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A DSC STUDY OF THE CONFORMATIONAL TRANSITION OF POLY-γ-BENZYL-L-GLUTAMATE*

J. SIMON** AND F. E. KARASZ
Polymer Science and Engineering, University of Massachusetts, Amherst, Mass. 01002 (U.S.A.)
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ABSTRACT

The thermally induced random coil-to-helix conformational transition of poly-γ-benzyl-L-glutamate (PBG) in dichloroacetic acid (DCA)–chloroform, DCA–1,2-dichloroethane (DCE), and DCA–1,1,2,2-tetrachloroethane (TCE) mixtures has been studied as a function of solvent composition. The experimental data were obtained using the Perkin–Elmer DSC-2 differential scanning calorimeter with a modified sample container. Results of a systematic investigation of the transition temperature and the heat of transition in the above solvent systems are presented. The data are interpreted in terms of current theory.

INTRODUCTION

Proteins, and their synthetic analogs, homopolypeptides, are polymers of α-amino acids. A common feature, and one that is central to the present discussion, is that many polypeptide chains in solution can undergo a reversible transition from the random coil conformation to the ordered or α-helical conformation[1]. The order–disorder transition of such polypeptides in solution is accompanied by measurable changes in their thermodynamic parameters, e.g. in enthalpy and heat capacity. If these quantities could be measured for a polypeptide in a single solvent, the thermodynamic analysis of such transitions would be relatively uncomplicated and the effect of varying the polypeptide side-chain structure, for example, could be directly ascertained. It is well known, however, that the transition temperature (T_c) for most polypeptides in a single solvent cannot be measured experimentally because of the high temperatures required, leading to problems of polymer solubility, degradation, and solvent vaporization. Consequently, in most cases of polypeptides soluble in organic solvents studied to date, binary systems have been used which in effect reduce T_c to an experimentally accessible range. These systems consist of a

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**Permanent address: Institute for General and Analytical Chemistry, Technical University, Budapest, Hungary.

non-interacting or inert (I) and an interacting or active (A) component, the latter capable of binding to the polypeptide. The measured transition enthalpy (ΔH_{cal}) thus contains contributions from the peptide–peptide and peptide–solvent interactions. The overall transition process can be described in two steps: (1) A quantity ΔH_1 associated with the heat of the hydrogen bond formation (per mole of residue) in the formation of a helical sequence from a coil sequence in the polypeptide. This is an exothermic process; (2) A quantity ΔH_2 associated with the interaction of a residue in the coiled or disordered conformation with the active solvent molecules. This is considered to be an endothermic process.

The overall molar enthalpy change can thus be expressed by the following equation:

$$\Delta H_{cal} = \Delta H_1 + F_c \Delta H_2 \qquad (1)$$

where F_c is the fraction of coil groups bound to A at the transition mid-point. It is well established that the measured enthalpy ΔH_{cal} is a function of solvent composition and hence of transition temperature. It may be noted also that such conformational transitions can be induced by at least two means: (a) isothermally, by varying the solvent composition, and (b) at a fixed solvent composition by varying the temperature.

Direct investigation of the thermally-induced transition was first carried out through heat capacity studies[2] and later isothermally by heat of dilution[3] and heat of solution measurements[4] using single and twin adiabatic calorimeters. In addition, the conformational transitions of polypeptides in mixed organic solvents can be studied at fixed solvent compositions using the differential scanning calorimetric (DSC) technique. The first attempts in the use of the DSC technique for this purpose were made by Steim[5], Karasz and Kagemoto[6], and McKnight and Karasz[7]. Recently the application of the related differential thermal analysis (DTA) technique for the determination of enthalpy changes accompanying the helix–coil transition in polypeptides has been reported[8].

There has been an increasing interest in extending the methodology of thermal analysis to a variety of biochemical systems in solution[9,10]. Experimental difficulties arise when attempting to measure the relatively very small values of ΔH_{cal} at ambient or higher temperatures. In order to obtain reproduceable results for this low energy transition, it is necessary to consider operating conditions that will optimize the signal-to-noise ratio. The signal is increased by employing larger samples, and by higher heating rates. The upper limit of the latter is determined by background noise related to temperature gradients within the sample. Noise levels can, however, be minimized by using reference samples well matched in heat capacity to the sample being measured and other standard measures.

In studies reported here a special sample pan has been constructed to accomodate a larger sample. Figure 1 shows the basic features of this and, depending on the corrosiveness of the solvent mixture being used, either gold (99.99% purity; 0.13 mm thick, United Mineral and Chemical Corp.) or aluminum sheet was employed to

construct the pans described below. The special DSC pan was constructed so as to fit the sample holders of the Perkin–Elmer DSC-2 used for all of the measurements reported here and had approximately three times the capacity of the sealable pans supplied by the manufacturer. The special pans containing the solution sample were hermetically sealed using a modified crimper of Perkin–Elmer design and with a pre-set sealing force delivered by means of a torque wrench. The encapsulation process was, in most cases, successful (as judged by lack of solvent weight loss) when care was taken to avoid wetting the rim of the sample pan.

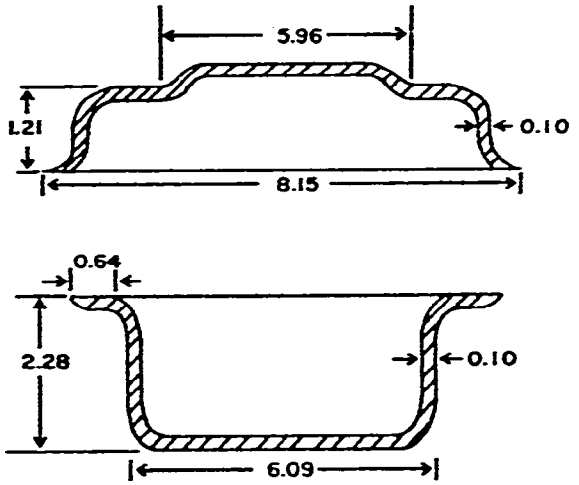

Fig. 1. Schematic drawing of a sealable DSC cell for liquid samples. Dimensions are in mm.

EXPERIMENTAL

An attached drybox accessory and refrigeration system for the Perkin–Elmer DSC-2 allowed measurements to be conveniently made to temperatures as low as 230 K. Samples were weighed into gold pans which were subsequently sealed as described above and weighed to $\pm 10\ \mu g$. Sample sizes were ordinarily of the order of 60–70 mg. All measurements were made at a heating rate of $10\,^{\circ}C\ min^{-1}$ and at instrument sensitivities of 0.5 or 0.2 mcal sec^{-1}. A sealed pan containing an equal quantity of the solvent mixture was placed in the reference sample holder. Prior to each measurement, the samples were heated to around the anticipated transition temperature to monitor any possible leakage from the sealed pans. This is essential to protect the apparatus from the corrosive effect of the active solvent used.

The solvent systems studies were: dichloroacetic acid (DCA)–chloroform; DCA–1,2-dichloroethane (DCE); DCA–1,1,2,2-tetrachloroethane (TCE). All solvents were of analytical grade and were used as received except for the DCA which was distilled at 65 °C and 8 mm pressure. The PBG sample was purchased from Pilot Chemicals, Inc., and had a viscosity average molecular weight of 500,000. All solutions were made up in the range of 2–3% (weight/volume) in PBG.

RESULTS AND DISCUSSION

Representative DSC traces for the transition region in the three solvent systems are shown in Fig. 2. From the curves the transition temperatures and enthalpies were obtained as a function of solvent composition. The conformational transition occurs

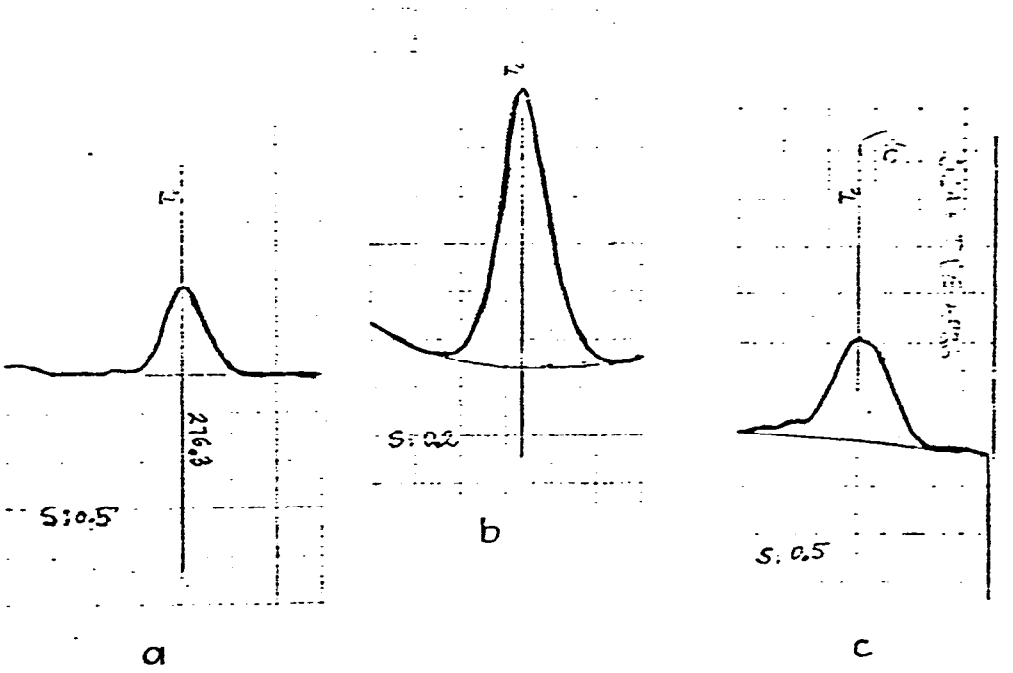

Fig. 2. Representative DSC curves: (a) PBG–DCA–$CHCl_3$ system; (b) PBG–DCA–DCE system; (c) PBG–DCA–TCE.

with a half-width of 7–15°C and with a well-defined peak identified as the transition temperature, T_c. A small shift in the equilibrium baseline after the transition is an indication that the heat capacity of the sample has changed. The correct baseline for the peak area estimates was obtained by extrapolating the heat capacities of the initial and final states to the transition temperature. This procedure introduces an error in the evaluation of ΔH_{cal}, although the reproducibility of each run is excellent. It is estimated that the accuracy of the ΔH_{cal} value is between 5–10%, which falls into the range of errors reported in the literature[11].

The transition temperatures and enthalpies for the three systems are shown in Figs. 3–5 as a function of solvent composition in terms of the mole fraction of active solvent, x_A. There is a sharp increase in T_c with increasing active solvent concentration for all three systems. The T_c versus x_A curves represent phase boundaries between the helical and coiled conformations of the polypeptide in which the upper temperature domain corresponds to the ordered conformation and the lower region to the disordered one. In the ΔH_{cal} versus x_A curves (Fig. 6), increasing concen-

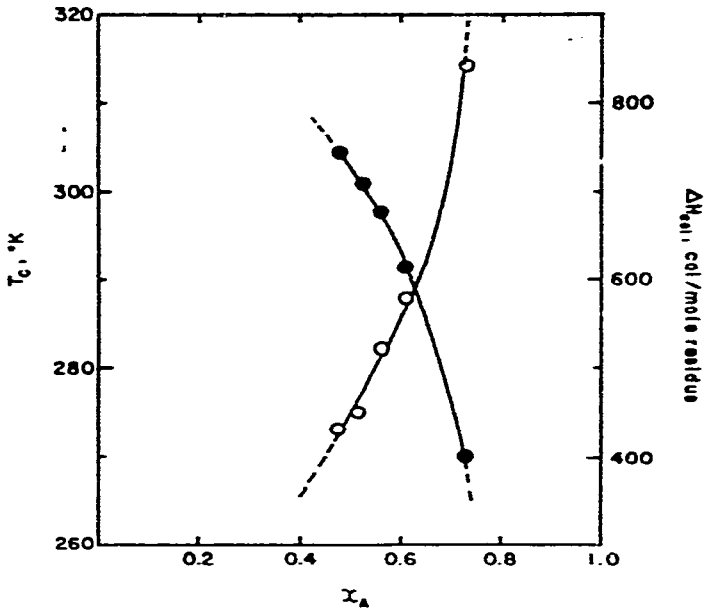

Fig. 3. Transition temperature (○) and transition enthalpy (●) as a function of mole fraction of active solvent, x_A, for DCA–$CHCl_3$–PBG system.

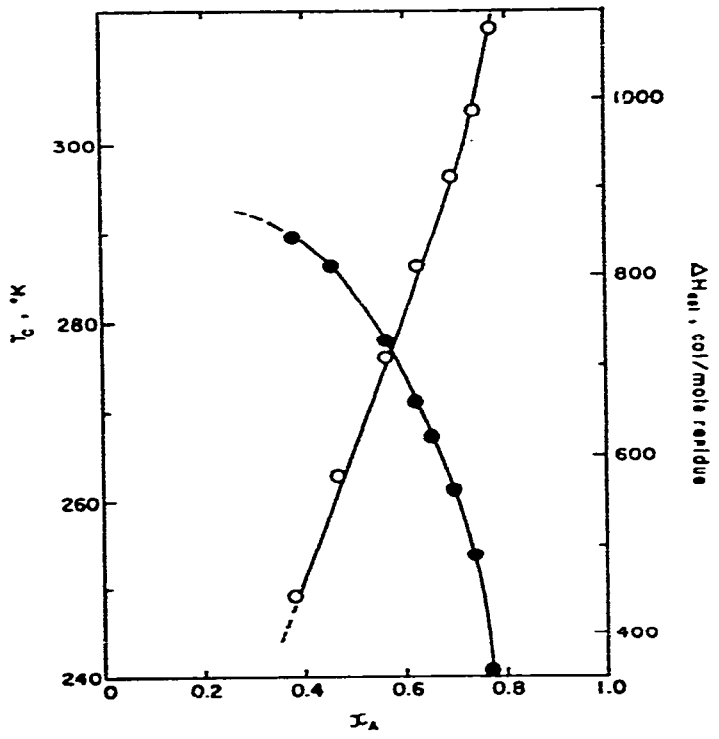

Fig. 4. Transition temperature (○) and transition enthalpy (●) as a function of mole fraction of active solvent, x_A for DCA–DCE–PBG system.

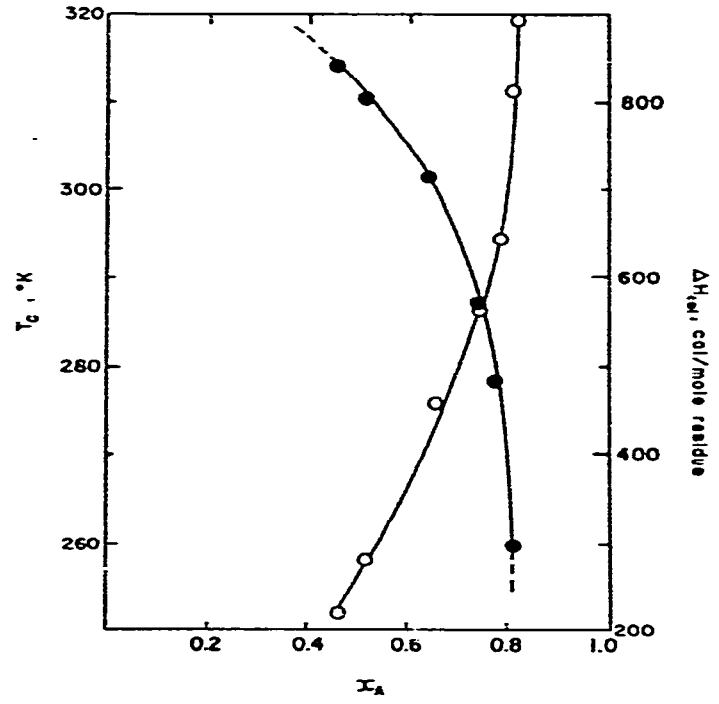

Fig. 5. Transition temperature (○) and transition enthalpy (●) as a function of mole fraction of active solvent x_A for DCA–TCE–PBG system.

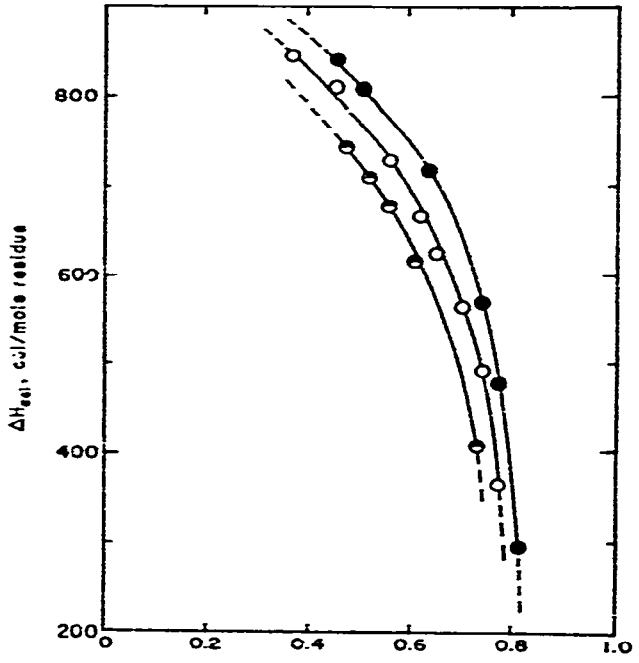

Fig. 6. Comparison of ΔH_{cal} for the systems studied: (a) ◓ PBG–DCA–$CHCl_3$; (b) ○ PBG–DCA–DCE; (c) ● PBG–DCA–TCE.

trations of active solvent result in a decrease in the ΔH_{cal} values[12], as is the case for ΔH_{cal} versus T_c data.

The data obtained by the DSC method support the basic concept of the helix-coil transition analysis. As the binding fraction parameter F_c decreases with increasing temperature, the contribution of the second term in eqn (1) to the overall enthalpy change also decreases. The value of ΔH_{cal} would then be expected to decrease with increasing temperature as has been found to be the case for the above-mentioned systems. In the experimentally accessible temperature range studied (where the interaction of coil groups with the active solvent is the major enthalpic contribution), an endothermic effect was observed with ΔH_{cal}, decreasing with increasing temperature.

The effect of solvent composition on transition temperature and enthalpies has been considered recently[12,13]. As already discussed, the measured calorimetric heat, ΔH_{cal}, is the sum of two contributions in which the fraction of peptide sites bound to active solvent, F, varies with temperature and solvent composition according to:

$$F = \frac{x_A}{x_A + K_2} \tag{2}$$

where K_2 is the temperature dependent equilibrium constant for the binding process. This fraction is usually evaluated (F_c) along the phase boundary representing the midpoint of the transition, i.e. at $T = T_c$, $x_A = x_{A,c}$. In all cases considered here, F_c decreases with increasing temperature; and inasmuch as ΔH_1 and ΔH_2 are of opposite sign and $|\Delta H_2 \Delta > |\Delta H_1|$, ΔH_{cal} can change sign for some $0 < F_c < 1$. This corresponds to the point at which the observed thermal transition changes direction from an "inverse" (coil-to-helix) to a "normal" (helix-to-coil) process.

It is assumed as a first approximation that the activity of the active solvent is unaffected by the presence of the second, inert, solvent species; in other words, for a given active solvent and polypeptide all inert solvent combinations should yield the same results. This, however, is not the case as can be observed in Figs. 3–5 for T_c versus x_A. Similar effects have been observed previously[14], and are probably due to changes in active solvent activity on addition of various so-called inert solvents. The same effect is observed for the ΔH_{cal} data as well, and is discussed in detail elsewhere[15].

CONCLUSIONS

As far as the measuring technique is concerned, there are some advantages of which the following are worth mentioning: (1) Small amounts of polypeptides or proteins only are required; (2) Both the transition temperature and transition enthalpy can be obtained from each run with results comparable in accuracy to those using more traditional techniques; (3) Relatively large temperature ranges can be scanned with modest expenditures of time; (4) The method can be employed over a wide temperature range including the subambient, the latter being a particular unexplored aspect of conformational transitions studies.

ACKNOWLEDGEMENTS

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