5. Conclusions

In this work a specific group of protein dehydrins was analyzed for which it is believed to play an important protective role during cold and water-deficit stress in plants. Despite many studies that have been done till now, the cryoprotective activity of dehydrins from *Arabidopsis thaliana* has not been proven yet, and it still remains not fully answered what mechanism enables dehydrins to protect the cell membranes in the low temperature conditions.
A humble move further in discovering and building a clearer picture in this field of knowledge tries to give this work too. The results that we have obtained are summarized below:

1. The cryoprotective activity has been proven for 3 from 4 analyzed recombinant dehydrin proteins (LTI29, COR47 and ERD14) expressed in *E.coli* by using a freezing test. For the protein RAB 18 in this test a low cryoprotective activity has been shown.

2. It is determined that due to the process of heat treatment most of the proteins from bacterial supernatant become denaturalized, while the dehydrins remain heat stable. This has been shown to be an important step in the purification of dehydrins. Also the yield of proteins was on the satisfactory level although the difference in the yield among the analyzed proteins has been noticed.

3. Cryoprotective active dehydrins are partially additive, do not inhibit the activity of each other while analyzed in the freezing test. Similarly, it was noticed in the case when CPP has been analyzed in the combination with other cryoprotective active dehydrins.

4. Western blot analyses point out on the bounding ability of dehydrins with the thylakoid membrane which is in correlation with their cryoprotective activity (LTI 29). Cryoprotective inactive dehydrin RAB 18 did not bind with the thylakoid´s membrane, according to our results, respectively.

5. By using of Boyle van't Hoff analyse the thylakoid membrane permeability has been examined in the presence of dehydrin proteins. It has been shown that the cryoprotective dehydrins increase the membrane permeability in the freeze-thaw cycle. This might be a possible mechanism by which the dehydrin proteins cryoprotectively affect the cell membranes.

6. The difference in thylakoid´s sedimentation was noticed in the presence of CPP and LTI29 separately, when these proteins are incubated on 0°C during a longer period of time. In the presence of added LTI29 the thylakoid sedimentation is hindered, contrary to CPP, what leads to the conclusion about the different mechanism of activity of these two proteins. These have also been shown by Boyle van't Hoff analyses during a longer incubation time at 0°C.

7. The influence of the LTI 29 dehydrin on the thylakoid membrane's permeability was analyzed in the LSS (light scattering system) system. The results obtained coincide with the results from the Boyle van't Hoff analyses, meaning that cryoprotective dehydrins increase the membrane's permeability. Similar result was obtained when we examined the cryoprotective CPP protein by LSS method.

8. The presence of added CPP in the thylakoid suspension immediately changes the light scattering signal (increase). This can be attributed to the thylakoid's aggregations in the presence of CPP observed by light microscope analyses.

9. The presence of Ca^{2+} and Mn^{2+} ions has an influence on the apparent LSS signal. These ions increase the LSS signal in the presence of CPP, while in the presence of LTI29, according to our results, LSS signal remains unchanged. This further supports the conclusion that the mechanism of the activity of CPP and the dehydrins is different.