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THE HEAT OF DEHYDRATION OF HALOPHILIC BACTERIA AS MEASURED BY DIFFERENTIAL SCANNING CALORIMETRY (DSC)

B.Z. GINZBURG *

Physiological Laboratory, Cambridge University, Cambridge, (Gt. Britain)

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ABSTRACT

The heat of dehydration of two species of halophilic bacteria and of red cell pellets was measured by DSC. The molar heat of dehydration of *H. marismortui* was found to be 50.4 kJ mole^{-1} H_2O, whereas that of *H. halobium* was 46.2 kJ mole^{-1} H_2O, and that of human red blood cell 40.6 kJ mole^{-1} H_2O. The molar heat of dehydration of *H. marismortui* has been shown to be composed of three fractions; the second one has a molar heat of 58 kJ mole^{-1} H_2O and the third one 108 kJ mole^{-1} H_2O.

INTRODUCTION

The importance of the interaction of water molecules inside any biological system cannot be exaggerated, yet their description and understanding are still far from completion. In his recent book, Pethig [1] states: "If there are uncertainties regarding the molecular characteristics of the bulk of liquid water, then the situation for the aqueous state in biological systems poses even greater problems." An important still unanswered question in this field is how far the effect of macromolecular surfaces, such as those of proteins, extends into the bulk of the water; in other words, the range of change in water structure.

It is agreed by many recent reviewers in the field (e.g. ref. 2) that, on the whole, most of the water in biological systems, or in hydrated biopolymers, is quite mobile and behaves like normal liquid except for a small fraction, 0.2—0.5 mole of H_2O per 100 g of biopolymers, which is bound specifically to certain groups of the polymers. There is another quantity of about 1—3 mole H_2O/100 g of biopolymer which is influenced, but with the properties of liquid water. Thus as a generalization it can be stated that only a small proportion of cellular water, much less than 10%, seems to be affected by the macromolecules inside the cell. Yet we have come across a system which we think to be an exception to the rule. This unique system is a halophilic bacteria isolated from the Dead Sea [2—6].

Halophilic bacteria from the Dead Sea, *Halobacterium marismortui* (*H.*

* On leave from the Institute of Life Science, The Hebrew University of Jerusalem, Israel.

maris. hereafter), have several unusual properties. For instance, there is very high selective discrimination between K^+ and Na^+. The selectivity $[(K^+_{in}/Na^+_{in})/(K^+_{out}/Na^+_{out})]$ can exceed 20 000. This high selectivity is maintained even with no measurable output of metabolic energy under conditions when there is a relatively fast communication of ions between the outside and inside phases. A model to explain this situation [5] suggests that the cell water of *H. maris.* could be regarded as a two-phase system. One phase consists of a modified form of non-specific bound water ("structured water" — sw) while the other phase has properties close to that of bulk water (w). It was suggested that the Na ions do not enter the sw phase, due to unfavourable ΔG of hydration, whereas K^+ ions are accumulated in the sw phase as ion-pairs either with chloride or with carboxylate of the amino-acid residues in a region of low dielectric constant.

A possible prediction of such a model is that there would be amplification in the molecular interactions of some of the water molecules, either by increased protein—water, ion—water and/or water—water interactions. This might be reflected in an increase in the heat of dehydration of such a system. A study of dehydration has recently been made on various powders of proteins and polypeptides to obtain the heat of dehydration [7—12]. It seems that such thermodynamical methods might be complementary to the many sophisticated dynamical methods (NMR, dielectric relaxation, IR, etc.) applied to study the state of water in biological systems.

In this work we measured the heat of dehydration of pellets of various cells. The excess of molar heat of dehydration over that of the media has been found to increase in the order erythrocytes—*Halobacterium halobium*—*H. maris.*; this suggests that the interactions within the water framework are stronger in halophilic bacteria than in erythrocytes.

MATERIALS AND METHODS

H. maris. was isolated from the Dead Sea in 1965 and has been maintained ever since on agar slants in our laboratory in Jerusalem. Methods of culture of *H. marismortui* have already been described [3].

H. halobium was supplied by Dr. Allard of the Biochemistry Department of Cambridge University, and was grown in his laboratory.

Fresh human blood cells (RBC) were washed twice with isotonic saline solution, centrifuged down and used 1—3 h later.

The Perkin-Elmer differential calorimeter, model DSC-2, was used to determine the heat of dehydration of the cells' pellets.

All cells were centrifuged down in 1.5 ml polyethylene tubes in an Eppendorf microfuge for 3—4 min, the supernatant sucked off and the tip cut with a razor blade. Two to six mg of pellet were put into aluminium pans (Perkin-Elmer 219-0062) which were completely sealed except for a pinhole of 0.3 mm diameter drilled in the cover of the pan. This pinhole retarded the loss of water and vapour and prevented any appreciable build-up of pressure inside the closed pan; this conclusion can be drawn from the observation that the boiling point of pure water in the pan did not exceed 373 K by

more than 0.5—1°C. The pan was weighed first empty, then after sealing, and finally after heating in the calorimeter. The difference between the two latter weights was taken as the weight of water evaporated or desorbed.

The pans were introduced into the calorimeter, which was then closed. The machine was continuously flushed with N_2 gas. Heating was started a few minutes later. In the scanning mode a recording was made of the heat needed to maintain a constant rate of increase in temperature. The calorimeter response of DSC-2 can be assumed to be constant over the entire temperature range [13]. After completion of the heating-run, the sample was cooled and reheated over the same temperature range, care being taken not to change the position of the pan inside the calorimeter. The purpose of this second run, done with dry material only, was to obtain a baseline without recourse to any arbitrary calculations; the problem of defining the baseline is not trivial for quantitative measurements of enthalpy [13,14]. In these measurements we worked at constant pressure; thus heat and enthalpy are equal. The heat or enthalpy of water desorption (or dehydration) was defined as the area between the curves obtained from the first and second runs. The area is almost closed (e.g. Figs. 1—3; [14]).

For the isotherm mode [15] at zero time the initial heating rate is set very fast (80°C min^{-1}) and thus an apparent given temperature of the measured pan is achieved in a relatively short time, and is maintained until no water is left in the pan. This can be seen from the trace falling to a constant level.

The area of the power—time curve (thermogram) was measured with an Albitt planimeter. Each area was measured two to three times, so as to ensure reproducibility of 1%. Most areas were between 25 and 50 cm^2. The accuracy of the planimeter measurements was checked by measuring known areas (squares) of 25 and 50 cm^2 at least 10 times. The S.D. was less than 1% of the average.

The output of DSC-2 was calibrated in two ways. (1) The endothermic peak of melting of a pure sample of indium, supplied by Perkin-Elmer, was measured in a sealed aluminium pan. This was done with two different amounts of indium (7.35 mg and 15.8 mg) at several sensitivities and rates of heating. (2) Known amounts of water (9.9 mg and 19.9 mg) were sealed into aluminium pans and heated over a range of temperatures. As the C_p of water is constant between 273 and 350 K, the enthalpy of heating water between 333 and 343 K could be calculated.

Samples of indium: 0.189 ± 0.0012 cal cm^{-2} paper
0.185 ± 0.0011 cal cm^{-2} paper
Samples of water: 0.1916 ± 0.0014 cal cm^{-2} paper
0.1895 ± 0.001 cal cm^{-2} paper

The two methods of calibration agree to within 2%.

RESULTS

Figures 1—5 depict thermograms of the dehydration of pellets of the halophilic bacteria, *H. maris.* (obtained by DSC-2). The pellets consist of bacter-

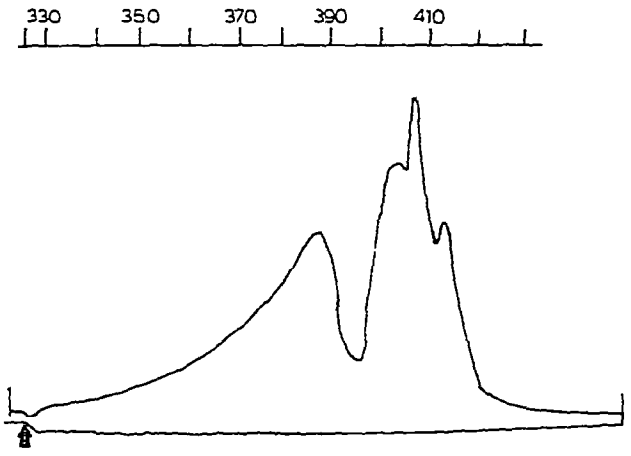

Fig. 1. Thermogram of the dehydration of *H marismortui* pellet. Scanning mode. 10°C min^{-1}; 10 mcal sec^{-1}. 2.58 mg H_2O; 1.76 mg dry weight. The baseline represents the heating of dried material.

ial cells and solution trapped between the cells. The solution composition is 3.5 M NaCl; 150 mM $MgSO_4$ and 10% yeast autolysate [3]. The volume of trapped solution occupies about 25—30% of the volume of the pellet. The composition of the bacteria (late logarithmic phase [3]) is given by weight relative to water: water 1.0; K^+ 0.213; Na^+ 0.06; Cl^- 0.174; protein 0.37; others (nucleic acids, lipid, carbohydrate etc.) 0.11.

From the areas of the thermograms of 18 cultures of *H. maris.* the molar heat (at constant pressure), or molar enthalpy ($\Delta\bar{H}$) which is involved in the dehydration process was calculated and is shown in Table 1. The baseline is obtained after heating the dried material [14,16]. It is assumed that the main thermal event is that of removing the water from the system and con-

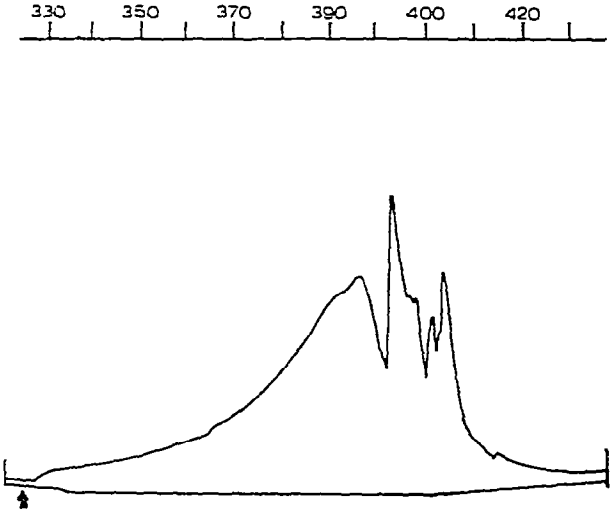

Fig. 2. Thermogram of the dehydration of *H. marismortui* pellet. Scanning mode. 10°C min^{-1}. 10 mcal sec^{-1}. 2.66 mg H_2O; 2.27 mg dry weight.

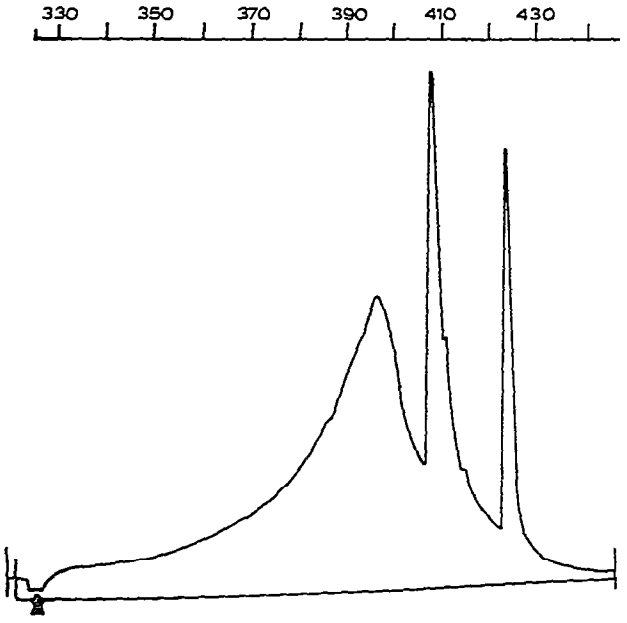

Fig. 3. Thermogram of the dehydration of *H. marismortui* pellet. Scanning mode. 10°C min^{-1}; 10 mcal sec^{-1}. 3.50 mg H_2O; 1.74 mg dry weight.

verting it into vapour. As can be seen from Table 1, the average molar heat of dehydration of *H. maris.* is 50.4 kJ $mole^{-1}$ H_2O. This is 10 kJ $mole^{-1}$ H_2O more than the heat of evaporation of pure water [16] measured under equivalent conditions, and about 6 kJ $mole^{-1}$ H_2O more than $\Delta H_{dehyd.}$ of concentrated solutions (3 M) of NaCl or KCl [16]. In comparison, we see in Table 2 that the molar heat of dehydration of pellets of red blood cells

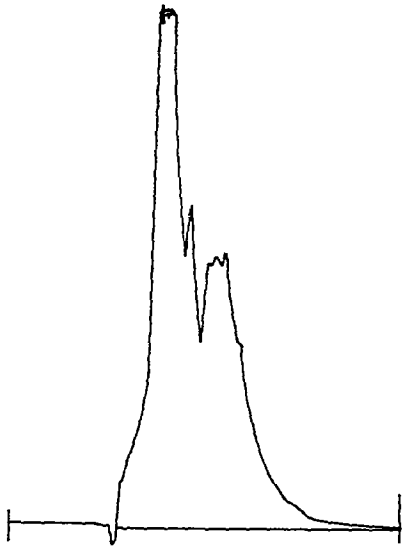

Fig. 4. Thermogram of the dehydration of *H. marismortui* pellet. Isothermal mode. 80°C min^{-1}; 403 K. 3.44 mg H_2O; 2.37 mg dry weight.

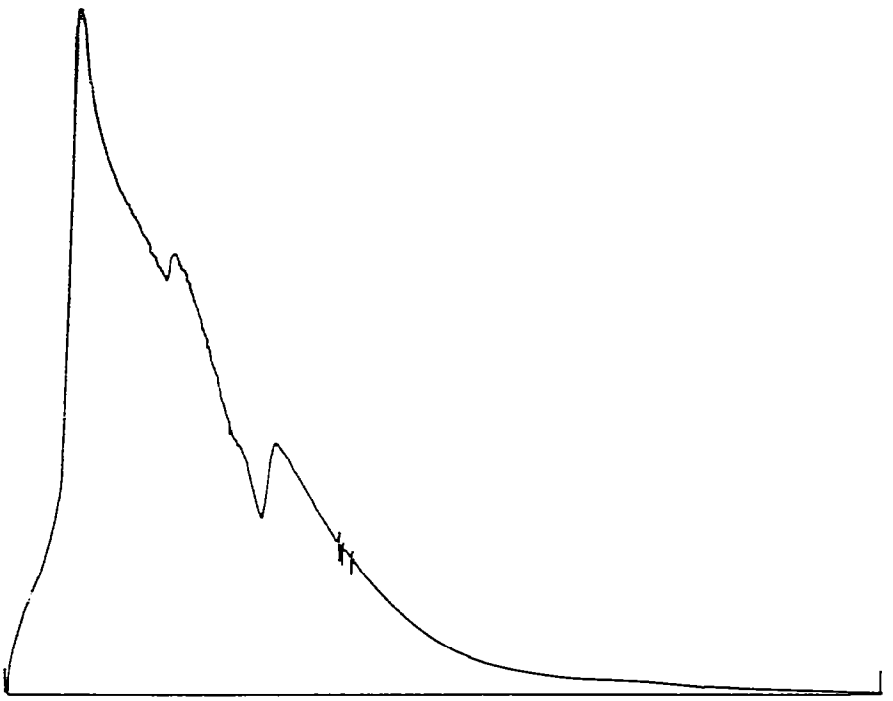

Fig. 5. Thermogram of the dehydration of *H. marismortui* pellet. Isothermal mode. 80°C min^{-1}; 395 K. 3.82 mg H_2O; 2.64 mg dry weight.

TABLE 1

The molar heat of dehydration (Joules) of *Halobacterium marismortui* per mole of water

Sample	$\Delta\overline{H}$ (kJ $mole^{-1}$ H_2O)	Water/dry wt.
691	52.19	1.31
696	47.00	1.21
591	50.01	1.35
592	49.03	1.64
369	45.87	1.48
397	51.51	1.31
491	49.48	1.47
494	48.50	1.32
391	52.34	1.44
392	48.12	1.47
393	49.40	1.47
394	45.71	1.45
381	61.61	1.23
382	49.78	1.38
383	48.80	1.43
384	53.62	1.54
182	45.78	1.4
183	58.85	1.23
	50.42 ± 0.85 (S.E.)	1.39

TABLE 2

The molar heat of dehydration ($\Delta\bar{H}$) from pellets of human red blood cells, *Halobacterium halobium* and *Halobacterium marismortui*

	Molar heat of dehydration (kJ mole^{-1} H_2O)
Human red blood cells	40.6 ± 1.43 [4] [a]
Halobacterium halobium	46.2 ± 0.96 [7]
Halobacterium marismortui	50.4 ± 0.85 [18]

[a] The number of replicates is given in brackets.

(RBC), which was obtained in the same way as that for the bacteria, is almost the same as of pure water. RBC can be considered to be a relatively concentrated protein solution, rich in haemoglobin, but of course does not have the concentration of ions comparable to that of *H. maris.* On the other hand, the molar heat of dehydration of another halophilic bacteria, *H. halobium*, is much nearer to that of a concentrated solution of NaCl or KCl. Yet, there is still an excess of heat of molar heat (1.7 kJ). It should be noticed that the molar heat of dehydration of bacteria is an average over all the water in the pellet. As at least 25—30% of the water is outside the bacteria, then the molar heat of dehydration of the cellular water of the bacteria is even higher than indicated in Tables 1 and 2. It should also be mentioned that there was no important difference in the results mentioned above, whether the DSC was employed in the scanning or isothermal mode.

A closer inspection of Figs. 1—5 gives more information on the thermal events of the dehydration of the bacteria. Figures 1—3 describe the scanning mode of the DSC, where the power output (at constant pressure) $\mathrm{d}H/\mathrm{d}t$, is plotted as a function of constant increase of temperature while water is escaping from the pellet. In another paper [16] we have shown that when the heating of pure water or NaCl solution is followed in the scanning mode, the trace on the recorder describes a monotonic increase of $\mathrm{d}H/\mathrm{d}t$ with increase of temperature. In contrast, with the bacteria the trace of the recorder shows three to four peaks. The first peak is at about 390 K, which corresponds to that of the 3 M NaCl solution [16]. The second peak is above 400 K and the third is at above 420 K. A similar trace with three peaks was observed in the dehydration of 3 M KCl solution [16], though there the peaks were closer to each other. In Figs. 4 and 5 we see the dehydration of the same bacterial systems, but in the isothermal mode. We observe here a kind of three-step function. These three steady-state levels can be likened to three levels of escaping tendencies of water, which are consistent with the apparent three to four populations seen in the scanning mode. These same phenomena were also observed in the case of the dehydration of KCl solution [16].

There is a small difference of 2—3% between the total $\Delta H_{\mathrm{dehyd.}}$ obtained by the isotherm or scanning mode. On the other hand, there are differences in the distribution of the heats of dehydration between the three fractions as obtained by the two modes of operation of the DSC (seen in Table 3).

The first fraction of $\Delta H_{\mathrm{dehyd.}}$ is less than 50% in the isothermal mode and

TABLE 3

The molar heat of dehydration (Joules) of *Halobacterium marismortui* per mole of water in different fractions (see text)

	Fraction			
	I	II		III
% Heat				
Isothermal mode	46.6	27.4		25.8
Scanning mode	67.3	15.4		17.0
$\Delta H_{dehyd.}$ from I [a]	44.51		63.03	
$\Delta H_{dehyd.}$ from II [a]	44.51	58.22		108.1

[a] See text.

67% in the scanning mode. The other two fractions are almost equal in the two modes.

We could not measure directly the molar heat of dehydration for each fraction, as there is no way of measuring $\Delta\bar{H}$ and weight change simultaneously. As we have argued elsewhere [16], there are two independent ways of calculating these parameters.

(a) We assume the first fraction to have a molar heat of dehydration ($\Delta\bar{H}_{dehyd.}$) of free solution of 3.5 M NaCl; then, according to Ginzburg [16] $\Delta\bar{H}_{dehyd.}$ is 44.5 kJ mole^{-1} H_2O. From the percentage of ΔH in this fraction we can calculate the amount of water in the first fraction. Then, by subtraction, we can determine the amounts of water in fractions II and III, and hence calculate the molar heat of dehydration, which was found to be 63 kJ mole^{-1} H_2O, or 18 kJ mole^{-1} excess molar heat of dehydration above that of the free solution of 3 M NaCl.

(b) It can be argued that the levels of the different fractions of the isothermal modes are reciprocally proportional to the molar heat of dehydration ($\Delta\bar{H}_{dehyd.}$) of the given fractions. We assume that fraction I consists of a free solution of 3.5 M NaCl [5,16], the molar heat of dehydration of which is known. It follows (Table 3) that fractions II and III have molar heats of dehydration of 58.3 kJ mole^{-1} H_2O and 108 kJ mole^{-1} H_2O, respectively.

DISCUSSION

It emerges from the data summarized in Table 2 that the overall average molar heats of dehydration of the pellets of the halophilic bacteria *H. maris.* and *H. halobium* are larger than that of the solution of 3.5 M NaCl they are immersed in. The excess of the molar heat of dehydration Δ ($\Delta H_{dehyd.}$) relative to that of 3.5 M NaCl solution is 6 kJ mole^{-1} H_2O and 1.7 kJ mole^{-1} H_2O for *H. maris.* and *H. halobium*, respectively. In contrast, $\Delta(\Delta H_{dehyd.})$ for human red blood cell pellets, relative to pure water, is nil (or a bit negative). The excess of molar heat of dehydration is distributed over all the water of the pellet, which is not uniform. About 30—50% of the water must be free water, including the water outside the cells, without any mole-

cular interaction with the cellular material, and thus have a molar heat of dehydration without excess. It is significant that we did not find any excess of molar heat of dehydration in the RBC pellet, even though they have a ratio of water: dry weight of 2 : 1. This is consistent with the finding of Pauly et al. [17]. We can state then that our main conclusion from this study is that there is an excess of molar heat of dehydration in the pellets of two halophilic bacteria, being larger in the pellet of *H. maris.* isolated from the Dead Sea. This difference between the two species of bacteria is consistent with the behaviour of K^+ ion in these two species [6]. Most of the K^+ is retained in *H. maris.* for a relatively longer time in the absence of output of metabolic energy, whereas only a small fraction of the K^+ in *H. halobium* is retained under similar conditions.

The excess of the heat of dehydration indicates the existence of an amplification of molecular interactions of the water in these cell systems, either directly with cell material like "hydration" of protein, ion etc., or increase of interaction between water molecules (water-structure-making) in the presence of the cellular components.

The next part of the discussion is more speculative, and depends on the interpretation of Figs. 1—5, where the dehydration thermograms indicate that there are at least three apparent populations of water, in both modes of operation of the DSC. In Table 3 two ways of calculating the molar heat of dehydration are presented.

I would like to combine these data with the model previously suggested by us explaining the selectivity properties of the system between Na^+ and K^+ [5,6]. The composition of the pellet of *H. maris.* is 1.4 g water and 1.0 g dry matter [0.4 g protein, 0.48 g salts (0.150 g NaCl; 0.330 g KCl)]. It is assumed that there are three kinds of water: (I) 0.7 g of water, half of it outside the cells and half inside. It consists mainly of a solution of 3.5 M NaCl and some other small solutes. It is not in contact with proteins or other macromolecules. The molar heat of dehydration is 44.5 kJ $mole^{-1}$ H_2O. (II) and (III) 0.7 g of water in which are dissolved all the proteins and the KCl. The water structure is modified. On average, it has (10 mole water)/(100 g protein + 1 mole KCl). The average excess heat of dehydration is 18 kJ $mole^{-1}$ H_2O. When calculated as two fractions the excess heats of dehydration are 13 and 63 kJ $mole^{-1}$ H_2O for fractions II and III, respectively. It would be reasonable to assume that fraction III would be the primary hydration layer, or the first monolayer of sorption onto the proteins. In other proteins Berendsen (1975) estimated it to be 0.2—0.5 mole/100 g protein, which fits estimates of Bull [18], Pauling [20] and most recent data [21,22]. There are fewer data on the excess heat of sorption of water to protein, especially on the primary hydration. Kuntz and Kauzman [23] calculated it to be up to 40 kJ $mole^{-1}$. Recently we obtained values above 45 kJ $mole^{-1}$ H_2O for BSA. These primary hydration or monolayers will take care of about 1 mole of water, and the main interaction must be a direct water—protein one. The remainder of the 9 moles of H_2O have to interact with the KCl and the protein. In concentrated KCl [16] we found that about 0.7 mole H_2O has a very high molar heat of adsorption [$\Delta(\Delta H) \sim 50$ kJ $mole^{-1}$ H_2O)] and about 5 mole H_2O have about the average excess of 13 kJ $mole^{-1}$. It turns out then

that 9 moles H_2O, which interact with KCl and not directly with protein (being two to five layers behind the first sheet), have an excess of 13—14 kJ $mole^{-1}$, which is one to two hydrogen bonds per mole of water. This is fraction II, which could be the non-specific hydration designated by Berendsen [19], but is modified here by the presence of massive amounts of K^+ and the carboxylates on the side chain of the halophilic proteins. Thus it can contain 9—10 moles H_2O/100 g biopolymers rather than 3 moles in the case of the more ordinary protein. It may be that the "increase" of structure in water is due to this ternary system of water, KCl and protein.

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REFERENCES

1 R. Pethig, Dielectric and Electronic Properties of Biological Materials, Wiley, New York, 1979, p. 106.
2 H.T. Edzes and H.J.C. Berendsen, Annu. Rev. Biophys. Bioeng., 4 (1975) 265.
3 M. Ginzburg, L. Sachs and B.Z. Ginzburg, J. Gen. Physiol., 55 (1970) 187.
4 M. Ginzburg, L. Sachs and B.Z. Ginsburg, J. Membr. Biol., 5 (1971) 78.
5 M. Ginzburg and B.Z. Ginzburg, Biomembranes, 7 (1975) 219.
6 M. Ginzburg, in S.R. Caplan and M. Ginzburg (Eds.), Energetics and Structure of Halophilic Microorganisms, Elsevier, North-Holland, Amsterdam, 1978, p. 561.
7 H. Morita, Biopolymers, 4 (1966) 215.
8 D. Puett, Biopolymers, 5 (1967) 327.
9 J.I. Bulgin and L.J. Vinson, Biochim. Biophys. Acta, 136 (1967) 551.
10 H.W. Hoyer and K.S. Birdi, Biopolymers, 6 (1968) 1507.
11 E. Berlin, P.G. Kliman and M.J. Pallansch, J. Cell Interface Sci., 38 (1970) 488.
12 B.Z. Ginzburg, in preparation.
13 J.L. McNaughton and C.T. Mortimer, in H.A. Skinner (Ed.), Differential Scanning Calorimetry, Int. Rev. Sci., Phys. Chem., Ser. 2, Vol. 10: Thermochemistry and Thermodynamics, Butterworths, London, 1975, Chap. 1.
14 M.J. Richardson, in I.W. Dawkins (Ed.), Polymer Characterization, Applied Science Publishers, Barking, 1979, Chap. 7.
15 H. Staub and M. Schnyder, Thermochim. Acta, 10 (1974) 237.
16 B.Z. Ginzburg, Thermochim. Acta, 46 (1981) 239.
17 R. Müller, G. Hasl and H. Pauly, Thermochim. Acta, 22 (1978) 339.
18 H.B. Bull, J. Am. Chem. Soc., 66 (1944) 1499.
19 H.J.C. Berendsen, in F. Franks (Ed.), Water, Vol. 5, Plenum Press, New York, London, 1975, Chap. 6.
20 L. Pauling, J. Am. Chem. Soc., 67 (1945) 555.
21 P.R.C. Gascoyne and R. Pethig, J. Chem. Soc., Faraday Trans. I, 73 (1977) 171.
22 S. Bose, P.R.C. Gascoyne and R. Pethig, J. Chem. Soc., Faraday Trans. I, 73 (1977) 1605.
23 I.D. Kuntz and W. Kauzman, Adv. Protein Chem., 28 (1974) 239.