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Cryoprotective activity of four dehydrins expressed in
E.coli and their influence on thylakoid membrane
permeability in comparison to cryoprotectin

Inaugural-Dissertation
to obtain the academic degree
Doctor rerum naturalium (Dr.rer.nat.)
submitted to the Department of Biology, Chemistry and Pharmacy
of Freie Universität Berlin

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Berlin, July 2007

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Date of defense: 12-Jul-2007

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Abbreviations

ABA	Abscisic acid
AFP	Antifreeze proteins
<i>A. thaliana</i>	<i>Arabidopsis thaliana</i>
APS	Ammoniumperoxodisulphat
ATP	Adenosine triphosphate
BCIP	5-Bromo-4-chloro-3-indolyl phosphate
BIS	Bisacrylamid
Bp	base pairs
BSA	Bovine Serum Albumin
CPP	Cryoprotectin
CTAB	Cetyl Trimethyl Ammonium Bromide
DEPC	Diethylpyrocarbonat
DHN	dehydrins
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
dNTP	2' Desoxyribonukleosid-5'-triphosphate
DTT	1.4-dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	Ethylenediamine tetraacetic acid
EtOH	Ethanol
IAA	Isoamylalcohol
IMAC	Immobilized metal ion affinity chromatography
IPTG	Isopropyl β -D-1-thiogalactopyranoside
kDa	Kilodalton
LEA	Late embryogenesis-abundant
LTP	Lipid transfer protein
MOPS	3-N-morpholinpropanesulfonic acid
MW	Molecular weight
NaAcetate	Na acetate
NBT	Nitrotetrazolium blue chloride
PAA	Polyacrylamide
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidone
SDS	Sodium dodecyl sulphate
TBE	Tris Borat-EDTA
TE	Tris EDTA
TEMED	N,N,N',N'-Tetramethylethylenediamine
TES acid	N-Tris-hydroxymethyl)methyl-2-aminoethanesulfonic
Tris	Trishydroxymethylaminomethane
OD	Optical density
ON	Over night

Summary

In this work the cryoprotective activity of four dehydrins expressed in *E.coli* were analyzed. These proteins play an important protective role during cold and water-deficit stress in plants.

We proved that three of four analyzed recombinant dehydrin proteins (LTI29, COR47 and ERD14) are cryoprotectively active in a "freezing test". For the dehydrin RAB 18 in this test a low cryoprotective activity has been shown.

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.

Also, the influence of the LTI 29 dehydrin on the thylakoid membrane's permeability was further analyzed in the LSS (light scattering system) system. The results obtained coincide with the results from the Boyle van't Hoff analyses, what has brought us to further understanding that cryoprotective dehydrins increase the membrane's permeability.

Difference in activity between dehydrin LTI29 and CPP was noticed when these two proteins were added separately in the thylakoid suspension during the long incubation time. According to these analyses dehydrins protect thylakoid membranes during long period of time, while CPP doesn't. Similarly, 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.

The presence of Ca²⁺ and Mn²⁺ 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, LSS signal remains unchanged. This further supports the conclusion that the mechanism of the activity of CPP and the dehydrins is different.

According to our Western blot analyses the dehydrin LTI29 is bound to the thylakoid membrane, while RAB 18 isn't. This points out that the binding ability of dehydrins could be included in complex mechanism of cryoprotective activity of dehydrins.

Zusammenfassung

In dieser Arbeit wurde die Frostschutzaktivität von vier in *E.coli* exprimierten Dehydrinen, analysiert. Diese Proteine spielen eine wichtige Schutzrolle während Kälte- und Trockenstress in Pflanzen.

Wir haben bewiesen, dass drei von vier analysierten rekombinanten Dehydrinen (LTI29, COR47 und ERD14) im „Frostschutztest“ Frostschutzaktivität haben. Für das Dehydrin RAB 18 ist in diesem Test eine niedrige Frostschutzaktivität gezeigt worden.

Die Permeabilität der Thylakoidmembran in Gegenwart der Dehydrine ist mittels Boyle van't Hoff Analyse geprüft worden. Es ist gezeigt worden, dass die frostschutzaktiven Dehydrine die Membranpermeabilität im Gefrier-Tau-Cyclus erhöhen.

Es handelt sich möglicherweise um einen Mechanismus, bei dem die Dehydrine frostschützende Wirkung auf die Zellmembran haben.

Weiterhin wurde auch der Einfluss des LTI 29-Dehydrins auf die Permeabilität der Thylakoidmembranen im LSS-System (light scattering system) analysiert.

Bei den Resultaten erhielten wir eine Übereinstimmung mit den Ergebnissen der Boyle van't Hoff- Analyse, was uns bestätigte, dass die kryoprotektiven Dehydrine die Membranpermeabilität erhöhen.

Bei langen Inkubationszeiten der Thylakoidsuspensionen mit LTI 29 oder CPP zeigten sich Aktivitätsunterschiede zwischen diesen beiden Proteinen. Entsprechend dieser Analyse schützen Dehydrine Thylakoidmembranen über einen langen Zeitraum im Gegensatz zu CPP.

Ein Unterschied wurde auch bei der Thylakoidsedimentation in Gegenwart von CPP und LTI 29, jedesmal separat zugegeben, festgestellt als diese Proteine bei 0°C während einer langen Zeitspanne inkubiert wurden.

In Gegenwart von LTI 29 wurde die Thylakoidsedimentation verhindert, im Gegensatz zu CPP, was uns zur Annahme von unterschiedlichen Mechanismen der Aktivität führt.

Die Gegenwart von Ca^{2+} und Mn^{2+} Ionen hat Einfluss auf das apparente LSS-Signal. Diese Ionen erhöhen das LSS-Signal in Gegenwart von CPP, während in Gegenwart von LTI 29 das LSS Signal unverändert bleibt

Dies unterstützt weiterhin den Schluss, dass der Mechanismus der Frostschutz-Aktivität bei CPP und den Dehydrinen, unterschiedlich ist.

Entsprechend unserer Westernblotanalysen ist das Dehydrin LTI 29 an die Thylakoidmembran gebunden, während dies bei RAB 18 nicht der Fall ist.

Das bedeutet, dass die Bindungsfähigkeit der Dehydrine in den komplexen Mechanismus der Frostschutzaktivität der Dehydrine eine Rolle spielen könnte.