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EFFECT OF SORBED WATER ON THE HEAT CAPACITY OF CRYSTALLINE PROTEINS

E. BERLIN, P. G. KLIMAN, and M. J. PALLANSCH
Dairy Products Laboratory, Washington, D. C. 20250 (U. S. A.)*
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SUMMARY

Heat capacity measurements by differential scanning calorimetry (DSC), performed with anhydrous samples of ovalbumin, yielded a value for C_p of 0.267 $\pm$0.033 cal/g/°C at 12°C; and the extrapolation of data from hydrated samples to zero water content yielded a similar value for C_p of 0.244 $\pm$0.011 cal/g/°C. The heat capacity of anhydrous β-lactoglobulin is 0.273 $\pm$0.027 cal/g/°C, and the value obtained by extrapolation of data for hydrated samples is 0.284 $\pm$0.019 cal/g/°C. A linear relation between specific heat and moisture content was observed with the hydrated samples which contained 0.03–0.21 g sorbed water per gram of protein. No temperature dependence of specific heat was observed in the interval scanned (0–25°C). The computed, apparent, partial specific heats of the proteins are 0.245 $\pm$0.010 cal/g/°C for ovalbumin and 0.283 $\pm$0.02 cal/g/°C for β-lactoglobulin; and the partial specific heat of the sorbate is 1.209 $\pm$0.103 cal/g/°C for water sorbed by ovalbumin, and 0.947 $\pm$0.137 cal/g/°C for water sorbed by β-lactoglobulin.

INTRODUCTION

The first published study on the specific heat of a protein was that of Kresheck and Benjamin[1], who reported the specific heat of egg albumin in dilute solutions to be 0.457 cal/g/°C at 25°C, and 0.47 cal/g/°C for the dry protein at low moisture content. Subsequently, Bull and Breese[2] measured the specific heat of egg albumin–water mixtures over a wider range of composition extending from zero water to zero protein content, and reported $C_p = 0.29$ cal/g/°C for the dry protein, a value substantially lower than that of Kresheck and Benjamin[1]. Most of these data, however, were obtained with dilute solutions and only few values were reported for samples with less than 20% water.

Further reports on protein heat capacities are limited to those of Hutchens, Cole, and Sout[3] on insulin and chymotrypsinogen, Jackson and Brandts[4] on chymotrypsinogen, and that of Schwartz and Murray[5] who presented data on human and bovine fibrinogen, and bovine serum albumin and ribonuclease, determined by DSC.

*Eastern Marketing and Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture.

The present work was undertaken to further study the applicability of DSC for specific heat measurements of solid proteins, particularly when containing known amounts of sorbed water. Measurements were therefore made of the heat capacities of two crystalline proteins, ovalbumin and bovine β-lactoglobulin, containing between 20 and 250 mg of sorbed water per gram of protein.

EXPERIMENTAL

*Materials**

The crystalline proteins examined in this study were purified preparations obtained from chemical supply houses (ovalbumin, crystallized twice, Mann Research Laboratories, lot No. U3465; β-lactoglobulin, crystallized three times, Nutritional Biochemicals Corporation) and were used as received or after adjustment of moisture content to values between 0 and 25% water by equilibration in humidostats at appropriate relative humidities, controlled with saturated salt solutions[6].

Calorimetry

Specific heats were determined using the Perkin–Elmer Model DSC-1B differential scanning calorimeter by a technique based on the methods of Wunderlich[7] and O'Neill[8]. First, empty aluminum sample and references pans were heated in the calorimeter from 0 to 25°C at a programmed rate of 5°C/min, to establish a baseline accounting for the asymmetry of the system as a function of temperature. The power output of the calorimeter was then calibrated by heating a 0.03 g sample of Al_2O_3 (standard sapphire plate supplied by the Perkin–Elmer Co.) in the sample pan from 0 to 25°C at 5°C/min. A protein sample, weighing approximately 10 mg, was then encapsulated in the sample pan and heated in the same manner.

The sample pans used in these determinations were hermetically sealed with the Perkin–Elmer volatile-sample sealer accessory to prevent the sorption or desorption of water vapor until after completion of the specific heat determination. After the scan, the moisture content of each sample was determined by puncturing the sample container and drying to constant weight under vacuum at ambient temperature. The dried sample was then scanned again in the DSC apparatus to directly determine the specific heat of the anhydrous protein.

Experimental specific heats were calculated from the amplitudes of the recorder tracings using sapphire specific heat data of Ginnings and Furukawa[9]. Apparent partial specific heats were determined using the equation of White and Benson[10], as employed by Bull and Breese[2] in the form

$$(1 + W_1)C_p = \bar{C}_{p2} + \bar{C}_{p1} W_1$$

where W_1 is the weight of water sorbed per gram of protein, C_p is the experimental

*Mention of specific commercial products does not imply endorsement.

specific heat for protein plus water, $\bar{C}_{p1}$ is the apparent partial specific heat of the sorbed water, and $\bar{C}_{p2}$ is the apparent partial specific heat of the protein.

RESULTS

Specific heat values for ovalbumin and β-lactoglobulin at 12 °C are presented graphically as a function of water content in Figs. 1 and 2, respectively. Least-squares analysis yielded the linear relation

$$C_p = 0.240 + 0.010 \text{ (g } H_2O\text{/g sample)}$$

for the ovalbumin data and the relation

$$C_p = 0.284 + 0.007 \text{ (g } H_2O\text{/g sample)}$$

for the β-lactoglobulin data. In these equations C_p represents the measured specific heat of the protein-sorbed water samples, and the intercept on the C_p axis at zero water content is the extrapolated value for the specific heat of the anhydrous protein.

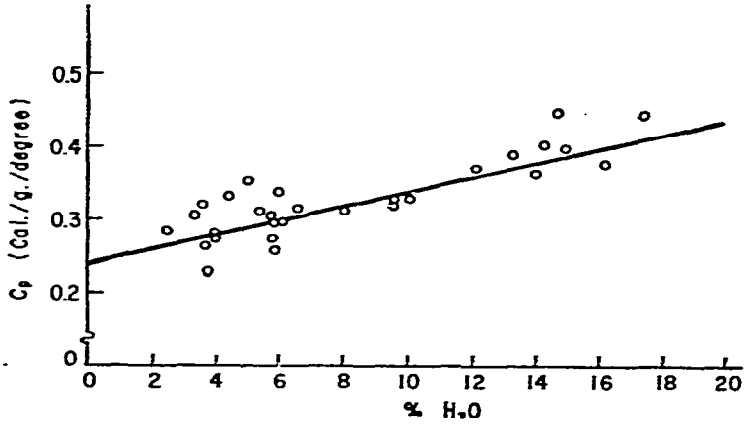

Fig. 1. Graph of C_p, the measured heat capacity of ovalbumin+sorbed water at 12 °C against percent moisture.

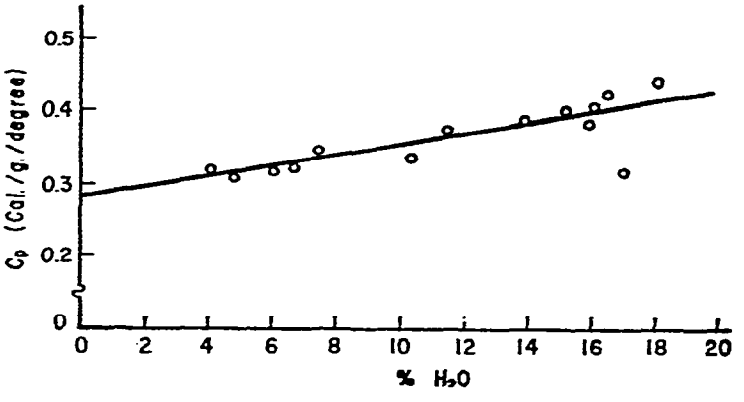

Fig. 2. Graph of C_p, the measured heat capacity of β-lactoglobulin+sorbed water at 12 °C against percent moisture.

All the observed C_p values for each protein have been included in the appropriate graph to indicate the degree of variation in these data; however, simple linear regression analysis[11] yielded the indicated straight lines with residual variances of 0.029 for ovalbumin and 0.020 for β-lactoglobulin. Though these figures indicate some scatter in the data, statistical treatment of these data employing the Fisher *t*-distribution showed that a significant linear relationship exists between the observed C_p and the water content of the samples at the 0.01 level of significance with ovalbumin and at the 0.10 level of significance for β-lactoglobulin.

The extrapolated value of $C_p = 0.244 \pm 0.011$ cal/g/°C for anhydrous ovalbumin is within experimental error of that observed directly with dry samples, $C_p = 0.267 \pm 0.033$ cal/g/°C. Similar agreement is apparent between the extrapolated value of 0.284 ± 0.019 cal/g/°C and the directly determined value of 0.273 ± 0.27 cal/g/°C for anhydrous β-lactoglobulin.

The data presented in Figs. 1 and 2 are only the values computed for 12°C, the mid-point of the scans; however, specific heats were computed at 5° intervals between 7 and 22°C. No significant variation in specific heat with temperature was observable in this range for either protein. The data at temperatures other than 12°C have therefore not been reported here in detail.

The calculated apparent partial specific heat values are: $\bar{C}_{p1} = 1.269 \pm 0.103$ cal/g/°C for the ovalbumin-sorbed water, and $\bar{C}_{p2} = 0.243 \pm 0.010$ cal/g/°C for ovalbumin; and $\bar{C}_{p1} = 0.947 \pm 0.137$ cal/g/°C for the β-lactoglobulin-sorbed water, and $\bar{C}_{p2} = 0.283 \pm 0.02$ for β-lactoglobulin.

DISCUSSION

Ovalbumin specific heat values reported in this paper tend to confirm the results of Bull and Breese[2] who reported C_p for dry egg-albumin to be about 0.29 cal/g/°C, and are at variance with the data of Kresheck and Benjamin[1] who reported a value of 0.47 cal/g/°C. As others[2,4] have pointed out, the value of Kresheck and Benjamin[1] was based on a single determination, while that of Bull and Breese[2] was based on an extrapolation from eleven different values. Our results, which are within experimental error of the data of Bull and Breese, were based on twenty-nine determinations with water-containing samples and twenty-two direct determinations with the dry protein. Bull and Breese reported $\bar{C}_{p2}$ as 0.282 ± 0.61 cal/g/°C and $\bar{C}_{p1}$ as 1.247 ± 0.023 cal/g/°C, which values are within experimental error of our ovalbumin data.

Bull and Breese attributed this high heat capacity value for the sorbed water, when compared with bulk-liquid water, to the high exothermic heat of adsorption of water vapor. Heating a sorbate–sorbent combination at constant pressure usually results in desorption of any physically adsorbed gas or vapor, if the system is initially at equilibrium. Since this desorption enthalpy is always endothermic, it will appear as an increase in the heat capacity. Dash, Peierls, and Stewart[12] have pointed out that it is a common experimental nuisance for heat capacities of physically adsorbed films

to be complicated by unwanted desorption effects, which cause the coverage to be temperature dependent and the total heat capacity to increase above the isosteric value.

Schwartz and Murray[5] used open sample pans in their DSC measurements of the specific heat of several proteins, and they found water desorption to be a significant problem. On repetitive scans with the DSC-1B instrument, they found the heat capacity value constantly decreasing, which they correctly associated with the presence of less water in their samples on each succesive scan.

The data reported in this paper were obtained with ovalbumin samples encapsulated in containers sealed to specifically avoid desorption interferences during the measurements. The samples were encapsulated at ambient temperature (23–27°C), then cooled to 0°C in the DSC-1B and then heated to 25°C. Desorption of water vapor while heating is not likely, since the samples contained less than the equilibrium mass of water which would be sorbed at any temperature and vapor pressure combination during the scan. Furthermore, additional water sorption during the initial cooling of the sealed sample would be negligible, as too little water vapor would be available. The maximum quantity of water which could be present in an otherwise empty 20 μl capsule at 100% relative humidity is only four μmoles, and even less water would be present when a 10–15-mg protein sample is present, and P/P_0 is less than unity. From the present work it may therefore be concluded that measurements of the heat capacities of wet protein crystals made with the differential scanning calorimeter are satisfactory only within the indicated error limits. The errors indicated are the standard deviations in each case. Using the DSC apparatus for protein specific heats does, however, necessitate determinations with a large number of samples.

The observed isosteric sorbate heat capacity values, $\bar{C}_{p1}$, for the water are compatible with the notion that such water exists in some associated form involving multiple hydrogen bonding[13,14]. The excess heat capacity of liquid water over that of ice or water vapor is sometimes called "structural" heat capacity, and is attributed to the thermal breakdown of the associated structure present in the liquid[15]. Water molecules bound to isolated specific sites on a protein surface should not exhibit such a structural heat capacity contribution, hence the elevation of $\bar{C}_{p1}$ for the sorbed water over the specific heat of ice or water vapor may be taken as evidence for the association of sorbed water into a structured hydration shell. The data indicate a possible higher degree of association in ovalbumin-sorbed water than in β-lactoglobulin-sorbed water. The excess heat capacity reported here should be attributed to the thermal breakdown of such complex structures, rather than vaporization of the sorbed water, as these values are reported as free of desorptive heat capacity contributions.

Hutchens, Cole, and Stout[3] reported the partial heat capacity of water bound by insulin and chymotrypsinogen to be temperature dependent. At temperatures above 0°C, the heat capacity of the bound water lies between that of ice and liquid water, when 4% of water is present in bovine zinc insulin, and when 10.7% of water is present in chymotrypsinogen A. At 25°C the heat capacity of water bound to chymotrypsinogen A was the same as that of liquid water. Consideration of these

data and comparison of our results with two different proteins suggest possible differences in the heat capacity value for water bound by different proteins.

REFERENCES

1 G. C. Kresheck and L. Benjamin, *J. Phys. Chem.*, 68 (1964) 2476.
2 H. B. Pal and K. Breese, *Arch. Biochem. Biophys.*, 128 (1968) 497.
3 J. O. Hutchens, A. G. Cole, and J. W. Stout, *J. Biol. Chem.*, 244 (1969) 26.
4 W. M. Jackson and J. F. Brandts, *Biochemistry*, 9 (1970) 2294.
5 A. A. Schwartz and M. Murray, *Biophys. Soc. Abstr., 14th Ann. Meet.*, Baltimore, 1970, p. 234a.
6 L. B. Rockland, *Anal. Chem.*, 32 (1960) 1375.
7 B. Wunderlich, *J. Phys. Chem.*, 69 (1965) 2078.
8 M. J. O'Neill, *Anal. Chem.*, 38 (1966) 1331.
9 D. C. Ginnings and G. T. Furukawa, *J. Amer. Chem. Soc.*, 75 (1953) 522.
10 P. White and G. C. Benson, *J. Phys. Chem.*, 64 (1960) 599.
11 J. E. Freund, P. E. Livermore, and I. Miller, *Manual of Experimental Statistics*, Prentice-Hall, Englewood Cliffs, N. J., 1960, p. 123.
12 J. G. Dash, R. E. Peierls, and G. A. Stewart, *Phys. Rev. A*, 2 (1970) 932.
13 J. Steinhardt and J. A. Reynolds, *Multiple Equilibria in Proteins*, Academic Press, New York, 1969, p. 162.
14 W. S. Brey, Jr., M. A. Heeb, and T. M. Ward, *J. Colloid Interface Sci.*, 30 (1969) 13.
15 H. J. C. Berendsen, in A. Cole (Ed.), *Theoretical and Experimental Biophysics*, Marcel Dekker New York, 1967, p. 26.