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THE INFLUENCE OF SUGARS ON THE PROPERTIES OF FREEZE-DRIED LYSOZYME AND HAEMOGLOBIN

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ABSTRACT

To learn more about the role of the sugar trehalose in anhydrobiosis, a study has been made of the effect dehydration has on properties of the proteins lysozyme and haemoglobin. Solutions containing just the proteins or the proteins and a sugar, either trehalose, galactose or glucose, were freeze dried. Differential scanning calorimetry of lysozyme showed that in going from dilute solution to the solid state the denaturation temperature increased by 70 °C and the denaturation enthalpy change halved. Unlike solutions where addition of sugar caused an increase in denaturation temperature (T_d), in the dry state the presence of sugar reduced T_d. The denaturation enthalpy changes in the presence of sugars ranged from values similar to those measured in dilute solution to values almost three times greater. Dehydration of oxyhaemoglobin solutions in the absence of sugars led to oxidation of the haem group and the formation of methaemoglobin. The presence of sugars either completely suppressed or markedly reduced haem oxidation during dehydration and reduced the oxidation rate when dry haemoglobin was stored. These results show that sugars can substitute for water and so enable proteins to retain in the solid state properties they usually exhibit only in aqueous solution. Two factors have been identified which may make trehalose of special value to anhydrobiotic organisms. Trehalose does not react with proteins at elevated temperatures, as reducing sugars do, and its solutions are more readily dehydrated.

INTRODUCTION

Water is commonly thought to be indispensable to the maintenance of life. However there are a small number of living organisms which are able to survive complete dehydration. This fact was recognized as long ago as the year 1800 when it was found that in a dried state tardigrades, small sand living organisms, were able to withstand temperatures well below freezing

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and as hot as boiling water [1,2]. Normal metabolic processes resumed in these organisms within 30 min of their rehydration even after decades of dry storage. In recent years the ability to survive complete desiccation, now called anhydrobiosis, has been shown to be a property possessed by a small but diverse group of organisms. These organisms include spores of certain fungi [3], macrocysts of the slime mold *Dictyostelium* [4], dry active Baker's yeast [5], brine shrimp cysts [6] and the dry larvae and adults of several species of nematode [7,8]. Survival of dehydration by some of these organisms has been correlated with the synthesis of trehalose during dehydration [7,9] or its degradation following rehydration [10]. The sugar trehalose is a non-reducing disaccharide of glucose. Trehalose has also been shown to be responsible for the behaviour of the desert dwelling 'resurrection' plant which shrivels to a dried brown husk in a drought but revives completely on watering, instead of sprouting again from ground level [9]. The repeated occurrence of trehalose in such a diverse group of anhydrobiotic organisms is a remarkable example of parallel evolution.

Recent work by Crowe et al. [11] has shown that dehydration causes irreversible damage to cell membranes. For example, when vesicles of calcium transporting membranes were dehydrated, the phospholipids formed complex crystalline phases, some of which were non-bilayer structures. Intramembrane particles that represent the calcium dependent ATPase were excluded from the crystals. Upon rehydration, vesicles showed evidence of morphological damage (including fusion and redistribution of intramembrane particles) and calcium transport activity was lost. By contrast, when the membranes were dried in the presence of trehalose at a concentration of at least 20% of the dry weight of the membranes (concentrations similar to those found in anhydrobiotic organisms) no evidence of phase transitions was seen during dehydration, and upon rehydration, vesicles were similar morphologically and functionally to freshly prepared ones. However, as well as the plasma membrane, other structures and complex molecules within the cell must be able to survive dehydration.

Sugars and polyols have been widely used by biochemists to stabilize biological molecules in solution. These additives have been shown to preserve enzymatic activities [12], inhibit irreversible aggregation [13] and increase the temperature at which proteins are thermally denatured [14,15]. Very little use has been made of sugars to preserve the native structure of biological materials in the dry state. That such a practice can be of value has been demonstrated by Henderson and Unwin, who were able to obtain electron micrographs of purple membranes from halobacteria by drying them from glucose solutions prior to transfer to the vacuum chamber of their instrument [16].

Our broad aim is to establish how effectively sugars can replace water in biological materials and preserve their structural integrity in the dry state. To this end we have compared properties of proteins in aqueous solution

with measurements of the same property after the protein has been dehydrated, in isolation and admixed with sugars. For this initial study it was decided to study the thermal denaturation of lysozyme and the spectrum of haemoglobin. A considerable literature on the solution properties of these two proteins is already in existence. The proteins have been dehydrated in the presence of three sugars, trehalose, galactose and glucose.

EXPERIMENTAL

Materials

Chicken egg white lysozyme was obtained from Sigma Chemical Co. as a three times crystallized, dialysed and lyophilized powder (Grade 1). Human adult haemoglobin (HbA) was isolated and purified from out-dated units of red blood cells by standard procedures [17]. Soluble HbA was dialysed against Tris buffer (0.05 M Tris, 0.15 M NaCl, pH 7.4) and stored at 4°C. Stock HbA solutions were approximately 5mM in haem as measured by the absorbance of the carbon monoxy form (HbCO) at 540nm. α,β-Glucose and α,β-galactose were purchased from Fluka, AG and α,α-trehalose from Aldrich Chemical Company.

Methods

Calorimetry

Samples for calorimetry were made by dissolving lysozyme in distilled water (pH 5.5). The concentration of the solutions was usually 10% w/w. Pure water was used in order that direct comparisons could be made with measurements on dry protein samples. It was suspected that the constituents of buffers would have different effects on the thermal characteristics of dry lysozyme and lysozyme in aqueous solution. Dry protein and sugar–protein mixtures were prepared by freeze drying the solutions and standing the powders obtained in a vacuum desiccator over phosphorus pentoxide. Samples were removed daily for thermal analysis until there was no difference between the thermograms recorded on three consecutive days. The sample was then presumed to have equilibrated and it is these results which are reported below. The storage periods required varied between one and two weeks. DSC measurements were carried out with a Perkin–Elmer DSC-2 instrument. The heating rate was 2.5°C min^{-1} and a full scale deflection on the recorder corresponded to a change in heat flow of 0.25 mcal s^{-1}. The instrument was calibrated by remeasuring the previously established temperatures and enthalpies of fusion of palmitic acid and indium. Protein (1 mg), dry sugar–protein mixtures (sufficient for there to be 1 mg of protein in the pan) and protein or sugar–protein solutions (15 microlitres) were sealed

inside aluminium calorimetry pans and their thermograms measured immediately. A blank run (sugar or water) was made at the conclusion of a series of sample runs. A planimeter was used to measure the area under the thermograms in the transition region and the enthalpy change of the transition (ΔH_{cal}) was calculated using a calibration constant and the molecular weight of lysozyme (14445). The transition temperatures (T_{tr}) reported are the temperatures of maximum heat flow. In these experiments there was little difference between this temperature and the temperature at which the transition was half complete. The entropy change, ΔS_{cal}, was obtained by dividing ΔH_{cal} by T_{tr}.

Spectrophotometric measurements of haemoglobin spectra

For spectrophotometric measurements haemoglobin and sugar–haemoglobin mixtures were dissolved in Tris buffer (0.05 M Tris, 0.15 M NaCl, pH 7.4). Spectra were recorded on a Pye–Unicam SP8-100 UV–visible spectrophotometer. Oxy-(HbO_2) and deoxyhaemoglobin (Hb) were oxidized to methaemoglobin (MetHb) by addition of an excess of potassium ferricyanide solution (200 mg $K_3Fe(CN)_6$, 140 mg KH_2PO_4 in 100 cm^3 water, pH 7.4). The concentrations of oxy-, deoxy- and methaemoglobins in solution were calculated from the spectrum of the solution using equations derived by Benesch et al. [18].

RESULTS

Calorimetric studies of lysozyme and sugar–lysozyme solutions

Table 1 contains the denaturation temperatures and denaturation enthalpy and entropy changes of lysozyme dissolved in water and in solutions of one of the sugars glucose, galactose or trehalose. The values obtained for the solutions which do not contain sugar are broadly in agreement with those reported by other authors [19–21] when allowance is made for the effects of pH and protein concentration [22].

The presence of one of the three sugars in a solution of the protein increases the denaturation temperature. When sugar and protein concentrations were equal the temperature increase averaged 2.7°C, (see *d–g*) while at a ratio of sugar to protein of 5 : 1, the average increase was 10.2°C (see *j–l*). Hence the sugars stabilise the native structure of the protein with respect to the denatured state. There does not appear to be any significant difference between the stabilising capacities of the individual sugars at a 1 : 1 ratio of sugar to protein, but at a 5 : 1 ratio, glucose and galactose are more effective than trehalose. While T_d rises progressively as the sugar-protein ratio is increased, the enthalpy and entropy changes initially rise, at 1 : 1, and then fall back to lower values, at 5 : 1, which in the cases of glucose and

TABLE 1

Denaturation temperatures and denaturation enthalpy and entropy changes of lysozyme in pure water and aqueous sugar solutions

Solution	Conc. lysozyme in water (w/w%)	Sugar	Ratio sugar to lysozyme (mol : mol)	T_d (°C)	ΔH_{cal} (kcal mol^{-1})	ΔS_{cal} (cal (mol K)$^{-1}$)
a	5	None	–	72.8	142.0	410.5
b	10	None	–	70.9	116.2	337.7
c	20	None	–	68.8	105.7	309.1
d	10	Glucose	1 : 1	73.8	118.9	342.7
e	10	Trehalose	1 : 1	73.6	119.6	344.9
f	10	Galactose	1 : 1	73.5	139.0	401.0
g	10	Galactose	1 : 1	73.6	133.0	384.2
h	5	Galactose	1 : 1	73.9	136.3	392.7
i	20	Galactose	1 : 1	75.3	101.0	289.6
j	10	Glucose	5 : 1	82.2	88.8	249.8
k	10	Trehalose	5 : 1	79.8	111.5	315.8
l	10	Galactose	5 : 1	81.5	90.4	254.9

galactose are significantly less than that measured for the protein in water.

Variation of the protein concentration (compare *a–c*) shows that the denaturation temperature and denaturation enthalpy and entropy changes decrease as the protein concentration is increased. The relationship between protein concentration and denaturation temperature is influenced by the presence of sugars. In galactose solutions the denaturation temperature is similar for 5% and 10% protein concentrations but rises by 1.8 °C when the protein concentration is increased to 20%, (compare *f–i*) unlike in sugar free solution where it falls. However the enthalpy and the entropy changes decrease as the protein concentration is raised, as they do in water, and by approximately the same percentages.

Calorimetric studies of dry sugar–lysozyme mixtures

Trehalose–lysozyme mixtures freeze dried rapidly and formed fine free running powders. Storage for a day or two over phosphorus pentoxide was sufficient for them to reach a state where they had reproducible thermograms. By contrast glucose mixtures did not immediately dry to free running powders. They were ‘sticky’ and appeared to be wet. Two weeks desiccation was required to transform them into free running powders which had a consistent thermal behaviour. The characteristics of galactose mixtures were intermediate between the other two.

Representative thermograms of dry lysozyme and sugar–lysozyme mixtures are shown in Fig. 1. All the thermograms except that of the 5 : 1

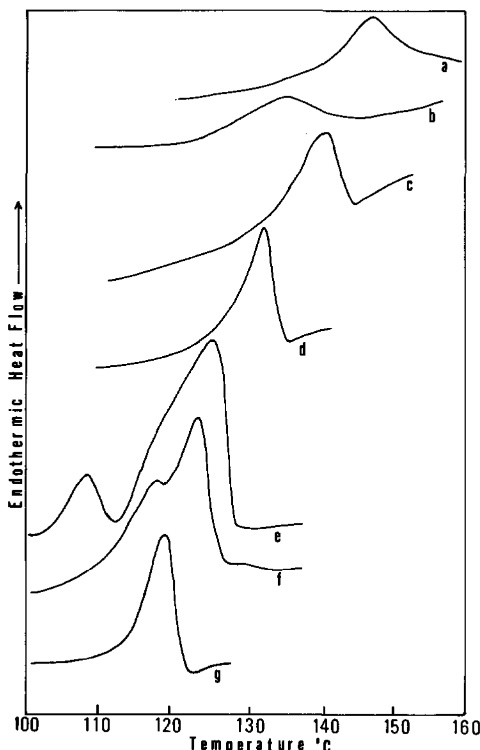

Fig. 1. DSC thermograms of lysozyme: (a) direct from supplier, (b) freeze dried, freeze dried with five times its weight of (c) trehalose, (f) glucose and freeze dried with an equal weight of (d) trehalose, (e) galactose and (g) glucose.

galactose–lysozyme mixture exhibit endothermic transitions. The 1 : 1 galactose mixture (see (e)) has two separate endotherms, the one at lower temperature being by far the weaker. The endotherm of the 5 : 1 glucose mixture (see (f)) has a 'shoulder' on its low temperature side. The thermogram of the 5 : 1 galactose–lysozyme mixture is dominated by a very strong exotherm in the temperature range in which the other mixtures undergo endothermic transitions (trace not shown). The pure dry sugars do not undergo thermal transitions below 150 °C.

The temperatures of maximum heat flow and the enthalpy and entropy changes associated with the transitions are set out in Table 2. The endothermic transition of pure dry lysozyme occurs at a temperature approximately 70 °C above the denaturation temperature of the protein in water (10% w/w). The transition takes place over a greater temperature range (i.e. with a lower co-operativity) and both ΔH_{cal} and ΔS_{cal} are significantly reduced (compare *g* with *b*, Table 1). ΔH_{cal} is approximately one half and ΔS_{cal} one third the values recorded for the denaturation of the protein in water (10% w/w solution). After freeze drying and storage over phosphorus pentoxide

TABLE 2

Transition temperatures and transition enthalpy and entropy changes of freeze dried lysozyme and sugar–lysozyme mixtures

Solution	Ratio sugar to lysozyme (mol : mol)	Sugar	Mean T_{tr} (°C)	S.D. (range)	Mean ΔH_{cal} (kcal mol^{-1})	S.D. (range)	ΔS_{cal} (cal $(mol\ K)^{-1}$)	Number of tests
a	1:1	Glucose	117.0	3.56	152.6	13.1	391.1	8
b	1:1	Trehalose	127.3	5.56	123.8	7.6	309.2	11
c	1:1	Galactose	128.1 [a]	3.25	368.0	22.1	917.2	6
			110.9	3.45	66.0	4.1	171.9	
d	5:1	Glucose	122.5	123.7–120.9	348.3	351.7–344.9	880.3	3
e	5:1	Trehalose	139.4	1.65	140.1	13.9	339.6	4
f	5:1	Galactose	See text					
g	Pure lysozyme		139.6	5.80	63.9	13.9	154.8	9
h	Pure lysozyme (freeze dried)		128.8	9.80	49.8	11.1	123.9	8

[a] Thermogram displays two separate endotherms.

the transition temperature of the lysozyme endotherm fell by approximately 10 °C and there was a further reduction in both ΔH_{cal} and ΔS_{cal} (see *h*). The presence of the sugars decreased the transition temperature, increased ΔH_{cal} and ΔS_{cal} and narrowed the temperature range over which the transition occurred. The ΔH_{cal} and ΔS_{cal} values for both trehalose and the 1 : 1 glucose mixtures are within the range of values found for lysozyme in aqueous solution. In the case of the galactose and the 5 : 1 glucose–lysozyme mixtures ΔH_{cal} and ΔS_{cal} are almost three times greater (compare *b*, *e* and *a* with *c* and *d*). A comparison of the sugar–lysozyme endotherms shows that the T_{tr} values of trehalose–lysozyme and, at least at 1 : 1, galactose–lysozyme are greater than glucose–lysozyme. It is clear that the enthalpy and entropy changes associated with these endothermic transitions and T_{tr} values vary between sugars more in the dry state than they do in water. Judging from the glucose and trehalose results it would appear that increasing the sugar–protein ratio from 1 : 1 to 5 : 1 increases T_{tr} by between 5 and 10 °C. The thermal denaturation of lysozyme in the solid state, with or without admixed sugars, is irreversible as calorimetric cooling curves show no evidence of thermal transitions.

When glucose and galactose samples were inspected after heating it was found that they were no longer white free running powders, but had become black-brown ‘resins’. To establish the temperature at which this change in state occurred, samples of each of the sugar–protein mixtures were packed into glass capillary tubes and observed as they were heated in a melting point apparatus. There were no significant changes in the appearance of trehalose–lysozyme mixtures even when they were heated to 170 °C. Both galactose and glucose mixtures turned brown, the galactose mixture starting at 120 °C and the glucose mixture at 140 °C.

Visible spectra of freeze dried haemoglobin and sugar–haemoglobin mixtures

The spectrum of haemoglobin dissolved in Tris buffer is shown in Fig. 2. When haemoglobin solutions are freeze dried, the colour of the protein changes from red to brown. By comparing the spectra in Fig. 2 ((a) with (c)) the changes in the spectrum responsible for the changes in the colour of the protein can be seen. The characteristic double peaked absorption lying between 500 and 600 nm is replaced by a broader complex absorption of lower extinction coefficient. The sharp strong absorption (Soret band) at 427 nm is blue shifted by 23 nm. Very similar spectral changes occur when potassium ferricyanide is dissolved in haemoglobin solutions. Potassium ferricyanide oxidises the iron atom of the haem prosthetic group from the $2+$ to the $3+$ state, i.e. it converts oxy- and deoxy-haemoglobins to methaemoglobin. Therefore the colour change observed during dehydration is due to the formation of methaemoglobin. However if either of the sugars (at weight ratios of 1 : 1 or 5 : 1 sugar–haemoglobin) is added to haemoglo-

Fig. 2. Visible spectra of (a) human adult haemoglobin (HbA), (b) HbA after treatment with $K_3Fe(CN)_6$ and (c) HbA after freeze drying. The spectra of (b) and (c) in the 400–500 nm range are identical. The spectra of (a) and (b) in the 400–500 nm range are shown half-scale.

bin solutions there is no apparent change in colour when water is pumped off.

Dry sugar–protein mixtures were mixed with KBr and compressed into transparent discs in order that their visible spectra might be recorded. These spectra and the corresponding spectra of solutions in Tris buffer were identical. The dry sugar–protein mixtures were stood under vacuum over phosphorus pentoxide and after three months their spectra were re-recorded. The percentages of oxy-, deoxy- and methaemoglobins in each mixture were calculated and the values obtained are listed in Table 3. In two instances the calculations yielded percentages which individually or in sum exceeded 100 and percentages which had negative values. This suggests that in these solutions species other than oxy-, deoxy- and methaemoglobin were present. In one case, fresh HbA treated with $K_3Fe(CN)_6$, it would not be surprising if a small percentage of haemichrome formed during oxidation. However the result for the 5 : 1 trehalose–haemoglobin mixture was not expected and is difficult to explain.

With one exception, the inhibition of oxidation during dehydration is independent of the type of sugar or its ratio to protein. The exception is the 1 : 1 trehalose mixture, where the amount of methaemoglobin formed, 37.1%,

TABLE 3

The compositions of fresh and freeze dried human adult haemoglobin

		Fresh HbA	Fresh HbA + $K_3Fe(CN)_6$	After freeze drying with (weight : weight)						
				No sugar	1:1 glucose	5:1 glucose	1:1 trehalose	5:1 trehalose	1:1 galactose	5:1 galactose
Initial measurements	%Hb	1.3	−5.0	4.5	6.1	8.1	0.2	5.9	6.8	7.5
	%HbO_2	83.3	−4.9	12.9	73.4	81.6	62.2	79.2	79.3	75.5
	%MetHb	15.4	109.9	82.6	20.5	10.3	37.7	15.0	13.9	17.1
After standing for three months	%Hb	2.5	–	1.5	3.5	3.0	0.6	−4.1	1.2	1.9
	%HbO_2	84.3	–	0.7	62.5	51.5	43.4	48.8	64.5	74.3
	%MetHb	13.2	–	97.8	34.0	45.5	56.1	55.3	34.3	23.8

is more than double the average for the other five mixtures. The average for these mixtures, 15.4%, is identical to the percentage of methaemoglobin in fresh HbA. While little or no oxidation seems to have taken place in these samples during dehydration, oxidation to methaemoglobin proceeds slowly on standing over phosphorus pentoxide. There seems to be no relationship between the sugar–protein ratio and the oxidation rate. Oxidation takes place more rapidly with the glucose mixture at the 5 : 1 ratio, while in the case of galactose it is the 1 : 1 mixture in which oxidation is most rapid. However there are significant differences between the capacities of the sugars to inhibit oxidation on standing. Averaged over both sugar–protein ratios, the percentage MetHb in the samples after three months is trehalose 55.7%, glucose 39.8% and galactose, 29.1%.

DISCUSSION

Calorimetry of sugar–lysozyme solutions

An extensive literature exists on the influence of carbohydrates on the properties of dissolved proteins. It has been found that many carbohydrates have a stabilising action on protein structure [21,23–28]. A protein in a polyol or saccharide solution denatures at a higher temperature than the same protein dissolved in pure water. The magnitudes of the increases in denaturation temperature measured by us are in general similar to those described by earlier authors. In addition we found that the sign of the difference between the denaturation enthalpy and entropy changes in water and sugar solutions depends on the sugar–protein ratio. At a 1 : 1 ratio of sugar to protein, ΔH_{cal} and ΔS_{cal} are greater than the values measured for protein dissolved in pure water, while at a 5 : 1 ratio both are less. Therefore, given the relationship $T_d = \Delta H_{cal}/\Delta S_{cal}$, it can be concluded that protein stabilisation occurs at the lower sugar–protein ratio because ΔH_{cal} increases more rapidly than ΔS_{cal}, and at the higher ratio, because ΔS_{cal} decreases more rapidly than ΔH_{cal}. Thus these sugars seem to inhibit the disordering of the system induced by heating, with the degree of inhibition increasing as the ratio of sugar to protein is increased. Other workers who have studied this phenomenon have reached similar conclusions and suggest that after denaturation the extent to which carbohydrate is excluded from the domain of the protein is increased [21,23–28]. Exclusion of carbohydrates from the domain of the protein is thought to occur because carbohydrates strengthen the hydrogen bond network of water and thereby inhibit its rearrangement around hydrophobic residues on the protein surface. This effect is more pronounced on denaturation because hydrophobic residues buried in the interior of the native protein molecule are exposed to water.

Lysozyme samples dissolved in concentrated monosaccharide solutions have significantly higher denaturation temperatures and exhibit lower enthalpy changes on denaturation than lysozyme dissolved in the corresponding trehalose solution. This suggests that monosaccharides are more effective at reinforcing the hydrogen bond network of water than disaccharides.

Calorimetry of dry sugar–lysozyme mixtures

Dry lysozyme undergoes a thermal transition at a temperature 70 °C above its denaturation temperature in water (at a 10% w/w concentration). Although there may be differences in detail between the mechanisms of these two transitions, it is highly likely that the transition in the dry state is also a thermally induced unfolding of the polypeptide chain and can thus be called a denaturation. Rupley et al. have shown that the conformation of lysozyme is unaffected by water removal [29,30]. In the solid state the polypeptide chains of a protein will be less flexible than in solution at the same temperature. Therefore a dry protein will reach the degree of flexibility necessary for unfolding of the polypeptide chains at a much higher temperature than a protein in aqueous solution.

The enthalpy change accompanying the denaturation of dry lysozyme is only half that recorded when the protein is denatured in aqueous solution (10% w/w). Evidently in the dry state there is a reduction in the strength of the forces which stabilise the native structure of the protein. Attention has already been drawn to the fall in the ΔH_{cal} of denaturation of lysozyme in solution caused by increasing its concentration. It is probable that if the solution concentration was further increased ΔH_{cal} would eventually approach its dry state value.

Comparison of the protein and sugar–protein mixture results shows that addition of sugar causes T_d to fall and ΔH_{cal} to rise. This implies, given the relationship $\Delta S_{cal} = \Delta H_{cal}/T_d$, that ΔS_{cal} increases more rapidly than ΔH_{cal} and demonstrates that a considerable disordering takes place when lysozyme is denatured in the presence of the sugars. The most likely explanation for this is that a network of water and sugar molecules hydrogen bonded to the protein surface is disrupted when the protein unfolds. The presence of sugars gives the protein structure a stability at least the equivalent of that it possessed in aqueous solution.

The readiness with which trehalose solutions freeze dry suggests that they have a higher vapour pressure than solutions of the other two sugars. This suggestion is consistent with the hypothesis put forward earlier to explain differences between the T_d and ΔS_{cal} values of lysozyme in different sugar solutions—that monosaccharides are more effective in reinforcing the hydrogen bond network of water than disaccharides. The differences in T_d between the sugar-protein mixtures are likely therefore to be the result of differences in residual moisture contents. The glucose mixture would have a

higher residual moisture content than the trehalose mixture. Madden et al., using tritiated water as a probe, have shown that when egg phosphatidylcholine is freeze dried in the presence of trehalose, the powder obtained contains 5% moisture [31].

The change in colour that occurs when glucose and galactose are heated with lysozyme is due to the "browning" reaction. Reducing sugars such as galactose and glucose react with the free amino groups of dry proteins. Trehalose is not a reducing sugar and therefore does not undergo this reaction. The browning reaction of the galactose mixture starts at a temperature 20 °C below the glucose mixture and this makes it impossible to record the denaturation endotherm of the 5:1 ratio mixture because it is overwhelmed by the exotherm of this reaction.

Characteristics of dry sugar–haemoglobin mixtures

Evidently dehydration of haemoglobin destabilises the 2+ state of the iron atom. Studies of lysozyme have shown that, on its dehydration, charges on carboxylate and ammonium groups are neutralised [29,30]. While this has no effect on its structure, it is possible that the structure of haemoglobin is more sensitive to the state of charge on the polypeptide chain and it is the neutralization of ammonium and carboxylate groups on dehydration which causes the oxidation of the iron atom. This hypothesis suggests a mechanism by which sugars inhibit the oxidation of haemoglobin. Sugar molecules surrounding ionic groups on the surface of the protein would provide a medium of sufficiently high dielectric constant that ammonium and carboxylate groups would remain ionised when water was removed.

It is clear from this work that sugars do have the capability to substitute for water and so enable proteins to retain in the solid state properties they usually exhibit only in aqueous solution. Galactose and glucose possess this capacity as well as trehalose. However in two respects trehalose differs from the monosaccharides; its solutions more readily dehydrate and it does not react with proteins (see also ref. 32). One or both of these factors may be the reason it occurs so often in anhydrobiotic organisms.

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