6 Summary

The evolution of the synthesis of ascorbic acid in the metazoa is not completely revealed yet. Hitherto, the ascorbic acid synthesis was assumed to have evolved repeatedly within different metazoan taxa. But as ascorbic acid is essential for the formation of collagen, the binding element of cells, this study investigated the hypothesis that the ability to synthesize ascorbic acid is a basic characteristic of the metazoa.

To test this hypothesis, basal taxa of the metazoa and taxa which are closely related to the vertebrates were examined. In the experimental part, an *in vitro* enzyme test and a HPLC method for the measurement of the ascorbic acid concentration were established. To validate the results of the enzyme test, a histochemical procedure was conducted by staining the enzyme, which is crucial in the ascorbic acid synthesis. In addition the genetic background of this enzyme was researched.

Ascorbic acid synthesis was found in the acrania *Branchiostoma lanceolatum*. This is the first proof of the ascorbic acid synthesis in the sister group of the vertebrata and thus implies that it can not solely be attributed to the craniota, but has to be considered as being developed earlier. The ascorbic acid synthesis was also found in basal taxa of the metazoa, such as sponges and cnidaria. The cnidaria *Alcyonium digitatum*, *Aiptasia sp.*, *Metridium senile*, *Sargatiogeton undatus*, *Thuiaria thuja* and the member of the porifera *Chondrosia reniformis* show the synthesis of ascorbic acid *in vitro*. Therefore it can be hypotesized that synthesis of ascorbic acid is an original characteristic of all multicellular animals. The positive results of the enzyme test in *A. digitatum* and *M. senile* were confirmed by the histochemical method. Additional with this method was the site of this synthesis localized in the gastrodem. No evidence of ascorbic acid synthesis was found in the cordata *Ciona intestinalis*. However, further experiments are necessary to clarify whether all tunicata are unable to perform the ascorbic acid synthesis or whether *C. intestinalis* may be an exception.

The sponge *C. reniformis* was able to synthesise ascorbic acid from either L-gulonolacton and L-galactonolacton as a precursor. However, *A. digitatum* and *M. senile* were found to specifically use only L-gulonolacton as a precursor.

The crucial enzyme in the ascorbic acid synthesis in vertebrates is the gulonolacton oxidase (GLO) that catalyzes the last step in this metabolic pathway. The cDNA with the database name ci0100132519 from *C. intestinalis* shows high similarity to the GLO gene in vertebrates. This sequence was used to design some primers. The primers were used in the investigation of some chordata, bryozoa and echinodermata whith PCR. However two

sequences were only identified in *C. intestinalis* and additional one sequence in *Clavelina lepardiformis*. The investigation of another 17 species of the deuterostomia did not give any results. The high difference between individuals of *C. intestinalis* from different populations showed that primers can only be used in closely related species.

Moreover several gene data bases were searched for similarities to the gulonolacton-oxidase-gene with a BLAST search. A sequence of *Ciona savignyi* was found and compared, together with the sequence of *C. intestinalis*, to the known GLO-sequences of the vertebrates *Rattus norvegicus*, *Mus musculus*, *Bos taurus*, *Sus scrofa*, *Gallus gallus* and *Scyliorhinus torazame* in a cladistic analysis. The data were assessed by a neighbor joining procedure and presented in a tree. The result corresponds to the current hypotheses. This shows furthermore that the GLO gene is useful in phylogenetic analysis.

The recent work shows that ascorbic acid synthesis of larger taxa such as the vertebrates derives from the one origin. The loss of this attribute has repeatedly taken place within groups. The initial hypothesis that the synthesis of ascorbic acid is a basic character of the metazoa can thus be accepted.