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Specific Heat Measurements on Lysozyme, Chymotrypsinogen, and Ovalbumin in Aqueous Solution and in Solid State

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Specific heats on solid lysozyme, chymotrypsinogen, and ovalbumin with different amount of water have been measured as a function of the water content. The $\bar{c}p°$ values obtained from extrapolation to zero water content are 1.192 ± 0.005 (lysozyme), 1.223 ± 0.004 (chymotrypsinogen) and 1.231 ± 0.005 (ovalbumin) in J K^{-1} g^{-1}. The specific heat for the water associated with the proteins is 30 % larger than the value for pure water.

Specific heat measurements were also carried out on aqueous protein solutions at different concentrations in order to obtain partial specific heat values at infinite dilution. The values found are 1.494 ± 0.007, 1.529 ± 0.015, and 1.534 ± 0.014 in J K^{-1} g^{-1} for lysozyme, chymotrypsinogen, and ovalbumin, respectively.

Specific heat values were calculated for solid lysozyme, chymotrypsinogen and insulin from the sum of the heat capacity values of the amino acids, corrected for the heat capacity contributions for the formation of the peptide bonds. The calculated values are within a few percent of the experimentally obtained values.

A similar analysis of the results from specific heat measurements on aqueous protein solutions indicates that a substantial part of the non-polar amino acid side groups are solvated by water.

The biochemical properties of protein are to a large extent governed by the mutual interaction between protein and water. One of the most important factors which stabilizes native proteins seems to be the interactions between water and the non-polar groups of the protein. Kauzmann [1] proposed that the stabilization effect is caused by the entropy gain when non-polar groups are taken out of contact with water to the inside of the protein. It was assumed that the hydrophobic side chains are buried in the interior of the protein. Klotz,[2] on the other hand, pointed out that the favourable enthalpy contribution from the solvation of non-polar groups could be an important stabilization factor. X-Ray investigations on several proteins have shown that nearly all ionic side-chains are exposed to solvent, and a substantial number of hydrophobic groups are accessible to solvent.[3]

There is an increasing interest for a quantitative characterization of these interactions and thermodynamic methods seem to be among those which are best suited for the purpose. From experiments with low molecular weight compounds it has been shown that hydrophobic groups in contact with water are associated with very large apparent heat capacity values. It is believed that the "excess" heat capacity is due to enthalpy of melting of water structures formed under the influence of the hydrophobic groups. Recent model compound experiments [4,5] indicate that the heat capacity values can be accounted for by simple additivity rules.

The analysis of the heat capacity of a protein in terms of the contributions from amino acid side groups, the effect of solvation and the polypeptide back bone can be shown to give information about the distribution of the non-polar groups between the interior and surface of a protein. Unfortunately, there are only a few globular proteins for which absolute specific heats are measured in aqueous solution or in the solid state.[6-9] Furthermore, most of the available heat capacity values are not precise enough to attempt to predict structural features.

The long range goal of this work is to make

correlations between heat capacities and structural features for proteins and protein constituents. Here we present a first exploratory study on the specific heats for a few globular proteins. The measurements have been made by a novel double drop heat capacity calorimeter.[10]

Specific heat measurements were made on ovalbumin, chymotrypsinogen, and lysozyme in dilute aqueous solutions at different concentrations. From these measurements partial specific heats at infinite dilution, $\bar{c}p^{\circ}_2$, were derived for the proteins. Measurements were also made on the proteins in their solid states at different water contents.

The observed cp° values of the solid proteins are compared with calculated cp values and the $\bar{c}p^{\circ}_2$ values obtained in aqueous solution are used to estimate the per cent of solvated hydrophobic residues which are solvated on proteins in aqueous solution.

EXPERIMENTAL PROCEDURE AND CALCULATIONS

Materials. Lysozyme (LYSF OCC, salt free) was obtained from Worthington Biochemical Corp. Ovalbumin was obtained from Miles-Servac (PTY) Ltd. Chymotrypsinogen (3× crystallized, salt free) was obtained from Nutritional Biochemical Corp. All proteins were used without further purification. Glass distilled water was used with dissolved gases removed by boiling.

Calorimetry. A newly developed double drop calorimetric method was used.[10] The calorimeter consists of two main parts: (1) a "furnace" for temperature equilibration of the sample ampoule and reference ampoule and (2) a receiver twin calorimeter of the heat conduction type. The calorimetric unit was kept at about 29.7°C and the furnace at 20.3°C. The ampoules were equilibrated in the furnace for about 30 min, after which they are dropped into the receiver calorimetric unit and the difference between the heat quantities transferred by the two ampoules measured. From the difference obtained with the sample ampoule filled and empty, and the measured temperature difference ($\pm 2 \times 10^{-4}$ °C) between the furnace and calorimeter, the mean specific heat (cp) for the temperature interval 20.3 – 29.7°C can be calculated.

Calibration of the calorimeter was performed with water. Results from test experiments indicate systematic errors to be less than 0.1 %.[10]

Heat capacity studies on proteins performed with scanning calorimeters [9,11] show that the partial specific heat values vary almost linearly with the temperature in the interval here concerned. Therefore the specific heat values given in the present study may be assumed to refer to the mean temperature 25°C. Variations in the mean temperature were small, ±0.01°C, and any corrections to 25°C were insignificant. The only correction applied on the experimental specific heat values was the effect caused by the displaced air,[12] which was of the same order of magnitude as the precision of the measurements.

Uncertainties given for the cp values are twice the standard deviation of the mean $s = 2\langle\delta^2\rangle^{\frac{1}{2}}/\{n(n-1)\}^{\frac{1}{2}}$. The partial cp values were calculated by using the linear regression analytical method.[13]

Procedure. All aqueous protein solutions were prepared from dry proteins, which had been stored in vacuum ($< 10^{-3}$ mmHg) over P_2O_5, at room temperature, for at least 4 days. The concentrations were determined on the weight basis. During the preparation of the solutions care was taken to avoid unwanted hydration of the dry proteins. Even short exposure of the proteins to the air gives errors in the partial specific heat of the proteins of the order of a few percent. All manipulations with the dry proteins were therefore performed under dry nitrogen atmosphere.

About 0.6 g of the protein solution was filled into the ampoule immediately after preparation. The sample ampoule and the reference ampoule, which in the present study was always empty, were placed in the calorimeter. The order of selection of the concentrations studied was random. 4 – 8 consecutive determinations were performed at each concentration.

Various desired water contents for the hydration of the solid proteins were obtained by placing the dry proteins in evacuated vacuum desiccators over aqueous sulfuric acid of the appropriate concentration. The proteins were allowed to equilibrate for at least 24 h. The hydrated samples (0.2 – 0.05 g) were then filled into an ampoule, which was sealed by a plunger system. The plunger could be pressed against the proteins to achieve a minimum of vapor space without compression of air. After 4 – 8 consecutive cp measurements on each sample, the water contents were determined by weight difference before and after drying in an oven at atmospheric pressure and 105°C for 24 h.

Calculations. Specific heats were measured for the aqueous protein solutions at several concentrations and for the solid proteins at several water contents. From the measured cp values as a function of the concentration the contributions to the total cp from water and protein were calculated. The relationship between the partial specific heats is given by eqn. 1 (*cf.* Ref. 7)

$$(1 + W_2)cp = \bar{c}p_1 + W_2\bar{c}p_2 \qquad (1)$$

where W_2 is the quotient between the masses of the solute and the solvent ($W_2 = m_2/m_1$). Index 1 stands for the solvent and index 2 for the solute. In the solid samples the solvent is the protein and the solute is the water. cp is the measured specific heat, and $\bar{c}p_1$ and $\bar{c}p_2$ are the partial specific heats for the solvent and solute, respectively. Eqn. 1 shows that a plot of $(1+W_2)$cp against W_2 gives the $\bar{c}p_1$ value as the ordinate intercept and the $\bar{c}p_2$ value as the slope.

RESULTS

Measurements on lysozyme in aqueous solution were made at 14 different concentrations from 1.11 to 27.12 weight % lysozyme. The results are summarized in Table 1. The pH in this concentration range varied between 4.56 and 4.58. In Fig. 1a the results are plotted as described above. The experimental points were best fitted using two straight lines with a break between 9.5 and 10.1 weight %. According to eqn. 1 the partial specific heats for lysozyme and water are constant on both sides of the 10 % region. At that concentration there is a 2.1 % increase of the $\bar{c}p_2$ value and an increase of 0.05 % for the $\bar{c}p_1$ values.

The same effect occurs for aqueous ovalbumin solutions, which were measured in the range 0.58 – 12.44 weight % ovalbumin (Fig. 1b). The partial cp values are constant up to 4.3 – 4.8 %, after which an increase of 1.4 % and 0.04 % occurs for ovalbumin and water, respectively.

Table 1. Specific heat for aqueous lysozyme solutions at different concentrations and calculated apparent specific heat [a] for lysozyme.

Weight-% lysozyme	cp/J K^{-1} g^{-1}	ϕ_{cp_2}/J K^{-1} g^{-1}
1.11	4.1497 ± 0.0005	1.470 ± 0.045
2.45	4.1135 ± 0.0004	1.473 ± 0.016
3.63	4.0818 ± 0.0005	1.476 ± 0.013
4.79	4.0499 ± 0.0004	1.466 ± 0.009
6.11	4.0154 ± 0.0004	1.489 ± 0.006
6.60	4.0014 ± 0.0005	1.476 ± 0.007
9.47	3.9253 ± 0.0004	1.492 ± 0.004
10.11	3.9128 ± 0.0004	1.538 ± 0.004
13.08	3.8322 ± 0.0004	1.528 ± 0.003
17.18	3.7263 ± 0.0004	1.540 ± 0.002
21.90	3.6001 ± 0.0004	1.533 ± 0.002
27.12	3.4606 ± 0.0003	1.528 ± 0.001

[a] $\phi_{cp_2} = \dfrac{Cp - m_1\bar{c}p^\circ_1}{m_2}$; where Cp is the total heat capacity and $\bar{c}p^\circ_1$ is the specific heat for pure liquid water.

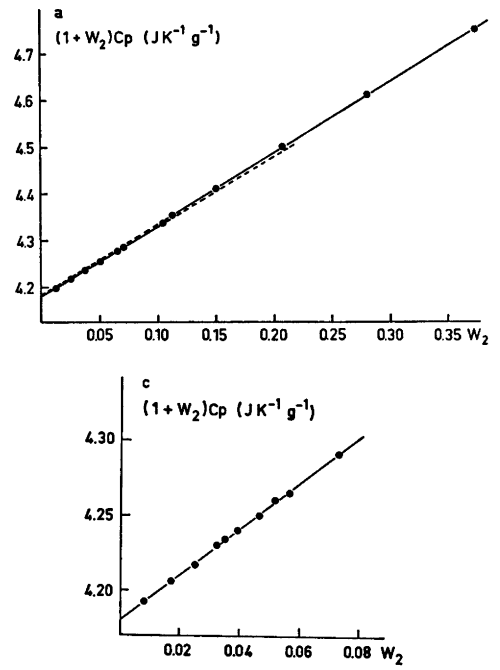

Fig. 1. Result of specific heat measurements on aqueous protein solutions as a function of the concentration: (a) lysozyme, (b) ovalbumin, (c) chymotrypsinogen.

In the measured concentration range there was a decrease of the pH from 6.3 to 6.1.

Fig. 1c shows the results from cp determinations on aqueous chymotrypsinogen in the interval 0.78 – 6.79 weight %, with a pH change from 4.06 to 4.10. In this region the $\bar{c}p$ values were constant, within the experimental errors.

Results from aqueous protein measurements extrapolated to zero concentration and the values found in the literature are summarized in Table 2.

Measurements on solid protein samples were carried out at six different water contents on each protein. Fig. 2 a, b, and c show the results for lysozyme, ovalbumin, and chymotrypsinogen, respectively. It is seen that the three curves show the same characteristic pattern: the experimental results from samples with less than 5 % and more than 15 % water fall on the same straight line, but the values between these limits fall below the extrapolated lines. The deviation from linearity is more pronounced for ovalbumin (*ca.* 3 %), than for lysozyme and chymotrypsinogen (*ca.* 2.0 – 1.5 %). Partial specific heats for the three proteins are the same on both sides of this interval.

The partial specific heat values extrapolated to zero water content from the solid protein experiments together with values found in the literature are summarized in Table 3.

Table 2. Partial specific heats of infinite aqueous dilution of lysozyme, chymotrypsinogen and ovalbumin at 25°C.

Protein	This study Water $\bar{c}p°_1$/J K^{-1} g^{-1}	Protein $\bar{c}p°_2$/J K^{-1} g^{-1}	Lit. Protein $\bar{c}p°_2$/J K^{-1} g^{-1}
Lysozyme	4.1792 ± 0.0004	1.494 ± 0.007	1.30 ± 0.04[a]
Chymotrypsinogen	4.1808 ± 0.0006	1.529 ± 0.015	1.60 ± 0.05[b]
Ovalbumin	4.1778 ± 0.0004	1.534 ± 0.014	1.91 ± 0.05[c]
			1.66 ± 0.09[d]

[a] Ref. 11. [b] Ref. 9. [c] Ref. 6. [d] Ref. 7. (mean temp. 11°C).

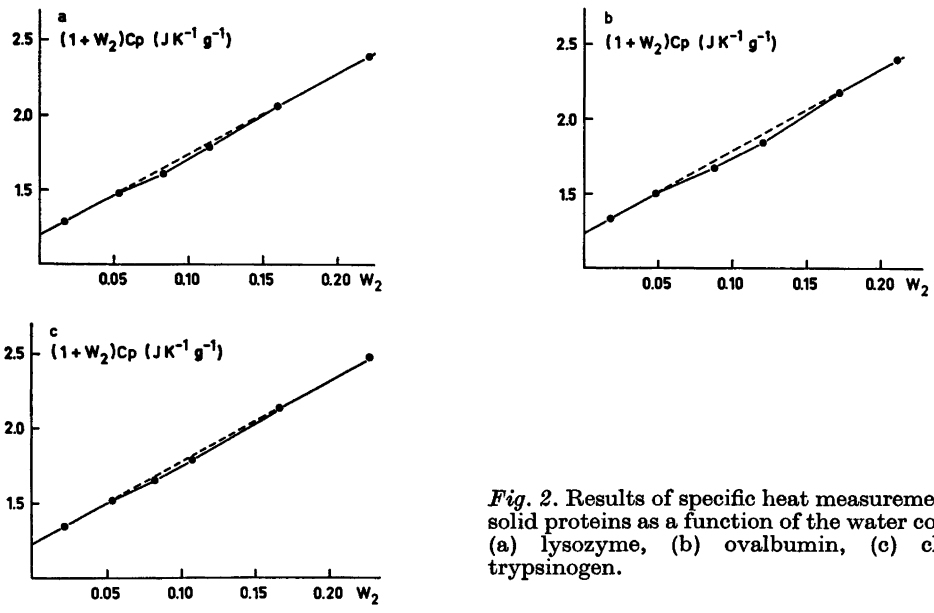

Fig. 2. Results of specific heat measurements on solid proteins as a function of the water content: (a) lysozyme, (b) ovalbumin, (c) chymotrypsinogen.

Table 3. Partial specific heats at zero water contents of solid lysozyme, chymotrypsinogen and ovalbumin at 25°C.

Protein	This study Protein $\bar{c}p°_1$/J K^{-1} g^{-1}	Water $\bar{c}p°_2$/J K^{-1} g^{-1}	Lit. Protein $\bar{c}p°_1$/J K^{-1} g^{-1}
Lysozyme	1.192 ± 0.005	5.42 ± 0.03	
Chymotrypsinogen	1.223 ± 0.004	5.46 ± 0.03	1.293 ± 0.003[a]
Ovalbumin	1.231 ± 0.005	5.53 ± 0.04	1.97 ± 0.08[b]
			1.18 ± 0.25[c]

[a] Ref. 8. [b] Ref. 6. [c] Ref. 7.

DISCUSSION

Hutchens *et al.*[8] suggested that it is possible to calculate the standard entropies for proteins from their amino acid composition and gave entropy calculations on solid proteins. The specific heats for the solid proteins (Table 3) and for the crystalline amino acids [14] on the average differ only slightly from each other, which seems to support the idea that heat capacities can also be calculated from the amino acid composition. With use of eqn. 2

$$n \text{ amino acids (c)} \rightarrow \text{protein (s)} + (n-i)H_2O \text{ (l)} \quad (2)$$

where n is the total number of amino acids and i is the number of polypeptide chains in the protein, it is possible to calculate the contributions from the peptide bonds to the total heat capacity of the protein. If ΔCp stands for the average molar heat capacity contribution from one peptide bond, then the ΔCp can be written by use of eqn. 2 as

$$\Delta C\text{p} = C\text{p}°(\text{protein}) + (n-i)C\text{p}°(H_2O) - \sum_i^n C\text{p}_i(\text{amino acids})/(n-i) \quad (3)$$

ΔCp values for three dipeptides, lysozyme, and chymotrypsinogen were calculated by use of eqn. 3. The heat capacity values for the amino acids and the dipeptides were taken from Ref. 14, where the data for L-Lys and L-His were estimated from the values given for L-Lys.HCl and L-His.HCl by subtracting 34.14 J K^{-1} mol^{-1}, which is the difference between D-Glu.HCl and L-Glu. The amino acid compositions for lysozyme and chymotrypsinogen given in Ref. 15 were used. The ΔCp values calculated in this study and the corresponding entropy data by Hutchens [8] are summarized in Table 4. In both cases a relative constant value for ΔCp or ΔS can be ascribed to the formation of the peptide bond. This points out that it may be possible to calculate the heat capacity for a solid protein from its amino acid composition with an accuracy of a few percent. Rearranging eqn. 3, using the ΔCp = 40.6 J K^{-1} mol^{-1} calculated from the two proteins and the Cp° = 75.3 J K^{-1} mol^{-1} for water leads to eqn. 4

Table 4. Calculated heat capacity and entropy changes [a] for forming peptide bond in the solid state at 25°C.

	ΔCp[b]	ΔS[b]
Lysozyme	40	
Chymotrypsinogen	43	38
Insulin		39
Gly-Gly	41	43
Ala-Gly	36	51
Leu-Gly	32	36

[a] Values taken from Hutchens *et al.*[8] [b] In J/K × (peptide bond).

$$c\text{p}°(\text{protein}) = \sum C\text{p}(\text{amino acid}) - (n-i)34.7 \text{ (J K}^{-1}\text{ g}^{-1})/\text{M} \quad (4)$$

The eqn. 4 was used to calculate the specific heat for insulin from its amino acid composition. The value obtained was 1.213 J K^{-1} g^{-1} compared with the measured value of 1.255.[8] This agreement is not surprising because if one calculates specific heat using eqn. 4 for all the various polypeptides made up of single amino acid units, the mean value falls within 1.2 ± 0.2 J K^{-1} g^{-1}. From this it can be assumed that the specific heat for most solid proteins is close t 1.2 J K^{-1} g^{-1}.

The specific heat for water bound on the solid proteins (Table 3) is about 30 % higher than for pure liquid water. This large value is surprising, because the specific heat of pure water is by itself "abnormally" high, caused by the gradual rupture of the hydrogen bonds with increasing temperature. The qualitative explanation could be that the water bound to the protein makes double hydrogen bonds at specific hydrophilic sites on the protein, such as was found in the collagen-water system by NMR-studies,[16] and that the rupture of the hydrogen bonds between water and protein falls off more rapidly than in pure liquid water with increasing temperature.

The specific heat decrease in the middle adsorption range (Figs. 2a, b, c) is probably due to specific heat change of the bound water, since from the argument above the specific conformation in the solid protein does not seem to affect the heat capacity values significantly. According to the adsorption mechanism proposed by Berlin *et al.*[17] the water is bound in double layers in the middle range due to the saturation of the specific water sites. This could lead to the decrease of the specific heat of bound water to a value closer to that of pure liquid water. The adsorption of more water was proposed to induce a conformational change which leads to an increase of available water sites, and the specific heat of water therefore increases to the same value as that found at low water content.

Partial specific heat of the protein in aqueous solution. In Fig. 1a, b, and c it can be seen that the partial specific heats are constant up to a rather high concentration. No obvious explanation can be offered for the abrupt changes in the c_p values for lysozyme and ovalbumin in the middle range. For the following discussion the $\bar{c}p^\circ$ values are obtained from the data below the breaking points (Table 3).

The derived $\bar{c}p_2^\circ$ values could be affected due to association reactions, which have been found to occur at higher pH from several sedimentation and chromatographic investigations on lysozyme [18-24] and chymotrypsinogen.[25-27] The effect on C_p caused by the association can be separated in two components: (1) direct changes in the specific heat caused by aggregation, and (2) change in the equilibrium of the association reaction induced by the temperature change. The effect 1 can be assumed to decrease the total specific heat due to the hydrophobic character found for the association reaction, whereas effect 2 always causes a positive c_p effect since an increase of temperature must cause a shift in equilibrium in the endothermic direction, requiring a positive absorption of heat. Since the observed $\bar{c}p_2$ is independent of concentration it is reasonable to assume that effects 1 and 2 are too small to be significant, and the observed $\bar{c}p_2^\circ$ values are equal to the true inifinitely dilute value.

The most striking result from the c_p measurements is the large Δc_p for the transferring of a solid protein to aqueous solution. The Δc_p°(solv) values for the proteins are 0.302 ± 0.008, 0.306 ± 0.015, and 0.303 ± 0.015 J K^{-1} g^{-1} for lysozyme, chymotrypsinogen, and ovalbumin, respectively, or an increase of 25 % in c_p for the process of solvating a protein. It is seen that nearly identical Δc_p°(solv) values were obtained for the three proteins.

Calculation of partial specific heats of proteins. In this section an attempt is made to analyse the obtained $\bar{C}p^\circ_2$ values by use of model compound data and simple additivity rules. In simple terms the different contributions to the $\bar{C}p^\circ_2$ values can be separated into several groups and are tabulated in Table 5.

A. Contributions from the back bone. From the observed independency of the heat capacities of the solid proteins on degree of hydration, it is assumed that the C_p contributions from the back bone do not change with the solvation of the protein. The C_p° for the back bone is estimated by using eqn. 4 on n glycine residues. The ΔC_p for solvating the terminal amino and carboxylic acid is taken to be zero.[4]

B. Contributions from solvated polar groups. All polar groups are assumed to be solvated.[3] The $\bar{C}p^\circ_2$ values for alcohol, amino, carboxylic, and amide groups given in Ref. 4 are used. The $\bar{C}p^\circ_2$ value for guanidine and imidazole groups are estimated by simple additivity rules from the values of the functional groups in Ref. 4. The phenolic group on tyrosine is estimated from values given in Ref. 28.

C. Contributions from non-polar groups. This group includes all the non-polar amino acid side chains, indole, mono- and disulfide groups, and

the non-polar parts of the polar side chains. The $Cp°_2$ values from these groups can be written as follows

$$\bar{C}p°_2 = Cp° + \alpha \Delta Cp(solv) \quad (5)$$

where $Cp°$ value is calculated from solid amino acid values [14] by subtracting the value for glycine, $\Delta Cp(solv)$ is the change when the non-polar groups are transferred to aqueous solution and α is the degree of solvation of the non-polar groups ($\alpha = 1$, when complete solvation). The ΔCp values were calculated for the different species through simple additivity rules from values given in Refs. 4, 14, 28. The ΔCp values for mono- and disulfide groups are not possible to estimate, but as the groups are probably not hydrated, the ΔCp is assumed to be zero. The ΔCp for the NH in indole is estimated to be zero.[4]

D. Contributions from ionisation. The $\bar{C}p°_2$ values for the polar groups all refer to the non ionic forms.[4] It is known that ionisation reactions occur with a decrease in the heat capacity.[29] Corrections for the ionisation of groups in the dissolved proteins were estimated from the expected pK values for the different side groups, the pH of the solution, and a mean ΔCp for ionisation reactions given in Ref. 30. It was assumed that lysozyme has 25 ionic groups at pH = 4.6 and chymotrypsinogen 27 at pH = 4.1.

The estimated contributions to the total $\bar{C}p°_2$ are summarized in Table 5.

The $\bar{C}p°_2$ can be written as the sum of all the different contributions

$$\bar{C}p°_2 = Cp(backbone) + \bar{C}p°_2(polar) + Cp° (non\text{-}polar) + \alpha \Delta Cp(solv) + \Delta Cp(ion) \quad (6)$$

The eqn. 6 with $\alpha = 1$, assuming all of the non-polar groups solvated, is used to calculate the theoretical heat capacities for completely solvated lysozyme and chymotrypsinogen (Table 6). From Table 6 it is seen that the calculated values exceed the experimentally obtained values with 70 – 80 %. This result suggests that the actual degree of solvation, α, is

Table 5. Contributions from the different groups to the total heat capacity of a protein in aqueous solution.

A. *Contributions from the back bone* $Cp°$ (back bone)				
$Cp°$(back bone) = n99.20 – (n – i)34.7 (J K^{-1} mol^{-1})				Eqn. 4
B. *Contributions from solvated polar groups* $\bar{C}p°_2$(polar)				
Alcohol	$\bar{C}p°_2$(polar) =	64	(J K^{-1} mol^{-1})	Ref. 4
» (phenol.)		–30		28[a]
Amino		58		4
Carboxylic		87		»
Amido		86		»
Guanidino		140		Estimated
Imidazole		110		»
C. *Contributions from non-polar groups,* $\bar{C}p°_2 = Cp°(non\text{-}polar) + \alpha \Delta Cp(solv)$				
Non cyclic aliphatic (CH_2 increment)	$Cp°(non\text{-}polar)$ =	23.5	(J K^{-1} mol^{-1})	Ref. 14[a]
Cyclic aliphatic (» »)		17.3		»
Aromatic (–CH=, »)		13.5		»
Benzene		80.8	(J K^{-1} mol^{-1})	Ref. 14[a]
Indole		115.9		»
Monosulphide		114.3[b]		»
Disulphide		17.4[b]		»
Non cyclic aliphatic (CH_2 increment)	$\Delta Cp(solv)$ =	65.7	(J K^{-1} mol^{-1})	Ref. 4,14[a]
Cyclic aliphatic (» »)		54.5		Estimated
Aromatic (–CH=, »)		43.2		Ref. 4,14,28[a]
D. *Contribution from ionisation* ΔCp(ion)				
	$\Delta Cp(ion)$ =	–167	(J K^{-1} mol^{-1})	Ref. 30

[a] Calculated from values given in the references. [b] Simple additivity rules do not seem to be applied for the mono and disulphide groups. However the errors for these values are compensated in the final calculation.

Table 6. Total contributions from the different species to the heat capacity (J K^{-1} mol^{-1}) of lysozyme and chymotrypsinogen.

	Lysozyme	Chymotrypsinogen
Observed, $\bar{C}p^\circ_2$	21 385	39 243
Calculated contributions from		
Back bone, Cp°(back bone)	8 354	15 835
Polar groups, Cp°_2(polar)	5 289	8 040
Non-polar groups, Cp°(non-polar)	6 927	12 867
Solvation of non-polar groups, ΔCp(solv)	18 669	35 613
Ionisation, ΔCp(ion)	−4 175	−4 509
Complete solvated, $\bar{C}p^\circ_2$(solv)($\alpha = 1$)	35 064	67 846
The degree of solvation, α.	0.27	0.20

much less than 1. The difference between the calculated ($\alpha = 1$) and the observed heat capacity values can be used to estimate the degree of hydration of the non-polar groups from

$$\alpha = 1 - \frac{\bar{C}p^\circ_2(\text{calc},\alpha=1) - \bar{C}p^\circ_2(\text{obs})}{\Delta Cp(\text{solv})} \qquad (7)$$

Values for α were calculated for lysozyme and chymotrypsinogen and the results are summarized in Table 6. For lysozyme the value for α was found to be 0.27. This value agrees with the proposed solvation picture given by Klotz.[3] The value of $\alpha = 0.26$ for lysozyme obtained from static accessibility calculation from X-ray data made by Lee and Richards [31] seems to be surprising since the solvation of polar groups is calculated to be only 0.49, whereas in above calculation a value of 1 has been assumed. The value of α obtained for chymotrypsinogen in this study is 0.20 which is smaller than found for lysozyme, which seems to be reasonable from the fact that larger molecules can easily bury greater fractions of the non-polar groups.

With the above method it is also possible to estimate the change of solvation upon denaturation. The ΔCp values found for the thermal denaturation of lysozyme [11] and chymotrypsinogen [9] indicate that the solvation increases to the values 0.35 and 0.34 for the respective proteins.

Finally, the values calculated above for the degree of solvation, α, can also be compared with the values derived from the very approximate α_i values given by Tanford.[32] Tanford gives values of the degrees of solvation of 0.3 for native and 0.4 for incomplete (thermal) denaturation of small protein molecules. The above results confirm these estimates.

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