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HEAT CAPACITY OF SOLID PROTEINS BY THERMAL ANALYSIS

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Abstract

In a continuing effort to better understand the thermodynamic properties of proteins, solid state heat capacities of poly(amino acid)s of all 21 naturally occurring amino acids, 4 copoly(amino acid)s and about 10 proteins have been analyzed by now using the Advanced Thermal Analysis System, ATHAS. The experimental measurements were performed with adiabatic and differential scanning calorimetry from 10 to about 450 K. The heat capacities of the samples in their pure, solid states are linked to an approximate vibrational spectrum by making use of known group vibrations and a set of parameters, Θ_1 and Θ_3, of the Tarasov function for the skeletal vibrations. Good agreement is found between experiment and calculation with root mean square errors mostly within ±3%. The experimental data were analyzed also with an empirical addition scheme using data for the poly(amino acid)s. Based on this study, vibrational heat capacity can now be predicted for all proteins with an accuracy comparable to common experiments. Furthermore, gradual transitions, indicative of molecular motion prior to devitrification, melting, or decomposition, can be identified. The new experimental data compared here with the prior samples are: bovine β-lactoglobulin, chicken lysozyme and ovalbumin.

Introduction

Proteins are one of the most important classes of biological macromolecules in nature. A comprehensive understanding of proteins requires, both, the knowledge of structure *and* energetics. Despite the tremendous progress made in the structure analysis with, for example over 6000 protein X-ray crystal structures listed in the Protein Data Bank at Brookhaven National Laboratory, little quantitative information is available on the energetics, and no consistent interpretation of the existing data had been developed earlier. Over the years, we have in our laboratory collected a significant amounts of thermodynamic information on synthetic polymers, which forms the basis of the analysis of molecular motion. A natural extension of this work is to remedy the shortage of information in the field of proteins. An Advanced Thermal Analysis System (ATHAS) was developed for this evaluation of the thermal properties and is the basis of a critically evaluated data bank of heat capacities.[1] Detailed thermodynamic information exists now for about 250 linear macromolecules and related small molecules. As building blocks for the study of proteins, the heat capacities of poly(amino acid)s of all 21 naturally occurring amino acids and 4 copoly(amino acid)s have been analyzed with respect

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to their molecular motion in the solid state.[2-4] The agreement of the experimental data with predictions was ±2% or better poly(amino acid) homopolymers and copolymers. Furthermore, heat capacities of some proteins in, both, our own work and the literature have been calculated with ATHAS to within ±3% compared to the measurement.[5-6]

The ATHAS scheme permits to link the macroscopic heat capacities of solids to their microscopic cause, the vibrational motion. As temperature increases, large-amplitude motion is initiated in the form of conformational motion (internal rotation) and, for small molecules, also translation and rotation. This large-amplitude motion begins either at a well-defined phase transition (melting, glass transition, or disordering transition) or gradually over a wide temperature range. Once the vibrational heat capacity is known at low temperature, it can be safely extended to higher temperatures to identify even gradual changes in the heat capacity. Endothermic and exothermic gradual transitions, indicative of molecular motion and ordering prior to or within the glass transition or on decomposition are expected for some of the proteins and were found already for two poly(amino acid)s.[7] Recently, it could also be shown that more complicated molecules, particularly those which display mesophases, may gain large-amplitude motion in the crystalline state at temperatures far below the disordering transitions.[8]

In the literature, there are only few reported measurements of the heat capacity of anhydrous, solid proteins.[9-11] However, in a broad array of papers, the thermal properties of hydrated proteins and protein in solutions are examined. The main effort in these papers is centered around the helix/coil transition and the hot and cold denaturation. It has been shown that the denaturation effect reduces and occur at higher temperatures with decreasing hydration.[12] Many researchers think that the denaturation effect arises from protein-water interactions rather than from changes of the proteins themselves. To resolve such complex questions quantitatively, it is crucial to establish proper reference "base lines" from data of anhydrous proteins. For example, the heat capacity of hydrated collagen has a complicated temperature dependence that is very difficult to interpret without a reference based on the heat capacity of the anhydrous form.[13]

Experiments

The representative proteins were selected for our study because of their well established structures, stability and availability. Samples were purchased from Sigma Chemical Company and used without further purification.

The measurements were carried out on two different instruments. The very low temperature data from 5-330 K were obtained with an adiabatic calorimeter.[5] Experiments from 220-420 K were carried out with differential scanning calorimetry (DSC). A Perkin-Elmer DSC 7 with mechanical refrigeration was used in standard, and dynamic configurations (DDSC). Dry nitrogen purging gas flowed through the DSC cell at 20 mL/min. Sample masses ranged from 5-15 mg.

Matched aluminum sample pans weighing 25.3 ±0.05 mg were selected. The DSC is also equipped with a dry box to avoid signal instability due to drafts or changes in room temperature. Low pressure dry nitrogen gas is kept flowing at constant rate through the dry box to prevent condensation of atmospheric moisture. Successive runs of baselines and sapphire (Al_2O_3), as a standard,[14] were made to calibrate the measurement at every temperature. The heating rate used was 10 K/min. Prior to each measurement the sample is dried by heating to and holding at 390 K (steady baseline/constant weight). The data generated are averages of at least three runs.

Calculations

In the *ATHAS* computation scheme the vibrational spectra of solid polymers are separated into group and skeletal vibrations ($N = 3 \times$ *number of atoms* $= N_g + N_s$). The number and types of group vibrations, N_g, are derived from the chemical structure as a series of single frequencies and box-distributions over narrow frequency ranges taken from normal-mode calculations on isolated chains or suitable low molecular mass analogs. All relevant group-vibration frequencies were collected earlier.[3] The skeletal vibrations, N_s, are not well represented by present day normal-mode calculations, but can be approximated for linear molecules by fitting the experimental, low-temperature heat capacities to a Tarasov function with the frequencies ν_1 and ν_3 (expressed by the corresponding temperatures, Θ_1, and Θ_3, where $h\nu/k = \Theta$ with h and k representing Plank's and Boltzmann's constants). Most sensitive in the 0–50 K temperature region, the parameter Θ_3 governs the contributions of a quadratic frequency distribution, largely representative of the intermolecular vibrations; while in the 100–300 K region, Θ_1 does the same for a constant frequency distribution (box), largely representative of the intramolecular chain vibrations.[15,16] This approximate vibrational spectrum, consisting of group and skeletal vibrations, is inverted to heat capacity at constant volume, C_v. To convert between C_p and C_v, we use the standard thermodynamic relationship or a modified Nernst-Lindemann approach that was proven applicable for polymers.[17]

The low-temperature experimental C_p from 5 to 300 K, converted to C_v, and decreased by the group vibrational contributions was analyzed as the skeletal C_v by fitting it to the Tarasov function. The fitting procedure is newly constructed from a standard routine for energy minimization.[18] Figure 1 is an example. When low temperature C_p data of the sample are unavailable, but Θ can be estimated, this procedure can easily be adapted to fit only Θ_1 while Θ_3 is fixed.

A purely empirical addition scheme was also developed in our laboratory, based on group contributions of the chain elements.[19] This treatment is particularly useful for the description of the heat capacity of solid copolymers above 100 K and for liquid polymers over the whole temperature range. With the thermodynamic data of all poly(amino acid)s readily available,[2,3] proteins, which are, in a way, random copolymers made of the 21 naturally occurring amino acids in the L-configuration, are ideal for such an approach. The amino acid compositions were

obtained from Ref. 20 and the atlas of protein sequence 1967-1968;[21] all data were confirmed with the Swiss-Prot protein sequence database on the Internet.[22]

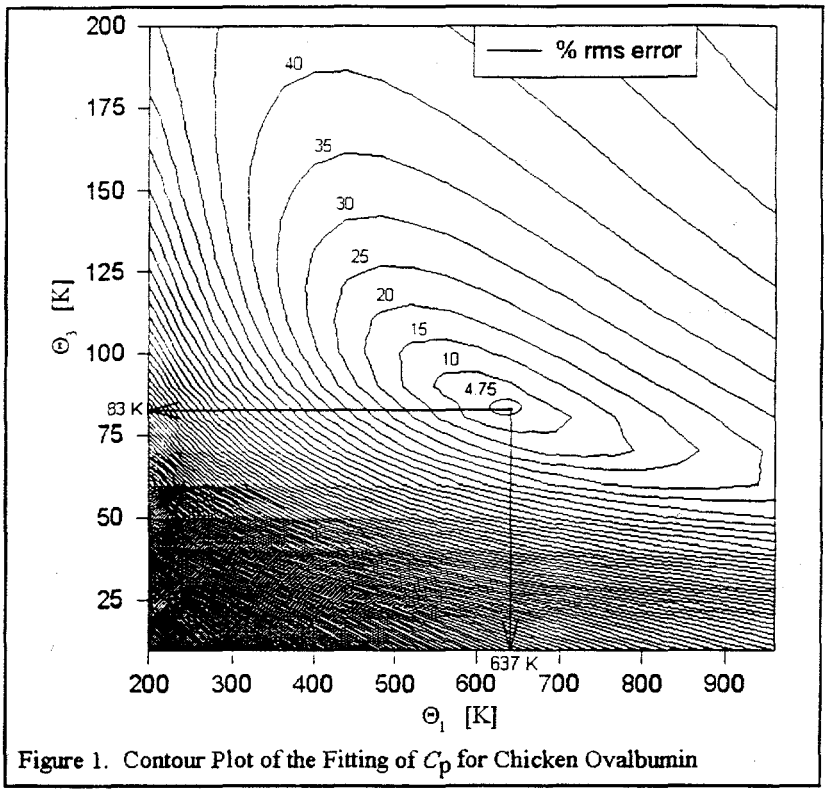

Figure 1. Contour Plot of the Fitting of C_p for Chicken Ovalbumin

Results

The measured experimental results are tabulated at regular temperature intervals along with the rest of the detailed analysis.[23] Representative results from the calculation are shown as in Fig. 2 for chicken ovalbumin together with the experimental data. Two sets of experimental data (points) and calculations (lines) based on the vibrational spectrum and on the empirical addition scheme based on the known heat capacities of poly(amino acid)s, are compared for this classic globular protein. Good agreement is reached in all cases. Key physical parameters are also shown in the bottom right corner of the figure. The average and rms percentage errors of the fit are $-1.06 \pm 4.8\%$. Values of N_s, Θ_1, Θ_3, errors and temperature range for some representative poly(amino acid)s and the newly analyzed proteins in fitting to the Tarasov functions,[5,6] are listed in the table below.

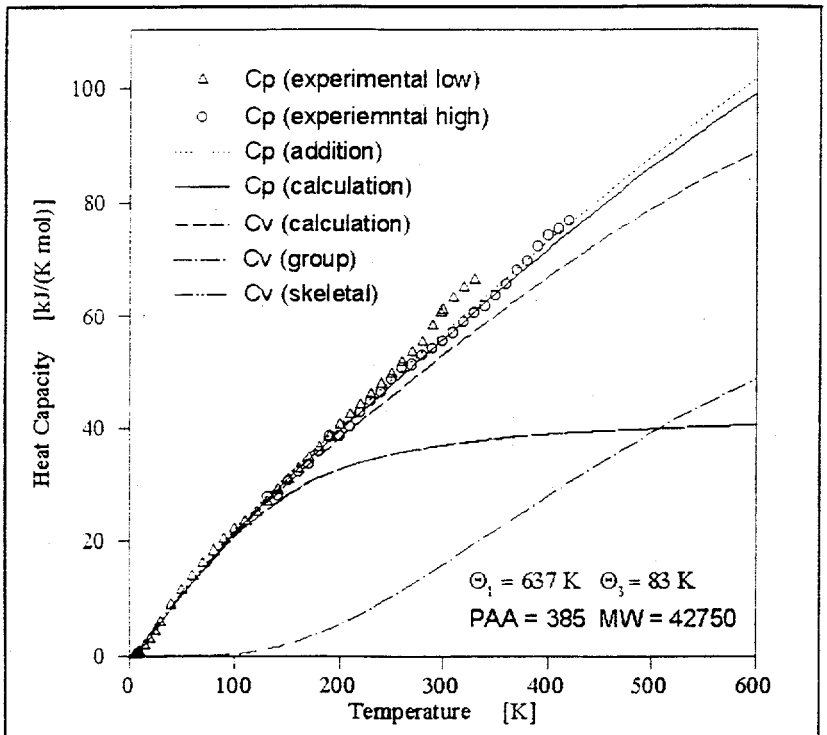

Figure 2. Heat capacities and Contributions for Chicken Ovalbumin

Biopolymer	N_s	Θ_1 (K)	Θ_3 (K)	% av & rms	T (K)
polyalanine	9	634	58	−0.5 ± 1.3	60 - 390
polyglycine	6	750	91	−1.5 ± 5.2	1.4 - 390
polyvaline	14	664	65	0.7 ± 2.6	2 - 390
polymethionine	15	542	83	−0.5 ± 1.6	5 - 200
polyphenylalanine	11	396	67	1.4 ± 3.5	5 - 300
lactoglobulin	2188	586	91	−0.47 ± 4.5	7 - 200
ovalbumin	5008	637	83	−1.06 ± 4.8	5 - 200
lysozyme	1665	618	79	0.42 ± 5.1	7 - 200

A surprising observation is that the two parameters for poly(amino acid)s and proteins cluster about 616 K for Θ_1 and 82 K for Θ_3 as shown in Fig. 3. This may

largely be due to the averaging effect of the multitude of amino acids contained in the proteins. It clearly implies, that rather reliable estimates can be made for the thermal properties of other proteins based on the knowledge of a limited number of samples, improving on the empirical copolymer addition scheme.

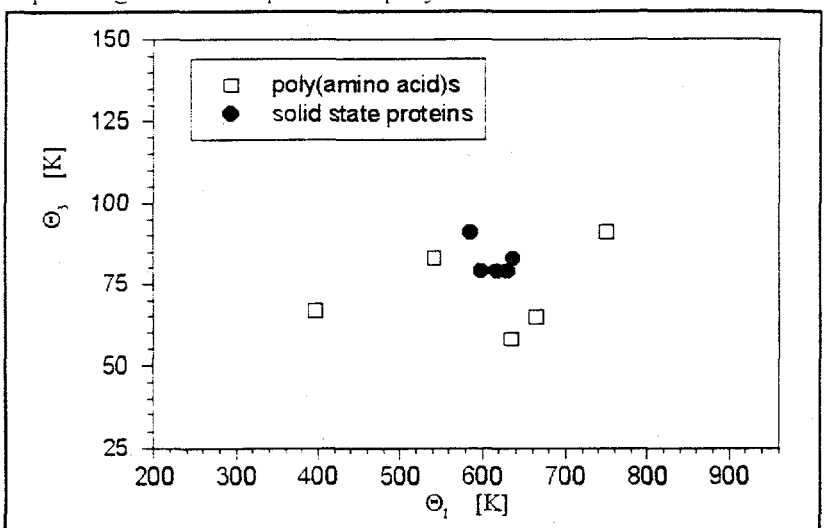

Figure 3. Distribution of Θ-Temperatures for Poly(amino acid)s and Proteins

Discussion

The experimental heat capacities shown in Fig. 2 are compared to the calculated results from 0–600 K. The heat capacity of all samples gradually increases over the temperature interval studied. Of all proteins investigated, only the heat capacity of anhydrous α-chymotrypsinogen A and insulin were the subject of an earlier study.[9] The results by Hutchens *et al.* match well to our calculated and measured data.[5] The discrepancies between various sets of experimental data are about 2–3%. As an approximation, an average over all sets is compared to the calculation and used as recommended experimental data in the ATHAS data bank.

An earlier attempt to calculate the heat capacity of α-chymotrypsinogen A had been made.[24] These data can, however, not be compared to ours since the author has used the Debye model that is inappropriate for linear polymers. Indeed, these calculations deviate largely from the measured heat capacities. The new computations are comparable to other proteins studied by this method more recently.[5] The value of Θ_3 is a measure of the intermolecular vibrational frequencies and seems not to differ much, pointing to similar interactions. Although Θ_1 and Θ_3 should not be effected much by the exact amino acid sequence of a protein, they ought to be related to the same parameters, Θ_1 and Θ_3, of its constituent poly(amino acid)s. To support this argument, the average of Θ_3 is 73 K for the 5 poly(amino acid)s, polyglycine (Θ_3 = 91 K), polyalanine (58 K), polyvaline (65 K), polymethionine (83 K) and polyphenylalanine (67 K), for which low-

temperature C_p values are available.[2,5,25] Although Θ fitted with a constant Θ may differ some with values based on fitting from low temperature C_p, it is a good approximation for the temperature range of most interest, 200-500 K. The largest sensitivity in the Θ-to-C_v inversion is at the point of inflection of the Tarasov function at about $\Theta/4$ to $\Theta/5$.[26] At higher temperatures, the sensitivity of the inversion decreases and approaches zero above the Θ temperature as C_v approaches $N_s \times R$, a constant (Dulong-Petit's rule). With unlimited number of proteins, our goal is to search and predict intrinsic relationships between any protein's structural information and physical properties by selectively studying representative samples. The optimization of Fig. 1 reaches always one unique global minimum, demonstrating the physical relevance of the two parameter description.

With the knowledge of heat capacities from absolute zero of temperature, we can calculate the basic thermodynamic functions like enthalpy H, entropy S, and Gibbs free energy G. These functions can serve as a beginning for the understanding of the energetics of biopolymers like proteins. The data tables and corresponding curves, as well as tables of the computed C_p and the recommended experimental C_p for the proteins can be inspected and reproduced from the ATHAS data bank available through the World Wide Web on the Internet.[1]

This series of studies represents the first systematic analysis of protein heat capacities. Based on the approximate vibrational spectra, we can now calculate heat capacities and the integral functions enthalpy, entropy, and Gibbs function for all proteins of known compositions to an accuracy comparable to common thermal experiments.

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