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Galactose-Specific Lectins Protect Isolated Thylakoids against Freeze-Thaw Damage

Dirk K. Hincha*, Irina Bakaltcheva[1], and Jürgen M. Schmitt

Institut für Pflanzenphysiologie und Mikrobiologie, Freie Universität, Königin Luise-Strasse 12–16, W-1000 Berlin 33, Germany

We have measured freeze-thaw damage to isolated spinach (*Spinacia oleracea* L.) chloroplast thylakoid membranes in the presence of different galactose-specific seed lectins to determine whether the binding of proteins to the membrane surface can lead to cryoprotection. Of the seven lectins investigated, five were protective to different degrees and two showed no measurable effect. Protection was afforded by a reduction of the solute permeability of the membranes. This reduced the solute influx during freezing and thereby osmotic rupture of the thylakoid vesicles during thawing. Using model membranes and fluorescently labeled lectins, we could show that the proteins bound exclusively to the digalactosyl lipids in the membranes. Binding was a prerequisite for the protective effect, because the presence of up to 5 mM galactose in the samples completely inhibited both binding of the lectins to thylakoid and model membranes and cryoprotection. The degree of binding was, in contrast, not related to the cryoprotective efficiency of different lectins; cryoprotection was a function of the hydrophobicity of the proteins.

A role for specific proteins in stress resistance of plants has been implicated in a variety of cases. The synthesis of stress-related proteins has been shown for several plant species during exposure to cold (Guy, 1990; Thomashow, 1990), heat (Vierling, 1991), salinity (Höfner et al., 1987; Hanson, 1992), and drought (Close et al., 1989) stress and in seeds during maturation drying (Bewley and Oliver, 1992). For a protein fraction from cold-acclimated leaves, a functional role in cellular stress resistance could be postulated from the fact that these proteins protected isolated thylakoid membranes against freeze-thaw damage. Because these proteins were already highly effective at very low concentrations, a colligative mode of action could be ruled out (Hincha et al., 1989, 1990). Therefore, it is tempting to speculate that these proteins alter the physical properties of the membranes by binding to specific membrane components. There is, however, no direct evidence for this yet.

Only in the case of chloroplast-specific heat-shock proteins was binding to thylakoid membranes shown under some conditions (Glaczinski and Kloppstech, 1988; Adamska and Kloppstech, 1991). In this case, however, no unequivocal evidence for a protective effect on the membranes has been obtained so far (Vierling, 1991).

In the present paper, we have attempted to clarify the basic question of whether the binding of a large, hydrophilic protein to a membrane can protect the membrane against the stresses associated with a freeze-thaw cycle. Because thylakoids contain a high percentage of mono- and digalactosyl lipids (Quinn and Williams, 1983), we used several commercially available Gal-specific lectins to investigate possible cryoprotective effects and correlate them to lipid binding and physical properties of the proteins.

MATERIALS AND METHODS

Materials

All lectins, including the FITC-labeled proteins, soybean phosphatidylcholine, and TNS (K-salt), were purchased from Sigma Chemical Co. and were used without further purification. D-[U-^{14}C]Glc (specific activity 304 mCi mmol^{-1}) was supplied by Amersham, and liquid scintillation cocktail (Rotiscint 2200) was from Roth.

Thylakoid Isolation and Freezing

Chloroplasts were isolated from the leaves of nonhardy spinach plants (*Spinacia oleracea* L. cv Monnopa) as described recently (Hincha and Schmitt, 1992a). Thylakoids were washed three times in 10 mM $MgCl_2$/20 mM K_2SO_4. Samples (0.4 mL) contained approximately 0.5 mg of Chl mL^{-1} in 5 mM $MgCl_2$/10 mM K_2SO_4/150 mM K-glutamate/50 mM Suc (artificial stroma medium [Hincha and Schmitt, 1988]) and additional proteins and Gal as indicated in the figure legends. If not indicated otherwise, they were incubated for 3 h at −20°C and were rapidly thawed in a water bath at room temperature. After thawing, the membranes were sedimented by centrifugation (16,000*g* for 15 min), and the amount of the soluble lumenal protein PC was determined in the supernatants by radial immunodiffusion as described before (Hincha et al., 1985). Aliquots of unfrozen samples were lysed in 2% Triton X-100 to determine the total amount of PC in the samples. Freeze-thaw damage is expressed in the figures relative to samples without added lectin (= 100% freeze-

[1] On leave from the Institute of Cryobiology and Lyophilization, Sofia, Bulgaria, with financial support from the Hochschulsonderprogramm 2, Freie Universität Berlin.

* Corresponding author; fax 49–30–838–4313.

Abbreviations: DGDG, digalactosyldiacylglycerol; F_{max}, maximal fluorescence emission; FITC, fluorescein isothiocyanate; MGDG, monogalactosyldiacylglycerol; PC, plastocyanin; RCA, *Ricinus communis* agglutinin; TNS, 2-*p*-toluidinylnaphtalene-6-sulfonic acid.

thaw damage). This corresponds to a release of 35 to 45% of the total PC.

Glc Permeability Measurements

Thylakoids were washed three times in 5 mM NaCl. Samples (0.2 mL) contained approximately 0.4 mg of Chl mL^{-1} in 2.5 mM NaCl and 100 mM D-[U-^{14}C]Glc (specific activity 3.98×10^{-2} μCi $mmol^{-1}$). After different incubation times at 0°C, the membranes were separated from the solution by centrifugation (8 min at 16,000*g*). The pellets were resuspended in 200 μL of 100 mM unlabeled Glc and mixed with 4 mL of scintillation cocktail. Radioactivity was quantitated by liquid scintillation counting using a Beckman Instruments LS 3801 liquid scintillation system. A standard quench curve was determined with different concentrations of thylakoid membranes at a fixed amount of radioactivity. The Chl content (Arnon, 1949) in every sample was determined after resuspending the pellets, and the measured cpm were transformed to dpm using the quench curve. The amount of radioactivity remaining with the thylakoids at time = 0 (addition of radioactive Glc to the thylakoids and immediate centrifugation) was subtracted from the values obtained at subsequent times. During the observed 30-min period, radioactivity in the thylakoids increased linearly with time. Permeability coefficients (P) were calculated from these data using

$$P = J/(A \times \Delta C)$$

where J is the influx of Glc in mmol s^{-1}, ΔC is the concentration gradient of Glc across the thylakoid membranes in mM, and A is the membrane area (16.7 cm^2 μg^{-1} of Chl [Ball et al., 1985]). If we assume that the internal Glc concentration is 0 at the start of an experiment, the gradient is the same as the applied external concentration.

Binding of Lectins to Galactolipids

Spinach thylakoid lipids were isolated as described by Sprague and Staehelin (1987). Briefly, lipids were extracted from washed thylakoids in chloroform:methanol (1:2, v/v). The concentrated chloroform phase was applied to a column of silicic acid (SIL-LC; Sigma), activated with petroleum ether, and equilibrated in chloroform. Pigments were eluted with chloroform, MGDG was eluted with acetone:chloroform (1:1, v/v), and a mixture of DGDG, sulfolipid, and phospholipid was eluted with methanol:chloroform (1:1, v/v).

The two lipid fractions were concentrated by rotary evaporation, and aliquots (60 mg) were dried at 60°C under a stream of N_2 and stored under vacuum. For the preparation of vesicles, galactolipid fractions were dissolved in chloroform and mixed with an appropriate amount of phosphatidylcholine in the same solvent. In the case of the DGDG-containing fractions, it was assumed that approximately 50% of the lipid was DGDG (Quinn and Williams, 1983; Webb and Green, 1991). The lipids were dried again as described above, and residual organic solvent was removed under vacuum overnight. Lipids were hydrated at 10 mg mL^{-1} in 300 mM K-glutamate/100 mM Suc by sonication for 2 min to suspend the lipids in solution. Large unilamellar vesicles were prepared by extruding the lipid suspension 10 times through two polycarbonate filters of 100-nm pore size (Nuclepore) in a Lipex Biomembranes Extruder (Hope et al., 1985).

Samples (150 μL), containing 200 μg mL^{-1} of FITC-labeled lectin, 5 mM $MgCl_2$/10 mM K_2SO_4/150 mM K-glutamate/50 mM Suc, and lipid vesicles as indicated in the figure legends, were incubated for 2 h at 0°C. After incubation, the vesicles were removed by centrifugation (1 h at 52,000*g*), and FITC fluorescence in the supernatants was measured relative to samples containing no lipid in a Kontron SFM 25 fluorimeter at an excitation wavelength of 480 nm and an emission wavelength of 530 nm.

Titration of Hydrophobic Domains with TNS

Lectins (300 μg) in 1 mL of 100 mM Na-phosphate buffer (pH 6.5)/0.05% NaN_3 were placed in a cuvette in the fluorimeter with the excitation set to 350 nm and the emission to 423 nm. The temperature of the cuvette was maintained at 20°C. Titrations were performed by addition of small volumes of a 1-mM TNS solution in phosphate buffer. At the chosen wavelengths, TNS fluorescence is minimal in an aqueous solution but is greatly enhanced when TNS is transferred to a hydrophobic environment such as the hydrophobic domain of a protein (Roberts and Goldstein, 1982). Data were corrected for dilution during titration and for fluorescence of TNS in buffer solution without added protein. Results were analyzed as described by Wang and Edelman (1971). In a graph of reciprocal fluorescence versus reciprocal TNS concentration (formally analogous to a Lineweaver-Burk plot), the value of the y axis intercept will give the F_{max} when the dye concentration is made infinitely large. This can be used as a measure of the relative hydrophobic binding capacity of different proteins (Wang and Edelman, 1971).

RESULTS

Freeze-thaw damage to thylakoids both in vitro and in vivo is accompanied by the release of the electron transport protein PC from the intramembrane spaces due to transient rupture of the membranes (Hincha et al., 1987; Hincha and Schmitt, 1988). This leads to an inactivation of photosynthetic electron transport and light-driven ATP synthesis after a freeze-thaw cycle (for a review, see Hincha and Schmitt, 1992b). The prevention of the loss of PC from isolated thylakoids can be used as an indicator for the presence of cryoprotective proteins during freezing (Hincha and Schmitt, 1992b). If the membrane vesicles are suspended in a simplified artificial stroma medium (Hincha and Schmitt, 1988), the action of proteins can be assayed under conditions that are similar to those in vivo (Hincha and Schmitt, 1992a).

Figure 1 shows that under these conditions the Gal-specific agglutinin from *Abrus precatorius* seeds was clearly a cryoprotective protein. Similar to cryoprotective proteins from cold-acclimated cabbage leaves (Hincha and Schmitt, 1992a), it showed a linear dose-response curve. Cryoprotection depended on the binding of the agglutinin to sugar residues on the membrane surface. This was indicated by the fact that the presence of free Gal leads to a complete inhibition of the protective activity (Figs. 1 and 2). This inhibition was a linear function of the Gal concentration when the lectin concentra-

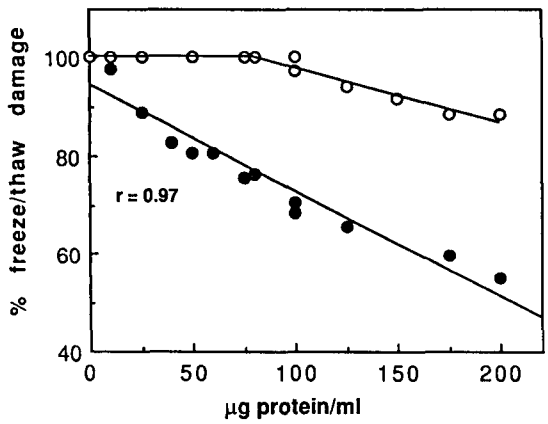

Figure 1. Cryoprotection of isolated spinach thylakoids by the Gal-specific agglutinin from *A. precatorius* seeds. Membranes were incubated for 3 h at −20°C with different protein concentrations in the absence (●) or presence (○) of 2 mM Gal. The data were compiled from two independent experiments. Freeze-thaw damage was measured as the release of the soluble protein PC from the thylakoid lumen. Loss of PC in the absence of added protein was taken as 100% freeze-thaw damage to facilitate comparisons between different experiments.

tion was held constant (Fig. 2). In contrast, Gal alone was protective. However, we reproducibly observed that in the presence of agglutinin and Gal at concentrations greater than 3 mM the protective effect of the sugar itself was inhibited (Fig. 2). Because the sugar was present in an approximately 1000-fold molar excess, this cannot be due to a depletion of free sugar in the solution, and no convincing explanation has been found.

We observed that the lectin strongly agglutinated thylakoids both in frozen-thawed and control samples. No agglutination could be observed in the presence of 5 mM Gal (data not shown), which indicates that the agglutinin readily bound to the membranes and that the inhibition of this binding by Gal was accompanied by an inhibition of cryoprotection.

In the artificial stroma medium used in these experiments, freeze-thaw damage shows a characteristic biphasic time dependence (Hincha and Schmitt, 1988). The *A. precatorius* agglutinin only influenced the second, slow phase of PC release (Fig. 3). The first, rapid phase was not influenced. A slow, linearly time-dependent release of PC is also evident at 0°C in the absence of ice formation (Hincha and Schmitt, 1988). This release was also reduced in the presence of 200 $\mu g\ mL^{-1}$ of agglutinin (Fig. 3).

We have presented evidence (Bakaltcheva et al., 1992; Hincha and Schmitt, 1992b) that the slowly proceeding release of PC is a result of the diffusion of solutes into the thylakoids that leads to osmotic swelling and vesicle rupture. The data presented in Figure 3, therefore, indicated that the binding of the *A. precatorius* agglutinin leads to a reduced solute permeability of the membranes at both −20 and 0°C.

This was experimentally tested for membranes suspended in a solution containing radiolabeled Glc and varying concentrations of lectin (Fig. 4). In these experiments, thylakoids were not frozen and thawed. Samples were incubated at 0°C for different times, and the diffusion of Glc across the membranes was measured. From these data, it is evident that the agglutinin reduced the Glc permeability of thylakoid membranes in a concentration-dependent way. Both PC release (Fig. 1) and permeability reduction (Fig. 4) were linear functions of lectin concentration. Also, reduced permeability was dependent on the binding of the lectin to the membranes, because in the presence of 5 mM Gal the protein showed no effect (Fig. 4).

The protective activity was not an exclusive property of the *A. precatorius* agglutinin (Fig. 5). Experiments with six other Gal-specific lectins showed that four were protective

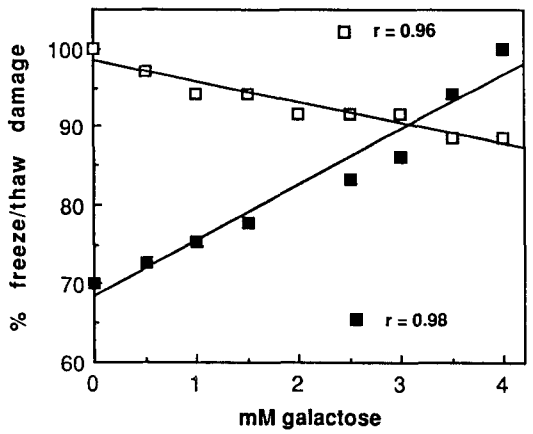

Figure 2. Inhibition of the cryoprotective activity of the *A. precatorius* agglutinin for thylakoid membranes by Gal. Samples contained either no (□) or 200 $\mu g\ mL^{-1}$ (■) protein. Compare Figure 1 for additional details.

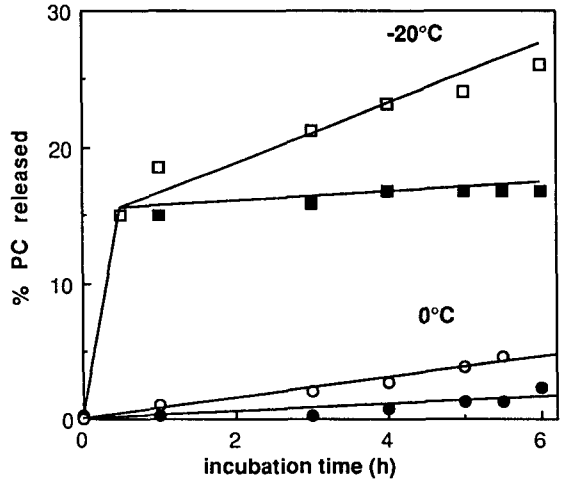

Figure 3. Release of PC from isolated thylakoids as a function of incubation time. Samples contained no (open symbols) or 200 $\mu g\ mL^{-1}$ (solid symbols) of *A. precatorius* agglutinin. The amount of PC released after different incubation times was corrected for the amount released directly after mixing the membranes with the incubation solutions (time = 0). The correlation coefficients were $r = 0.98$ (□); 0.96 (■); 0.99 (○); 0.85 (●).

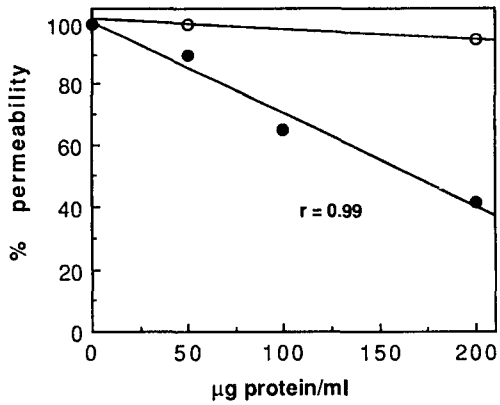

Figure 4. Reduction of the solute permeability of thylakoid membranes by *A. precatorius* agglutinin. Thylakoids were incubated at 0°C for up to 30 min in the presence of ^{14}C-labeled Glc (see "Materials and Methods" for details) with (○) or without (●) 5 mM Gal. One hundred percent permeability corresponds to a permeability coefficient of $P = 2.22 \times 10^{-9}$ cm s^{-1}. Data points represent the means from results of two independent experiments for each agglutinin concentration. SD values were between 8.1 and 15.7%.

to different degrees, whereas two had no measurable effect (Fig. 5, Table I). In all cases, protection could be completely suppressed by the addition of 5 mM Gal before freezing (data not shown).

A possible explanation for these results was that only the protective lectins were able to bind to the membranes and the inactive proteins were not. However, we found that the RCA_{120} very strongly agglutinated isolated thylakoids (data not shown), although it was not protective (Fig. 5). This, in conjunction with the observation that all protective activity could be inhibited by the presence of Gal, indicated that binding of the lectins to the membranes was a prerequisite to their protective action but that other factors decided whether a given protein was protective or not. Because thylakoids contain both mono- and digalactosyl lipids, it was possible that only binding to one group of galactolipids would confer cryoprotection to the membranes.

We investigated this by chromatographically separating MGDG from DGDG and the rest of the thylakoid lipids and reconstituting the two lipid fractions into phosphatidylcholine bilayers. These large unilamellar vesicles were incubated with fluorescently labeled lectins, and binding was determined by the ability of the vesicles to precipitate the fluorescent label (Fig. 6). The experiments showed that the lectins investigated bound almost exclusively to DGDG (Fig. 6A) but not to MGDG (Fig. 6B) or to pure phospholipid vesicles (data not shown). As in the case of cryoprotection and agglutination, binding to the vesicles was completely inhibited in the presence of 5 mM Gal (data not shown). The effect of added lipid on lectin binding was saturated at about 2 mg of lipid mL^{-1}. At higher lipid concentrations, no additional protein could be removed from the solution. We cannot explain this from the available data.

The degree of binding of the different lectins to DGDG does not correlate with their effectiveness as cryoprotectants. RCA_{120} showed the strongest binding (Fig. 6A) but no cryoprotection (Fig. 5C), whereas RCA_{60} and the *A. precatorius* agglutinin showed good cryoprotection (Table I) but much weaker binding (Fig. 6A). Therefore, another property of the lectins, in addition to sugar binding, must play an important role in cryoprotection. It has been shown for a variety of lectins that they have hydrophobic domains close to their sugar-binding sites (for a review, see Lis and Sharon, 1986). It seemed possible that hydrophobic interactions take place between the lectins and the membranes after binding has

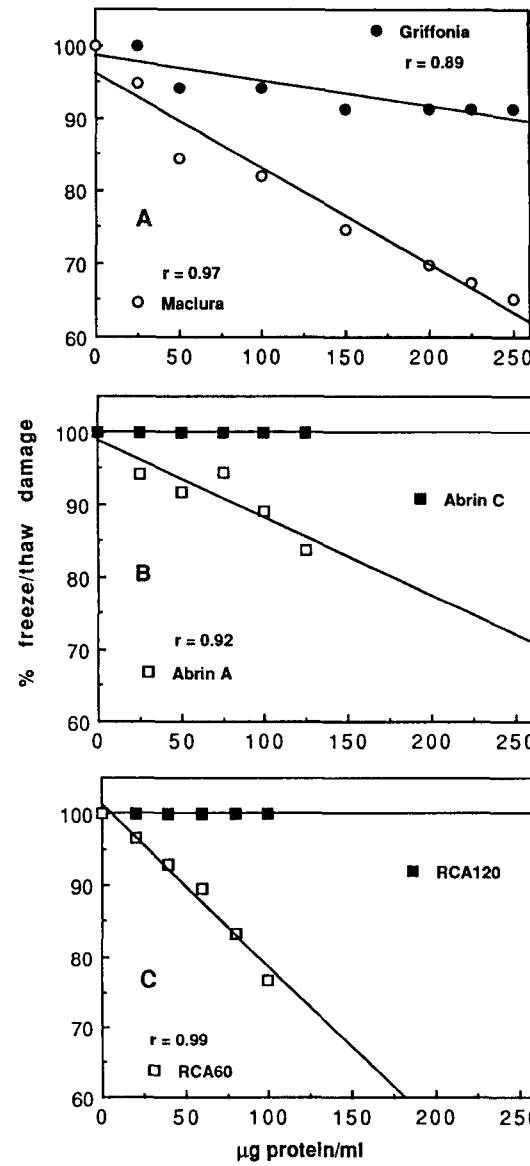

Figure 5. Comparison of the cryoprotective effects of different Gal-specific lectins for isolated thylakoid membranes. Experimental conditions were as described in Figure 1. The proteins were: A, seed lectins from *Griffonia simplicifolia* and *Maclura pomifera*; B, the toxins A and C from seeds of *A. precatorius*; C, the agglutinin (RCA_{120}) and toxin (RCA_{60}) from seeds of *R. communis*. Samples in A contained 1 mM $CaCl_2$ and 1 mM $MnCl_2$ in addition to the artificial stroma medium.

Table I. *Comparison of the cryoprotective efficiency of different Gal-specific lectins*

Lectin	Relative Efficiency[a]		kD
	Mass Concentration		
A. precatorius			
Agglutinin	0.216	0.289	134 (Olsnes et al., 1974)
Toxin A	0.108	0.065	60 (Wei et al., 1974)
Toxin C	0.0	0.0	64 (Wei et al., 1974)
G. simplicifolia	0.036	0.041	114 (Hayes and Goldstein, 1974)
M. pomifera	0.131	0.052	40 (Bausch and Poretz, 1977)
R. communis			
RCA_{120}	0.0	0.0	120 (Olsnes et al., 1974)
RCA_{60}	0.228	0.137	60 (Olsnes et al., 1974)

[a] The relative cryoprotective efficiency is the negative slope of the lines that were obtained by plotting the percentage of freeze-thaw damage as a function of protein concentration (compare Figs. 1 and 5). This is expressed on the basis of either protein mass ($\mu g\ mL^{-1}$) or molar protein concentration.

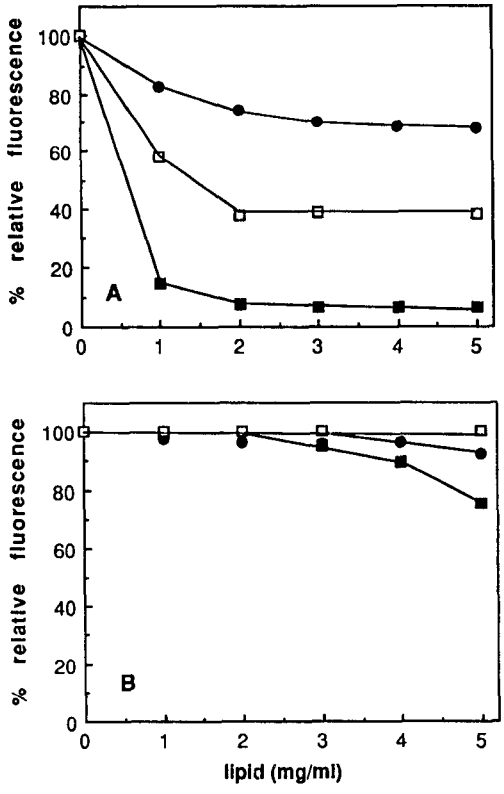

Figure 6. Binding of fluorescently labeled lectins to lipid vesicles. *A. precatorius* agglutinin (●), RCA_{120} (■), and RCA_{60} (□) were incubated at a concentration of 200 $\mu g\ mL^{-1}$ for 2 h at 0°C in an artificial stroma medium (compare Fig. 1) in the presence of different amounts of lipid. Lipids were added in the form of large unilamellar vesicles containing 20 weight percent DGDG (A) or MGDG (B). After incubation, the vesicles were removed from the solution by centrifugation, and fluorescence was measured in the supernatants.

been achieved and that cryoprotection was related to the different hydrophobicity of the proteins.

Titration of the hydrophobic sites of the lectins strongly supported this hypothesis (Fig. 7). Increasing amounts of TNS were added to the lectins in the cuvette of a fluorimeter. TNS is a ligand that has a high affinity for the hydrophobic domains of proteins. The transfer of TNS from an aqueous solution to a more hydrophobic environment strongly increases the quantum yield of its fluorescence emission (Roberts and Goldstein, 1982). Double-reciprocal titration curves as shown in Figure 7A can be used to quantify the relative hydrophobicity of different proteins (Wang and Edelman, 1971). From the y axis intercept of the curves, the F_{max} at an infinitely large TNS concentration can be calculated. When F_{max} as a measure of protein hydrophobicity was plotted as a function of the cryoprotective efficiency of the different

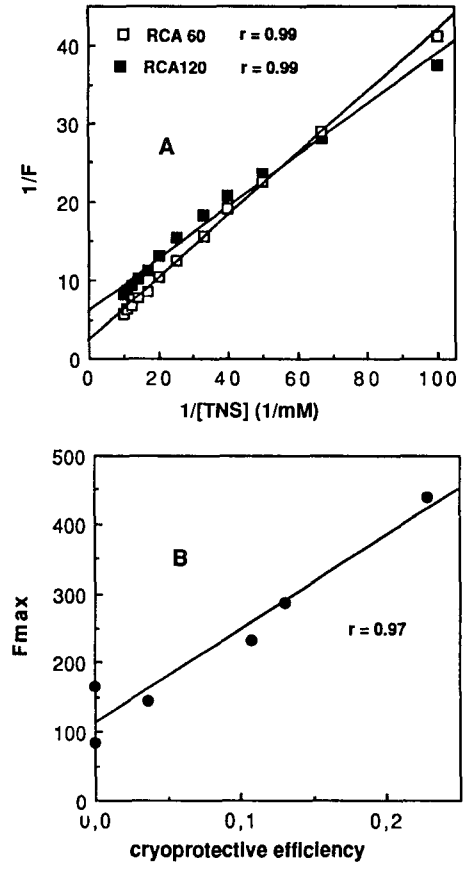

Figure 7. Fluorescence titrations of lectins with TNS to quantify the hydrophobic binding properties of the proteins. The protein concentration in the cuvette was 300 $\mu g\ mL^{-1}$, and TNS concentrations were varied from 10 to 100 μM. Fluorescence values (F) were analyzed by a double-reciprocal plot as shown for RCA_{120} and RCA_{60} in A. From the intercept of the straight line with the y axis, the F_{max} at an infinite TNS concentration can be calculated as a measure of relative protein hydrophobicity. In B, F_{max} is plotted as a function of the cryoprotective efficiency of the lectins listed in Table I except for the *A. precatorius* agglutinin, which precipitated in the presence of TNS. All titrations were done in triplicate and were averaged. SD values were between 1.5 and 9.6% of the means.

lectins (Table I), a linear correlation was obtained (Fig. 7B). These titrations were only performed with six of the seven lectins used in our study because the *A. precatorius* agglutinin precipitated already from solution in the presence of 10 μM TNS.

When we calculated cryoprotective efficiency on a molar rather than on a mass basis (Table I), we still found a linear correlation between F_{max} and the protective efficiency (not shown), although the correlation coefficient was decreased from 0.97 (Fig. 7B) to 0.93.

DISCUSSION

The aim of the present study was to clarify whether a biomembrane can be protected against freeze-thaw damage by binding a soluble protein to its surface. From the data presented in Figures 1 and 5, we conclude that this is indeed possible. Whether the cryoprotective leaf proteins we have isolated from frost-hardy cabbage plants (Hincha and Schmitt, 1992a, 1992b) protect thylakoid membranes by a mechanism similar to that described here for Gal-specific lectins is currently under investigation in our laboratory.

Another open question is whether the stabilization of cellular structures against environmental stress effects is one of the functions that lectins have in plants. Obviously, the seed lectins we have used in this study will not be involved in the frost tolerance of chloroplasts in leaves. So far, lectins have been implicated in the recognition processes between roots and symbiotic bacteria and as defense substances against fungal and bacterial pathogens (Etzler, 1985; Chrispeels and Raikhel, 1991). Because most cellular membranes such as the chloroplast envelope (Block et al., 1983), tonoplast (Haschke et al., 1990), and plasma membrane (Lynch and Steponkus, 1987) contain glycolipids, interactions of appropriate leaf lectins with these membranes are possible. Here, we have presented evidence for the first time that such an interaction may be beneficial for the stress resistance of a cell. However, it remains to be shown whether a lectin present in one of the cellular compartments increases the stress tolerance of that specific membrane.

The binding of lectins to glycolipids in both natural and model membranes has been investigated extensively (Grant and Peters, 1984). In agreement with the data in Figure 6, it has been shown for several lectins and lipids with corresponding mono- or disaccharide headgroups that binding is much stronger with the disaccharide glycolipids. This has been explained either with the greater affinity of most lectins for disaccharides as compared to monosaccharides or with the higher accessibility of the terminal sugar of a disaccharide headgroup as it protrudes further from the membrane surface (Grant and Peters, 1984). For the *A. precatorius* agglutinin and RCA_{120}, it has been shown that the sugar-binding sites comprise at least the recognition of specific disaccharide structures (Wu et al., 1992). Also, the fact that cryoprotection is not influenced by the size of the proteins (Table I) might favor the idea of preferential binding to disaccharides over accessibility related to steric hindrances.

An important observation made in our study is that binding of a lectin to the membrane is not sufficient to ensure cryoprotection. RCA_{120} shows stronger binding to DGDG than to RCA_{60} (Fig. 6A), but it is completely ineffective as a cryoprotectant, whereas RCA_{60} is highly effective (Fig. 5C, Table I). Also, the *A. precatorius* agglutinin shows weaker binding than RCA_{60} (Fig. 6A), but it has the same degree of cryoprotection (Table I).

In all experiments, both binding and protection could be shown to be a specific function of the sugar-binding properties of the lectins, because the presence of free Gal in mM concentrations inhibited cryoprotection (e.g. Figs. 1 and 2) and binding (data not shown). From these results, it is evident that binding of the lectins to the lipid headgroups is a prelude to the protection that is finally afforded by a different mechanism, and our evidence points to the hydrophobic domains of the lectins.

The presence of hydrophobic domains on a variety of lectins has been shown before by titration with ligands such as TNS (Roberts and Goldstein, 1983). We could show that such domains also exist in the lectins that we investigated (Fig. 7A) and that the differences in the hydrophobic binding properties of the lectins correlate well with their cryoprotective efficiency (Fig. 7B). Although the binding of lectins to membrane receptors such as glycoproteins and glycolipids has been studied in detail (Grant and Peters, 1984), little is known about the effects this binding has on the structure and physical properties of membranes. It has been shown that the binding of RCA_{60} to galactocerebroside headgroups in model membranes prepared from a mixture of cerebroside and phosphatidylcholine leads to an increase in acyl chain packing order as determined by Raman spectroscopy (Picquart et al., 1989).

We have shown, for the example of the *A. precatorius* agglutinin, that protection of thylakoids against loss of PC is afforded both at −20 and 0°C (Fig. 3). The effect on the slow, linearly time-dependent release of PC suggested that the binding of the lectin reduced the solute permeability of the membranes (Hincha and Schmitt, 1992b). This could be directly verified at 0°C with the help of radiolabeled Glc (Fig. 4). Again, this effect was completely inhibited by the presence of free Gal. This is the first direct evidence that the solute permeability of a membrane can be reduced by the binding of soluble proteins to lipid headgroups. An increase in acyl chain packing order, as has been shown for RCA_{60} (Picquart et al., 1989), could be evoked to explain this reduced solute permeability of thylakoid membranes in the presence of a cryoprotective lectin (Fig. 4). This possibility will be the subject of further investigations.

In conclusion, our data show for the first time that membranes can be protected from freeze-thaw damage by binding of lectins to galactolipid headgroups. After binding is achieved, hydrophobic interactions between membrane and protein determine the extent of cryoprotection.

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