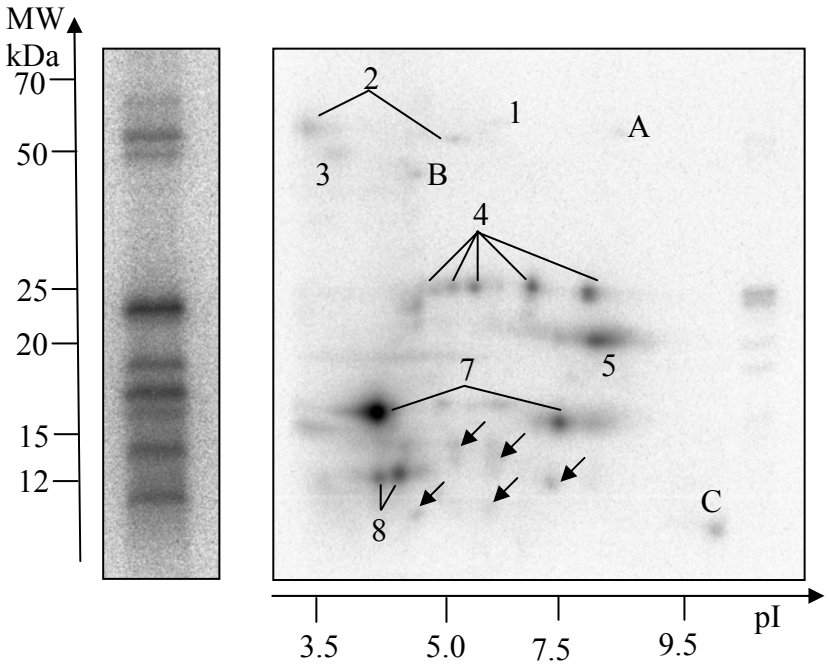

Figure 5-37 Autoradiogram of the selenoproteins in the nuclear lung fraction

The nuclei contained nine selenium - binding bands with molecular masses of (1) 74 kDa, (2) 60 kDa, (3) 55 kDa, (4) 25 kDa, (5) 20 kDa, (6) 18 kDa, (7) 15 kDa, (8)12 kDa. All bands detected in SDS-PAGE, except at 18 kDa, were resolved after 2D-PAGE into several spots

with different pI values. The 74 kDa proteins appeared on the autoradiogram in the acidic region. The protein nr. 3 was resolved into two spots both in the acidic region. The six spots with pI values between 5 and 8 came from the protein at 25-23 kDa. The 20 kDa protein occurred as one spot in a very basic region. The 15 kDa protein appeared as two spots in the acidic and neutral region and the protein nr. 8 with a molecular mass of 12 kDa was resolved into two spots, both in the acidic range. In the autoradiogram after 2D-PAGE more spots at 64 kDa (A), 40 kDa (B) and 10 kDa (C) and five further spots (marked with a arrow) with molecular masses smaller than 15 kDa and pI values between 5 and 7.5 could be detected.

Endoplasmic reticulum

The preparation of the smooth and rough endoplasmic reticulum was carried out as described in section 2.2.4.2.5. All fractions obtained were taken for electrophoresis. After the electrophoretic separation the selenoproteins were detected in the gel by autoradiography. The autoradiograms of lung homogenate (Lu-Hom), light mitochondria (Mt 1), mitochondria (Mt 2), smooth endoplasmic reticulum (ERS-S) in 1.3 M sucrose, smooth endoplasmic reticulum (ERS), rough endoplasmic reticulum (ERR), supernatant ($S_{100\ 000g}$) and wash fraction I (WI) are shown in the Figure 5-39. The purity of the endoplasmic fractions was improved using an immunoassay. The antibody used in this assay was anti-ER 72 kDa, a specific protein characteristic only for this cellular compartment. In this way the 72 kDa protein was found in the homogenate, the heavy mitochondrial fraction, and in both the smooth and rough endoplasmic reticulum fraction. It was not detected in the light mitochondrial and in the cytosolic fractions. The Western blot is shown in the figure 5-38.

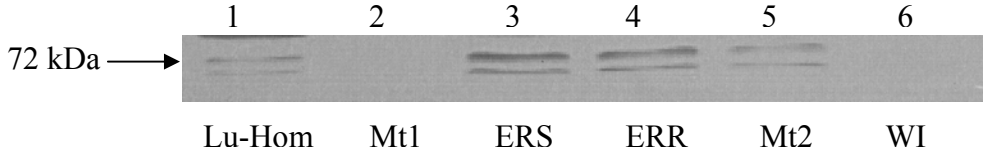

Figure 5-38 Western blot of the lung fractions: line 1 lung homogenate (Lu-Hom), line 2 light mitochondria (Mt 1), line 3 smooth endoplasmic reticulum (ERS), line 4 rough endoplasmic reticulum (ERR), line 5 mitochondrial fraction (Mt 2) and line 6 wash fraction I (WI)

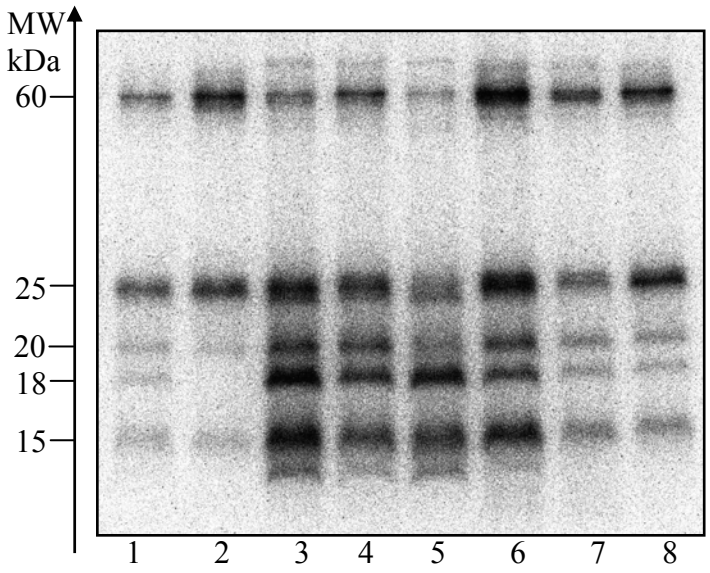

Figure 5-39 Autoradiogram of the selenoproteins in the lung homogenate (1), light mitochondria (2), mitochondria (3), smooth endoplasmic reticulum (4) in 1.3 M sucrose, smooth endoplasmic reticulum (5), rough endoplasmic reticulum (6), supernatant (7) and wash fraction I (8) of selenium-deficient rat lung after separation by SDS-PAGE.

For better characterization of the selenoproteins in the fraction of the ERR and ERS the two-dimensional electrophoresis was applied. The proteins were resolved in several subunits with different pI values. The figure 5-40 shows the autoradiogram of the ERS and ERR after 2D electrophoresis.

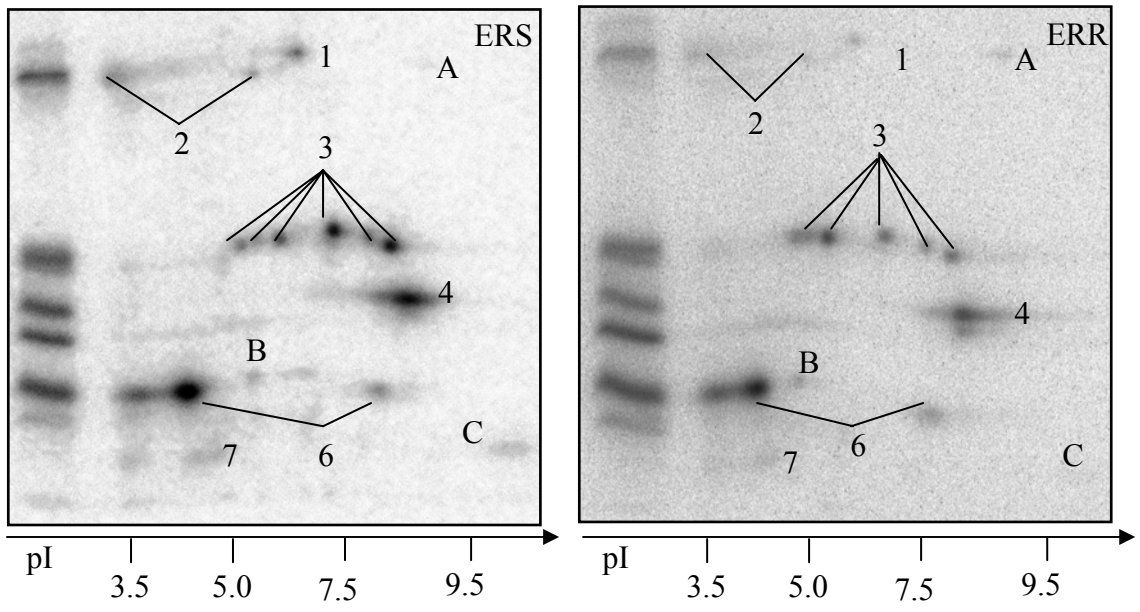

Figure 5-40 Autoradiogram of selenoproteins in the ERS and ERR fraction from rat lung after 2 D-PAGE.

After SDS-PAGE seven ^{75}Se-labeled bands could be detected in the ERS and ERR fractions. They had the following molecular masses (1) 74 kDa, (2) 57 kDa, (3) 25 kDa, (4) 20 kDa, (5) 18 kDa, (6) 15 kDa, (7) 12 kDa. Those selenium-containing proteins were also present in the autoradiogram of the nuclei proteins after 2D separation. The 74 kDa protein could be detected in slightly acidic pH area. The 57 kDa-band showed two spots, one in the very acidic and the other in the slightly acidic region. The 25 kDa band was resolved into six spots with different pI values of about 5 - 8, the 20 kDa protein appeared as one spot in the basic region. The 15 kDa band was resolved into two spots, one in the acidic and the other in the neutral region. The 12 kDa protein appeared as one spot in the acidic region. In the autoradiogram other spots could be detected, which were not observed after SDS-PAGE. They had molecular masses of about: (A) 65 kDa, pI value about 8, (B) 16 kDa, pI value 5, (C) 10 kDa, pI value 9.8. In the analyzed fractions the selenium-containing proteins at about 57 - 53, 25, 20, 18 and15 kDa were predominantly labeled.

Lysosomes

The lysosomal fraction was obtained using a method of Maunsbach. The preparation procedure is described in the section 2.2.4.2.7. The purity of the prepared lysosomes was tested using an electron microscope. The Figure 5-41 shows the picture of the lysosomes. The proteins present in the homogenates, mitochondria and lysosomes were then separated by SDS-PAGE and the selenoproteins present in these fractions visualized by autoradiography.

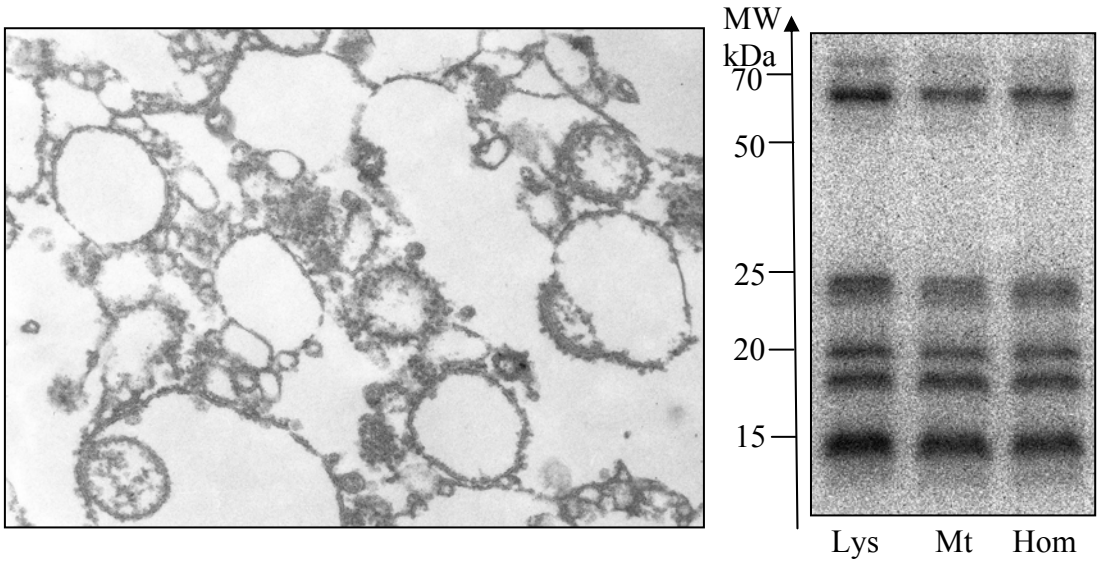

Figure 5-41 Lysosomes from the lung of the rat (left) and the autoradiogram of the selenoproteins in the lysosomes (Lys), mitochondria (Mt) and homogenate (Hom) of the selenium-deficient rat lung after separation by SDS-PAGE.

After SDS-PAGE seven ^{75}Se-labeled bands could be detected in the lysosomal fraction. They had the following molecular masses: 74 kDa, 57 kDa, 25 kDa, 23 kDa, 20 kDa, 18 kDa, 15 kDa, 12 kDa. The proteins at 18 kDa and 15 kDa were most strongly labeled.

Ribosomes

The ribosomes were purifiad as decribed in section 2.2.4.2.6. The picture below shows the ribosomes. The proteins of the homogenate, mitochondria, cytosol, wash fraction and ribosomes were separated by trcine SDS-PAGE and the selenium-containing proteins were then detected autoradiographically via the tracer. The autoradiogram is shown in the Figure 5-42. In the ribosomal fraction two selenium-containig proteins could be detected. They had molecular masses of 18 kDa and 15 kDa.

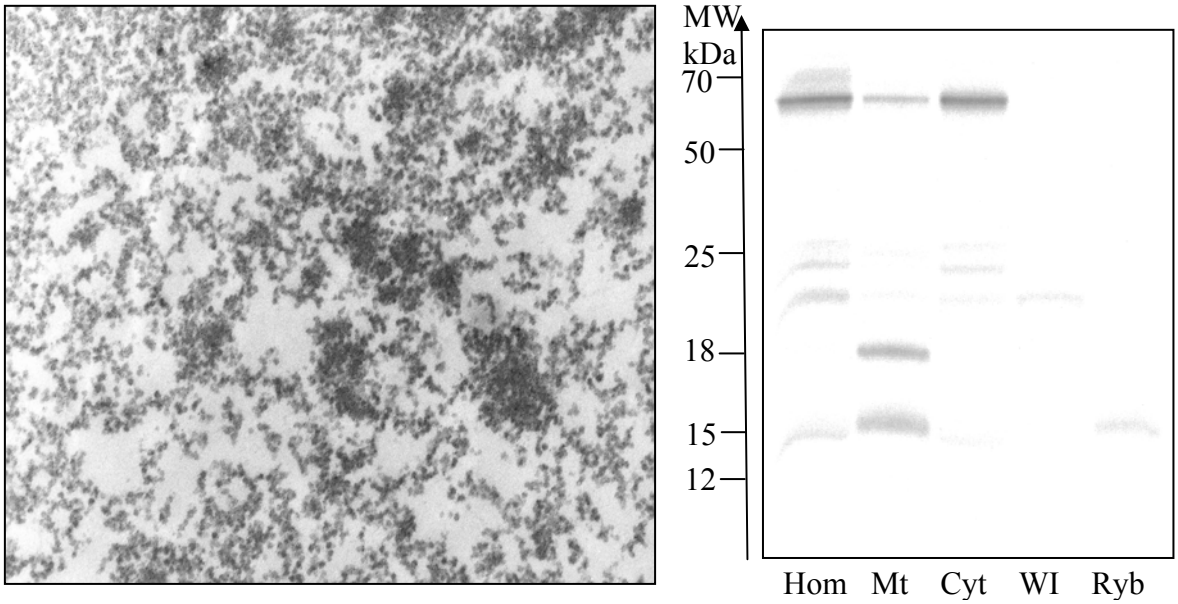

Figure 5-42 Ribosomes obtained from the lung of the rat- photo made of the electron microscope image (left) and autoradiogram of the selenoproteins in the homogenate (Hom), mitochondria (Mt), cytosol (Cyt), wash fraction (WI) and ribosomes (Ryb) after separation by tricine SDS-PAGE (right).

5.5 Summary

The protein separation by gel electrophoresis according to Laemmli has been successfully applied in selenium research. It has resulted in the detection of a large number of selenium-containing proteins. Unfortunately, this method is limited to the separation of proteins in the molecular mass range between 250 kDa and 8 kDa. It is also very difficult to separate different isomeric forms of the same protein. In the last case better results are obtained by 2D electrophoresis, which allows the separation of biomolecules according to their molecular mass and isoelectric point. These two characteristics are also helpful in the identification of the protein of interest

In this way more than 30 selenium-containing proteins could be distinguished in the lung and trachea samples. Some of them such as Gpx1, Gpx3, Gpx4, Trx1, SelP, SelT and Sel15 could be identified by means of immunoassays and localized in the cellular compartments.

There were several selenium-containing proteins with molecular masses below 12 kDa in the samples. The finding that selenium is also present in the mammalian organism in the form of several small proteins is of great interest with regard to the metabolism and function of this element. Because of their mobility small protein can quite easily pass the cell membrane. The newly found small proteins may play an important function in the transport in those tissues. By adding protease inhibitors to the sample and by sampling below 4 °C it was excluded that the small proteins are fragments of the larger compounds produced *in vitro* by accidental proteolytic decomposition.