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Specific effects of fructo- and gluco-oligosaccharides in the preservation of liposomes during drying

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The fructan family of oligo- and polysaccharides is a group of molecules that have long been implicated as protective agents in the drought and freezing tolerance of many plant species. However, it has been unclear whether fructans have properties that make them better protectants for cellular structures than other sugars. We compared the effects of fructans and glucans on membrane stability during air-drying. Although glucans of increasing chain length were progressively less able to stabilize liposomes against leakage of aqueous content after rehydration, fructans showed increased protection. On the other hand, glucans became more effective in protecting liposomes against membrane fusion with increasing chain length, whereas fructans became less effective. Fourier transform infrared spectroscopy showed a reduction of the gel to liquid-crystalline phase transition temperature (T_m) of air-dried liposomes by approximately 25°C in the presence of sucrose and maltose. For the respective pentasaccharides, the reduction of T_m of the lipids was 9°C lower for samples containing fructan than for those containing glucan, indicating increased sugar–membrane interactions for the fructan compared to the glucan. A reduced interaction of the longer-chain glucans and an increased interaction of the respective fructans with the phospholipid head groups in the dry state was also indicated by dramatic differences in the phosphate asymmetric stretch region of the infrared spectrum. Collectively, our data indicate that the fructo-oligosaccharides accumulated in many plant species under stress conditions could indeed play an important role in cellular dehydration tolerance.

Key words: desiccation/fructans/glucans/liposomes/oligosaccharides

Introduction

Fructans are a group of fructose-based oligo- and polysaccharides that are synthesized by many species of bacteria, fungi, and plants. They are classified into different families on the basis of their glycosidic linkages. Inulins contain β(2→1) linkages, levans β(2→6) linkages, and graminans contain both types of linkages. In plants, fructans serve a role as storage carbohydrates. In addition they have also been implicated in plant stress tolerance (Pollock and Cairns, 1991; Vijn and Smeekens, 1999). But while there is accumulating evidence for a functional role of specific proteins (Thomashow, 1999; Xin and Browse, 2000) and mono- and disaccharides (Crowe *et al.*, 1992) in cellular stress tolerance, the evidence for a functional role of fructans is still circumstantial. In several species fructans are either accumulated or modified in chain length during cold acclimation or desiccation (Pollock and Cairns, 1991; Livingston, 1996; Livingston and Henson, 1998; de Roover *et al.*, 2000). Additional evidence for a role of fructans in plant stress tolerance was provided by the fact that transgenic tobacco plants that accumulate low levels of a bacterial levan were slightly more tolerant of osmotic stress than were wild-type plants (Pilon-Smits *et al.*, 1995).

Because membranes are the primary targets of both freezing and desiccation injury in cells (Steponkus, 1984; Crowe *et al.*, 1992; Oliver *et al.*, 2001), a role of fructans in cellular stress tolerance should involve the stabilization of membranes under stress conditions. Recent reports indicate that a high molecular mass (degree of polymerization [DP] > 25,000) bacterial levan is able to directly interact with membranes (Demel *et al.*, 1998; Vereyken *et al.*, 2001). In addition, a cyclic bacterial fructan (cycloinulohexaose) protected liposomes during freezing and freeze-drying (Ozaki and Hayashi, 1996), and inulin preparations from chicory roots and dahlia tubers stabilized liposomes during freeze-drying (Hincha *et al.*, 2000).

This latter finding is particularly relevant because the chicory and dahlia inulin preparations of polysaccharides of a DP between 10 and 30 were protective (Hincha *et al.*, 2000), whereas the other investigated polysaccharides, hydroxyethyl starch and dextran, were completely ineffective in stabilizing membranes during freeze-drying (Crowe *et al.*, 1994; Hincha *et al.*, 2000). The available evidence suggests that this ineffectiveness is due to the inability of hydroxyethyl starch and dextran to interact with the membrane lipids in the dry state and depress the gel to liquid-crystalline lipid phase transition temperature (T_m) of the dry membranes (Crowe *et al.*, 1996, 1997; Hincha *et al.*, 2000; Tsvetkova *et al.*, 1998). This has been attributed to the large size of the polymers, which would sterically prevent them from interacting with membrane lipids.

It has, however, been shown that even oligosaccharides of different chain length vary significantly in their ability to stabilize membranes. Malto-oligosaccharides up to DP 3 (maltotriose) are good membrane protectants during freezing

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and drying, and homologous saccharides of DP 4 and above are much less effective (Miyajima *et al.*, 1986; Suzuki *et al.*, 1996; Nagase *et al.*, 1997). On the other hand, because the chicory inulins showed clear evidence of hydrogen bonding to phosphatidylcholine headgroups in dry membranes (Hincha *et al.*, 2000), the question arose of whether fructans might have fundamentally different properties than glucans that would enable them to interact much more effectively with membranes.

To answer this question, we have compared fructans and glucans of equal chain lengths for their ability to stabilize phosphatidylcholine liposomes during air-drying. We found that with increasing DP fructans reduced leakage of a soluble marker better than glucans, but that glucans afforded better protection against membrane fusion. Using Fourier transform infrared (FTIR) spectroscopy we were able to show that the ability of glucans to hydrogen bond to the head groups of dry lipids decreased dramatically with increasing DP, whereas chain length hardly affected the ability of fructans to interact.

Results

For a meaningful comparison of the effects of different glucans and fructans on membrane stability during drying, we first established the purity of the sugars. We used analytical high-performance liquid chromatography (HPLC) to analyze the chain-length distribution in the sugar preparations used in the subsequent experiments. Figure 1 shows that all oligosaccharide preparations contained the correct sugar with a purity of at least 95%. The fructo-oligosaccharides (Figure 1A) all contained small amounts of higher DP impurities, and the gluco-oligosaccharides (Figure 1B) mostly contained impurities of the next smaller sugar. A similar HPLC analysis of an inulin preparation from chicory roots has been published recently and showed the presence of fructans of a DP between 10 and 30, with no contamination by low-molecular-weight sugars (Hincha *et al.*, 2000).

All sugars except the inulin from chicory roots had a stabilizing effect on egg phosphotidylcholine (EPC) liposomes after drying and rehydration (Figure 2). The fructo-oligosaccharides showed increased protection with increasing DP. For the gluco-oligosaccharides, the effects were less variable and a clear influence of DP was not immediately evident.

When vesicle membrane fusion was measured under the same experimental conditions, a somewhat different picture emerged (Figure 3). Chicory inulin was again completely ineffective in membrane stabilization. The effectiveness of the other fructans in protecting membranes against fusion during drying decreased with increasing DP. The effect of the glucans on fusion did not show a clear dependence on DP.

To visualize the influence of DP on membrane protection by both classes of sugars, carboxyfluorescein (CF) leakage and

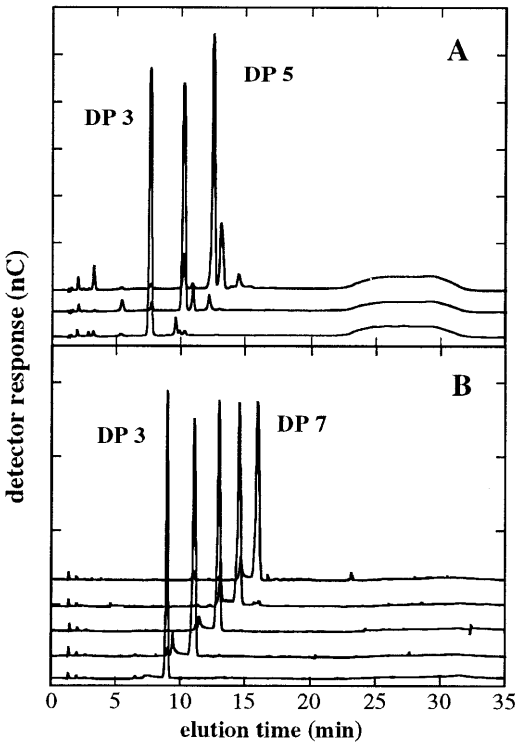

Fig. 1. HPLC analysis of the inulins (**A**) and glucans (**B**) used in the experiments reported in this paper. Sugars were analyzed by anion exchange chromatography. Longer elution times indicate a higher DP.

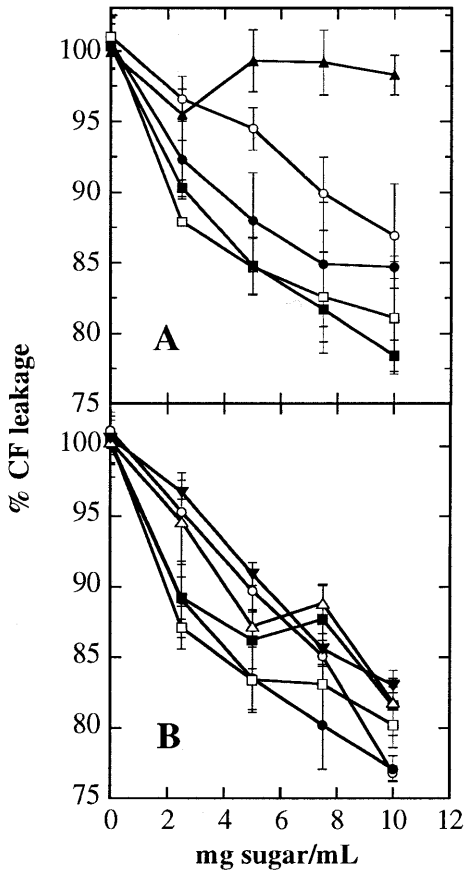

Fig. 2. Protection of large unilamellar liposomes from damage during drying by different inulins (**A**) and glucans (**B**). Leakage of CF from the vesicles was determined after air-drying and rehydration. The samples contained the indicated concentrations of sugars of different DPs. Open circles, DP 2; closed circles, DP 3; open squares, DP 4; closed squares, DP 5; open triangles, DP 6; closed triangles, base up, DP 7; closed triangles, base down, chicory inulin.

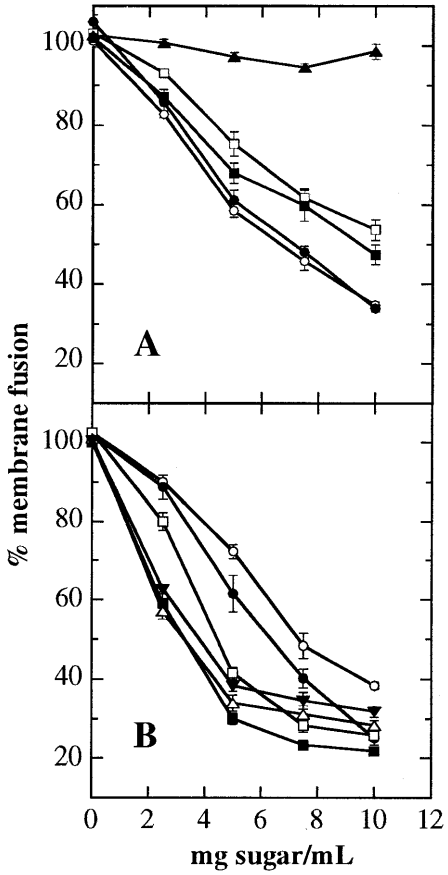

Fig. 3. Protection of large unilamellar liposomes from damage during drying by different inulins (**A**) and glucans (**B**). Damage after air-drying and rehydration was measured as vesicle membrane fusion. The symbols are the same as in Figure 2.

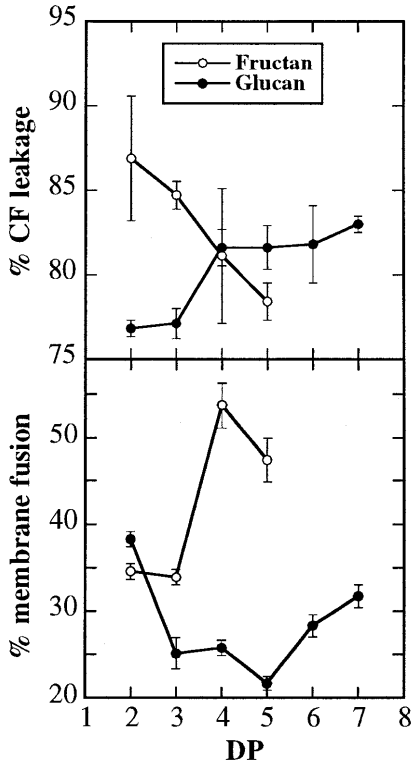

Fig. 4. Protective effect of different fructans and glucans on air-dried liposomes as a function of the DP of the sugars. The data from samples containing 10 mg/ml of the sugars (compare Figures 2 and 3) are plotted as a function of the DP of the sugars.

membrane fusion are plotted as a function of DP for samples containing 10 mg sugar ml^{-1} in Figure 4. It can be clearly seen that leakage and fusion followed completely different trends for both saccharide families. For the fructans, protection against leakage increased and protection against fusion decreased with increasing chain length. The glucans, on the other hand, showed decreased protection against leakage with increased DP, whereas protection against fusion showed an optimum between DP 3 and 5.

The data in Figure 4 seemed to indicate that increases in chain length may have opposite effects on leakage and fusion with glucans and fructans. A subset of the data shown in Figures 2 and 3 was therefore analyzed in more detail. There were data available for DP 2, 3, 4, and 5 for the respective fructans and glucans. Therefore, leakage (Figure 5A) and fusion (Figure 5B) data were plotted for the corresponding samples and subjected to linear regression analysis. A slope of 1 would indicate that at this DP glucan and fructan provided the same degree of protection; a slope higher or lower than one would indicate stronger protection by the glucan or fructan, respectively. When the slopes were then plotted as a function of DP, it was evident that with increasing DP glucans were progressively superior in protection against fusion and fructans superior in protection against leakage.

This finding was further corroborated by drying experiments, in which the liposomes were loaded with 10 mg ml^{-1} of the sugars and then protection by the corresponding external sugars was measured after drying and rehydration (Figure 6). A comparison with Figure 2 shows that protection was significantly increased when the fructans were also present inside the liposomes, the only exception being again the chicory inulin, which showed no protection. The protective effect of the glucans, on the other hand, was not further increased by the presence of the internal sugars. This could indicate that protection was predominantly due to reduced fusion, which would not be expected to be influenced by sugar present on the inside of the liposomes.

CF leakage during drying and rehydration is mainly due to two factors: vesicle fusion and gel to liquid-crystalline phase transitions of the membrane lipids (reviewed in Oliver *et al.*, 1998a, 2001). To gain further insight into the physical mechanisms underlying the functional differences between fructans and glucans, we used FTIR spectroscopy. We determined the phase transition temperature T_m of the dried EPC liposomes by monitoring the frequency of the CH_2 symmetric stretching mode around 2850 cm^{-1}, which increases by two to three wave numbers as the chains melt (Mantsch and McElhaney, 1991). Figure 7 shows that dry EPC in the absence of additional sugars had a T_m of 42°C, which is in good agreement with published data (Crowe *et al.*, 1997). In the presence of all

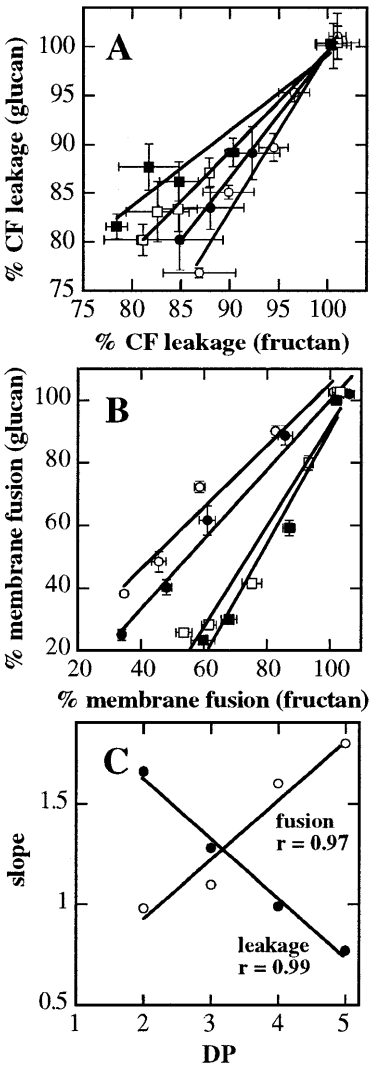

Fig. 5. Analysis of the differential effects of fructans and glucans on leakage and fusion of liposomes during air-drying. The data for this analysis were taken from Figures 2 and 3. This figure presents a correlation analysis of the leakage (**A**) and fusion (**B**) values measured in samples containing fructans and glucans of the same DP in different concentrations. The straight lines were fitted to the data by linear correlation analysis. The correlation coefficients (r) were between 0.99 and 0.96. A slope different from one indicates that the fructan and the glucan of this DP have a differential effect on leakage or fusion; open circles, DP 2; closed circles, DP 3; open squares, DP 4; closed squares, DP 5. (**C**) The dependence of these slopes on DP.

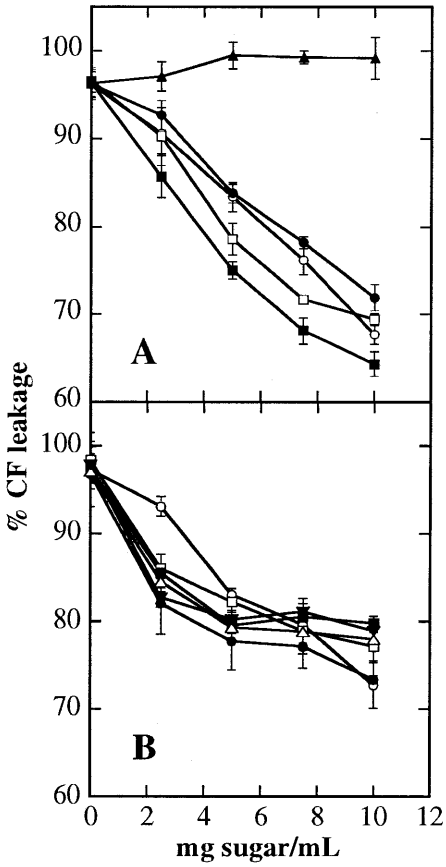

Fig. 6. Protection of large unilamellar liposomes from damage during drying by different inulins (**A**) and glucans (**B**). Vesicles were loaded with 10 mg/ml of the respective sugars; in addition the samples contained the indicated concentrations of the same sugars on the outside of the liposomes. Damage to the liposomes after air-drying and rehydration was determined as CF leakage. The symbols are the same as described in Figure 2.

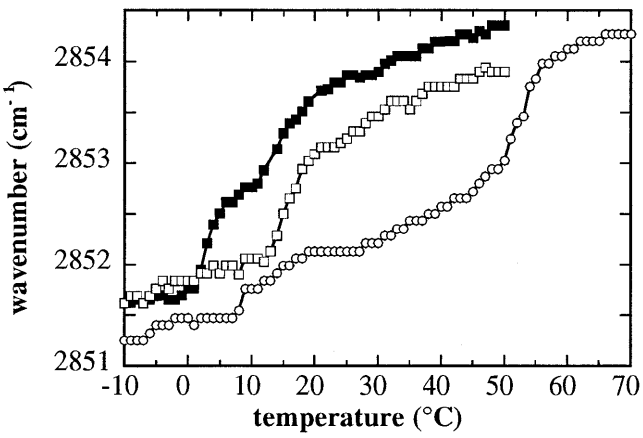

Fig. 7. Lipid melting curves of dry EPC liposomes as determined by FTIR spectroscopy. The wavenumber of the CH_2 symmetric stretch peak is plotted as a function of the sample temperature. T_m was determined as the midpoint of each melting curve. The samples contained either only EPC liposomes without additional sugars (open circles; T_m = 42°C), EPC liposomes and DP 5 inulin (closed squares; T_m = 10°C), or DP 5 glucan (open squares; T_m = 19°C) at a 1:2 mass ratio both inside and outside of the liposomes.

sugars, T_m of the dry lipid was strongly reduced. However, when the inulin and the glucan of DP 5 were compared, it was obvious that the reduction was much stronger with the fructan than with the glucan (Figure 7). An analysis that included all sugars, except the chicory inulin, used in the functional studies showed that with increasing DP, T_m steadily decreased in the case of inulins, whereas it increased in the case of glucans (Figure 8). The difference in T_m between the fructan and glucan of DP 5 was 9°C.

It has been suggested that the reduction in T_m observed in dry lipids in the presence of sugars is due to a direct hydrogen bonding interaction between the sugars and the phospholipid

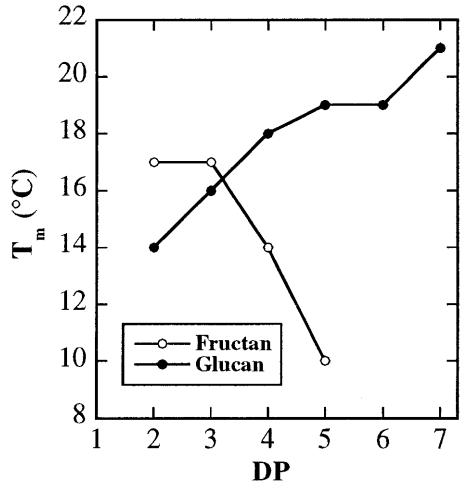

Fig. 8. T_m of dry EPC liposomes as a function of the DP of the sugars. T_m was determined from FTIR measurements of lipid melting curves as shown in Figure 7.

headgroups (reviewed in Oliver *et al.*, 1998a, 2001). This interaction can be detected as a shift in the frequency of the asymmetric phosphate vibration in the FTIR spectra around 1240 cm^{-1}. Figure 9 shows such spectra from dry EPC liposomes in the absence of additional sugars and in the presence of inulins and glucans of different DP. The EPC control samples showed a peak at 1259 cm^{-1}. This was strongly shifted in the presence of the disaccharides sucrose and maltose to 1241 and 1244 cm^{-1}, respectively. In the presence of longer-chain inulins, this frequency only slightly increased to 1246 cm^{-1} for DP 5. In the presence of glucans, however, the peak shifted strongly toward the control peak with increasing DP and only showed an additional low-field shoulder at approximately 1250 cm^{-1} with DP 6 and 7. On the other hand, the phosphate peaks in the presence of inulin, in addition to the main peak, all showed a low-field shoulder at approximately 1220 cm^{-1}, indicating additional very strong hydrogen bonding interactions.

Discussion

In the present article we have provided evidence that fructo- and gluco-oligosaccharides have fundamentally different properties regarding their ability to interact with membranes in the dry state. The most striking differences were revealed by FTIR spectroscopy in the ability of the different sugars to depress the T_m of dry EPC (Figures 7 and 8). These differences were not evident for the disaccharides, but became significant with increasing chain length. Fructans depressed T_m further with increasing DP, but T_m rose steadily with increasing DP for the glucans, from 14°C in the presence of maltose to 21°C in the presence of maltoheptaose (DP 7).

These differences were closely related to the differences observed in the ability of the sugars to form hydrogen bonds with the phosphate in EPC headgroups (Figure 9). While both disaccharides induced a downfield shift in the phosphate peak by at least 15 wave numbers, the difference between control samples containing only EPC and those containing glucans became progressively less with increasing DP, until the peaks almost coincided. The difference between the peak of control samples and of samples containing the DP 5 fructan, however,

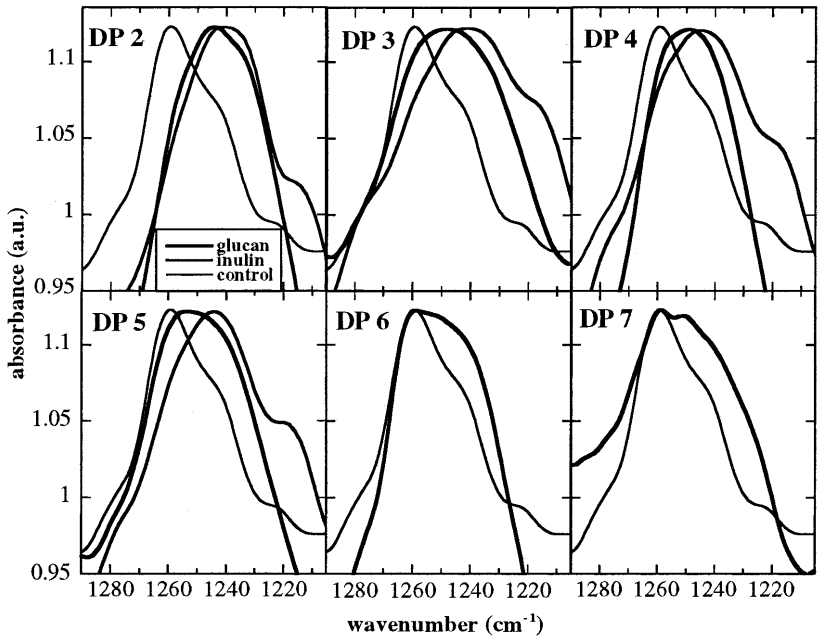

Fig. 9. FTIR spectra of the phosphate asymmetric stretch region in dry EPC liposomes. The samples contained either only EPC liposomes without additional sugars (control), or EPC liposomes and inulins or glucans of the indicated DP at a 1:2 mass ratio both inside and outside of the liposomes. Spectra were recorded at 50°C to ensure that the membranes were in the liquid-crystalline phase in all cases (compare Figures 7 and 8).

was still 13 wave numbers. In addition, all spectra from samples containing fructans showed a clear shoulder, centered around 1220 cm^{-1}, indicating very strong hydrogen bonding between the fructans and at least a subpopulation of the EPC head groups (Crowe *et al.*, 1996).

It is interesting to note that the inability of polymeric glucans to directly interact with membrane lipids has been previously attributed to the large size of these molecules (Crowe *et al.*, 1996, 1997; Tsvetkova *et al.*, 1998; Hincha *et al.*, 2000). We have presented evidence here that size-related effects of steric hindrance can not fully account for the observed effects of oligo- or polymeric sugars, as we have directly compared glucans and inulins of the same DP. Likewise, it has recently been shown (Vereyken *et al.*, 2001) that a bacterial fructan of DP > 25,000 was able to interact with fully hydrated lipid model membranes, but a smaller dextran was not. These findings indicate that much more specific (although currently not fully understood) properties of the different sugars than just size determine their ability to interact with membranes.

These differences in the ability of the different sugars to interact with membrane lipids and depress T_m were also reflected in their ability to stabilize liposomes during drying. The differences in CF leakage (Figures 2, 4, 6), however, were not as clear-cut as those observed by FTIR spectroscopy, because CF leakage is influenced not only by lipid phase transitions but also by vesicle fusion. In the concentration range used, the effects on fusion were much stronger than the effects on leakage (Figure 2). This is in agreement with previous findings (Crowe *et al.*, 1985), that much higher concentrations of the disaccharide trehalose were necessary to completely prevent leakage than were necessary to prevent fusion.

For the glucans, we found an increased ability to prevent fusion with increasing DP (Figures 4 and 5). In parallel, the ability to prevent leakage decreased. As a decrease in fusion should normally translate into a decrease in leakage, this could only mean that leakage due to lipid phase transitions increased very strongly with DP and thus surpassed the decrease in leakage associated with increased protection against fusion. This is in good agreement with the FTIR data, as discussed above. For the fructans, the reverse argument is applicable, as their ability to prevent leakage increased with increasing DP, although their ability to prevent fusion decreased.

The reduced ability of the inulins to prevent fusion with increasing DP is a surprising observation. In general, protection against fusion by sugars has been related to their ability to form glasses (vitrify) during drying (Crowe *et al.*, 1998). Because the propensity of oligomeric substances to vitrify has in many cases been found to increase with increasing DP (Levine and Slade, 1988; Slade and Levine, 1991), we would have expected increased protection against fusion from both fructans and glucans with increasing chain length. However, only the glucans, at least up to DP 5, fulfilled this expectation and for the glucans an increase in glass transition temperature (T_g) with increasing DP has indeed been reported before (Orford *et al.*, 1990; Slade and Levine, 1991). For the inulins, no conclusive data are available from the literature, as only commercial mixtures have been investigated and no information about composition and purity has been supplied (Schaller-Povolny *et al.*, 2000; Hinrichs *et al.*, 2001). With these preparations, however, the expected increase in T_g with increasing average DP was observed. It is therefore not clear at this point why the pure inulins (Figure 1) used in our experiments showed reduced protection against fusion with increasing DP.

Another unexpected finding was that the chicory inulin that had provided protection against leakage during freeze-drying (Hincha *et al.*, 2000) was completely ineffective during air-drying (Figures 2, 3, 6). The most likely reason is that the chicory inulin precipitated from solution during the slow air-drying process and thus was not available for membrane stabilization or glass formation. Visual inspection of the dried samples showed a powdery appearance, whereas all other sugars gave a clear, transparent film. We assume that in the freeze-drying process the chicory inulin was immobilized in the vicinity of the membranes during freezing in liquid nitrogen and therefore precipitation was prevented and protection became possible.

The fructan concentrations we have used in our fusion and leakage experiments may be expected to be present in plant cells, at least for example after cold acclimation (Livingston and Henson, 1998). Oligomeric glucans, on the other hand, have only been found in total concentrations between 1 and 2 mg ml^{-1} in plant cells (van de Wal *et al.*, 1998). Glucans could interfere with starch synthesis and probably other metabolic functions in plants and are therefore not expected to be accumulated to higher concentrations. In general, it may be expected that oligomeric or polymeric sugars that are not directly involved either in photosynthetic carbon fixation or glycolysis could be accumulated in plant cells to higher concentrations under stress than other sugars, without negative effects on primary metabolism and growth. We therefore conclude from our results that fructans may have unique properties that would make them ideal solutes to stabilize plant cells under stress. We are currently testing this hypothesis using transgenic plants that accumulate fructans in their leaves.

Materials and methods

Materials

EPC was purchased from Avanti Polar Lipids (Alabaster, AL) or Sigma. CF was obtained from Molecular Probes (Eugene, OR) and was purified according to the procedure described by Weinstein *et al.* (1984). N-(7-Nitro-2,1,3-benzoxadiazol-4-yl)-phosphatidylethanolamine (NBD-PE) and N-(lissamine Rhodamine B sulfonyl)-dioleoylphosphatidylethanolamine (Rh-PE) were purchased from Molecular Probes. Inulins from chicory roots, Suc, and all malto-oligosaccharides were purchased from Sigma; fructo-oligosaccharides were obtained from Gammazym (Gamma Chemie, München, Germany).

Sugar analysis

Malto- and fructo-oligosaccharides were analyzed by HPLC using a CarboPac PA-100 anion exchange column on the Dionex DX-300 gradient chromatography system (Dionex, Sunnyvale, CA) coupled with pulsed amperometric detection by a gold electrode. The column was equilibrated in 0.15 M NaOH and was eluted with a linear gradient of 1 M NaAc in 0.15 M NaOH as described in detail in previous publications (Hellwege *et al.*, 1997, 1998).

Preparation of liposomes

EPC was dried from chloroform under a stream of N_2 and stored under vacuum overnight to remove traces of solvent. Liposomes were prepared from hydrated lipids using a hand-held extruder with two layers of polycarbonate membranes with 100 nm pores (MacDonald *et al.*, 1991; Avestin, Ottawa, Canada).

Leakage experiments

For leakage experiments, an appropriate amount of lipid was hydrated in 0.25 ml of 100 mM CF, 10 mM TES, 0.1 mM ethylenediamine tetra-acetic acid (pH 7.4). After extrusion, the vesicles were passed through a NAP-5 column (Sephadex G-25; Pharmacia) equilibrated in TES-EDTA-NaCl (TEN) buffer (10 mM TES, 0.1 mM ethylenediamine tetra-acetic acid [pH 7.4], 50 mM NaCl), to remove the CF not entrapped by the vesicles. The eluted samples were then diluted with TEN to a lipid concentration of approximately 10 mg ml^{-1}. Liposomes (40 µl) were mixed with an equal volume of concentrated solutions of fructans and glucans in TEN and 20-µl aliquots were filled into the wells of 60-well microplates. The plates were dried in desiccators at 28°C and 0% relative humidity for 24 h in the dark.

Damage to the liposomes was determined after rehydration with 20 µl TEN buffer. For leakage measurements, 10 µl of sample were diluted in the wells of black 96-well plates in 0.3 ml TEN. Measurements were made with a Fluoroskan Ascent (Labsystems, Helsinki, Finland) fluorescence microplate reader at an excitation wavelength of 444 nm and an emission wavelength of 555 nm. Fluorescence of CF is strongly quenched at the high concentration inside the vesicles and is increased when CF is released into the medium. The total CF content of the vesicles (100% leakage value) was determined after lysis of the membranes with 5 µl 1% Triton X-100. The values were corrected for the quenching of CF fluorescence by Triton X-100. The figures show the means ± SD from three parallel samples. Where no error bars are visible, they were smaller than the symbols.

Fusion experiments

For liposome fusion experiments, two liposome samples were prepared in TEN. One contained 1 mol% each of NBD-PE and Rh-PE in EPC, the other contained only EPC. After extrusion, liposomes were combined at a ratio of 1:9 (labeled:unlabeled), resulting in a lipid concentration of 10 mg ml^{-1}. Liposomes (40 µl) were mixed with an equal volume of concentrated solutions of solutes in TEN and 20-µl aliquots were filled into the inside of the caps of 1.5-ml microcentrifuge tubes. Samples were dried as described above and were rehydrated by filling 1 ml TEN buffer into a tube and then quickly closing and inverting the tube. Membrane fusion was measured by resonance energy transfer (Struck *et al.*, 1981) with a Kontron SFM 25 fluorometer (Bio-Tek Instruments, Neufahrn, Germany) as described (Hincha *et al.*, 1998; Oliver *et al.*, 1998b). The figures show the means ± SD from three parallel samples. Where no error bars are visible, they were smaller than the symbols.

FTIR spectroscopy

Spectra were obtained from samples containing sugar and EPC liposomes at a weight ratio of 1:2, corresponding to the highest sugar concentration used in the leakage and fusion experiments. Liposomes were extruded in the presence of the sugars, so that the sugars were present on both sides of the membranes. Fifty microliters of sample were spread on a CaF_2 window and dried under the same conditions as described above. The window with the dried sample was fixed in a cuvette holder connected to a temperature control unit (Specac Eurotherm, Worthington, UK). The cuvette holder was placed in a vacuum chamber with windows which was placed in the infrared beam. Temperature was controlled by a liquid N_2 reservoir and an electrical heater. Temperature was measured with a thermocouple attached to the cuvette holder next to the sample. The sample was first heated to 50°C for at least 15 min under vacuum to remove residual moisture the lipid had taken up during sample handling. The effectiveness of this procedure was verified by the absence of a water band in the FTIR spectra at 1650 cm^{-1}. The sample was then cooled to –30°C and after a 5-min equilibration the temperature was increased at a constant rate of 1°C min^{-1}. Spectra were recorded with a Perkin-Elmer GX 2000 Fourier-transform infrared spectrometer, assisted by a computer equipped with the Spectrum 2000 software. The peak frequencies of the CH_2 symmetric stretch band around 2850 cm^{-1} were estimated by eye after normalization of absorbance and baseline flattening, using the interactive abex and flat routines, respectively (Tsvetkova *et al.*, 1998). T_m was estimated by eye as the midpoints of the lipid melting curves (Crowe *et al.*, 1997). The peaks from the phosphate asymmetric stretch vibrations of different samples were compared after normalization of absorbance (abex) and baseline flattening in the 1300–1200 cm^{-1} region. Because the peaks from samples containing inulins showed a strong broadening and the appearance of a shoulder when compared to controls and samples containing glucans, all phosphate peaks were further treated with the deconvolution routine included in the Spectrum software to enhance these spectral differences.

Acknowledgments

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Abbreviations

CF, carboxyfluorescein; DP, degree of polymerization; EPC, egg phosphatidylcholine; FTIR, Fourier transform infrared; HPLC, high-performance liquid chromatography; NBD-PE, N-(7-nitro-2,1,3-benzoxadiazol-4-yl)-phosphatidylethanolamine; Rh-PE, N-(lissamine Rhodamine B sulfonyl)-dioleoylphosphatidylethanolamine; T_g, glass transition temperature; T_m, gel to liquid-crystalline lipid phase transition temperature; TEN, TES-EDTA-NaCl; TES, CN-tris(hydroxymethyl)methyl-2-aminoethane–sulfonic acid.

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