guest residue in the copolymer is increased.[50] In a similar manner, PBLG in 82 wt % $HCCl_2COOH$ solution, which is the counterpart of PHPG in water, can be used as the host residue in copolymers containing potential helix makers as guest residues.

Acknowledgment. We thank Dr. Rudolf Andreatta for the synthesis of the PBLG samples used in this study and Mr. Hua Tjan for the micro-Kjeldahl analyses.

Helix–Coil Transition in Mixed Solvents. II. Calorimetric Study of Poly(γ-benzyl L-glutamate) in Dichloroacetic Acid–Dichloroethane Mixtures[1]

V. S. Ananthanarayanan,[2a] G. Davenport, E. R. Stimson,[2b] and H. A. Scheraga*

Department of Chemistry, Cornell University, Ithaca, New York 14850. *Received January 25, 1973*

ABSTRACT: The enthalpy change, $\Delta H°$, for the helix–coil transition of poly(γ-benzyl L-glutamate) in mixtures containing 70:30 and 82:18 weight ratios of dichloroacetic acid and dichloroethane, respectively, has been determined with the aid of a differential thermal analysis apparatus. The description of the apparatus and the details of analysis of the data are given. The values of $\Delta H°$ obtained in the two solvents compare fairly well with those computed from spectropolarimetric measurements in the preceding paper. Also, within the limits of the experimental error, it appears that $\Delta H°$ is independent of the concentration of the polypeptide over the range of concentrations studied.

In the preceding paper,[3] we indicated how we plan to use poly(γ-benzyl L-glutamate) (PBLG) as a host polymer in mixtures of dichloroacetic acid ($HCCl_2COOH$) and dichloroethane (Cl_2Et) for determining the Zimm–Bragg parameters[4] σ and s of guest amino acid residues in nonaqueous solvents. To apply the host–guest technique it is first necessary to determine the thermodynamic parameters for the thermally-induced helix–coil transition in the host homopolymer. This was done[3] for PBLG in $HCCl_2COOH$–Cl_2Et mixtures, by use of optical rotatory dispersion (ORD) measurements, and we report here a calorimetric determination of the enthalpy change ($\Delta H°$) for the same (inverted) transition, and compare it to the value obtained[3] from the temperature dependence of s.

Several calorimetric evaluations of $\Delta H°$ for the PBLG–$HCCl_2COOH$–Cl_2Et system have already been reported.[5-13] The motivation for this additional investigation is (1) to obtain independent data on the same systems used in our ORD study[3] to establish the thermodynamic parameters for the host homopolymer prior to further studies on the host–guest random copolymers, and (2) to demonstrate the applicability of a recently developed differential thermal analysis (DTA) apparatus for obtaining precise values of $\Delta H°$ for thermally induced helix–coil transitions. The DTA apparatus used here is of the conduction type,[14,15] and thus is simple in design and operation, and is low in cost compared to adiabatic-type calorimeters. The heat detectors used in our calorimeter are semiconductor thermoelectric generators and are superior to the thermopiles consisting of many wire-type thermocouples, used in earlier conduction-type calorimeters.[15] By making use of twin cells we have gained the advantages of a differential heat measurement. Another advantage of our calorimeter is that only about 40 mg of polypeptide (in ~1.5 ml), approximately 1/100th of the size sample usually used,[7] is required for good precision. These advantages have been gained without sacrificing precision and accuracy; as will be shown by the results reported here, the precision and accuracy in the measurement of heat change is comparable to those obtainable with an adiabatic-type calorimeter.[16]

The DTA apparatus is described, and then results are presented for the helix–coil transition in PBLG in 70 and 82 wt % $HCCl_2COOH$, respectively, in Cl_2Et.

Experimental Section

Materials. The PBLG samples were fractions I-2 and II-2 of the previous paper;[3] these have average degrees of polymerization ($\overline{DP}$) of 1550 and 1000, respectively. $HCCl_2COOH$ and Cl_2Et were the same solvents as in the earlier study.[3] *n*-Hexadecane was of Spectrophotometric grade from Aldrich Chemical Co.

The solutions of PBLG were prepared as described earlier,[3] and the concentration of polymer was determined by dry weight.

Description of Calorimeter. A schematic drawing of the calorimeter is shown in Figure 1A,B. The sample and reference cells (C_1 and C_2, respectively) were type 37 microcylindrical spectrophotometric glass cells, with long filling tubes, from Precision Cells Inc., New York. The separation of the internal faces of the cells was 5 mm and the internal diameter was 22 mm; the filling tubes were long (7 cm) and narrow (about 2-mm i.d.) to minimize evaporation of cell contents. The masses of the cells were matched to within 0.1 mg by grinding the faces of the cell. The

(1) This investigation was supported by research grants from the National Institute of General Medical Sciences of the National Institutes of Health, U. S. Public Health Service (No. GM-14312), and from the National Science Foundation (No. GB-28469X1).
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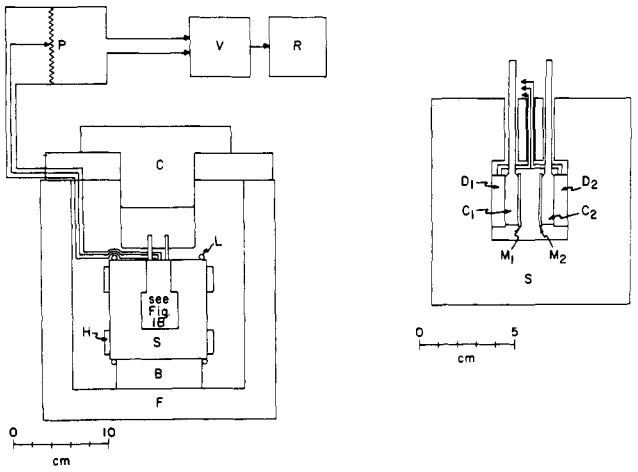

Figure 1. Schematic diagram of the calorimeter. (A, left) The calorimeter assembly and the recorder circuit: S, copper block; H, heaters; L, cooling coils; F, Dewar flask; C, Styrofoam lid; B, cork support; P, potentiometer; V, nanovoltmeter; R, recorder. (B, right) The calorimeter cells and detectors in the cavity of the copper block of (A). C_1, C_2, twin calorimeter cells; D_1, D_2, detectors; M_1, M_2, aluminum plates.

capacity of each cell was 1.54 ml when filled up to an inch below the top of the filling tube.

Two bismuth telluride alloy thermoelectric generators (type TH 0812/P, Asarco Intermetallics Corp., New York) (D_1 and D_2) were used as detectors, their outputs being connected in series, with polarities opposed, to a Keithley Model 148 nanovoltmeter (V) and Daystrom Model 6701 recorder (R). A 1000-ohm 10-turn potentiometer (P) was used to balance the outputs. An aluminum plate (M_1 or M_2) was placed on one face of each cell (covering the face completely), in order to increase the thermal conductivity (by way of spring clips, not shown) to the cells. The plates, cells, and detectors were mounted, with the aid of matched spring clips (not shown in Figure 1) and accurately weighed amounts of silicon grease on all abutting surfaces, in a cavity of a 30-lb copper block (S).

A mercury thermometer (not shown in Figure 1), electric heaters (H) and copper cooling coils (L) were attached to the outside of the copper block, which was then installed in a 4-l. Dewar flask (F) (Lab-Line Instrument Co., Melrose Park, Ill.) with a cork support (B). The Dewar flask was tightly sealed with a Styrofoam cover except for the central access hole to the copper block, which was covered with a tight-fitting, but removable, Styrofoam lid (C).

Procedure. A typical run is made as follows. The sample and reference cells are filled with 1.54 ml of the polypeptide solution and of the corresponding solvent mixture, respectively, by means of a long hypodermic syringe inserted through the filling tubes. The calorimeter assembly is then cooled several degrees below the temperature at which the transition is expected to begin, by passing cooled ethanol (−10°) through the cooling coils. Dry nitrogen (chilled by passing through a copper coil kept in a Dry Ice and ethanol mixture to avoid raising the temperature of the calorimeter assembly) is flushed during cooling through the calorimeter cavity and through the annular space between the copper block and the Dewar to remove all traces of moisture, and, after cooling, through the cooling coils to remove the ethanol. The calorimeter assembly is allowed to equilibrate thermally and then heated by passing current through the heaters. Typical heating rates are 0.7°/min and 0.175°/min.[17] The heating is continued until the temperature (measured with the thermometer) has reached a value of about 10° past the end of the helix-coil transition range (as observed from the tracing on the recorder chart, which is a plot of the difference in the emf output of the two detectors as a function of time). The instrument base line is then obtained in a separate experiment for each run by carrying out the above operations at the same heating rate over the same temperature range, but with both cells contaning the same solvent mixture. The computation of $\Delta H°$ (the heat of transition) is determined from these tracings,

(17) A given average rate of heating was selected on the basis of the considerations mentioned later (see Results). The rate of heating varied, though only slightly (±0.02–0.05°/min), with temperature.

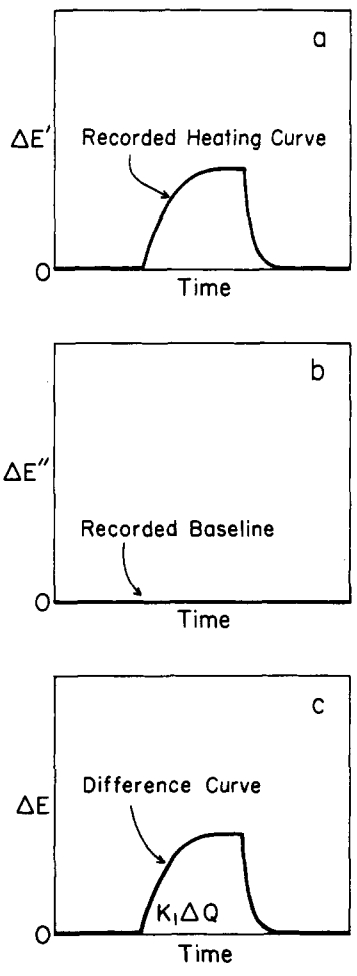

Figure 2. Schematic ΔE *vs.* t curves with water in both cells: (a) recorded curve when the water in the sample cell was heated ($\Delta E'$); (b) base line obtained by thermally equilibrating both cells (each containing water) ($\Delta E''$); (c) the difference between curves (a) and (b); $\Delta E = \Delta E' - \Delta E''$.

with the use of K_1 (a detector constant for D_1), as described in the next section.

The detector constant K_1 (see Appendix A) was obtained as follows. A resistance heater consisting of a short piece of manganin wire (40 gauge) was soldered to two enameled copper leads (no. 28), and the resistance of the heater was measured with a Biddle-Gray resistance bridge. The heater was immersed in water in the sample cell of the calorimeter, and an equal weight of water was placed in the reference cell. After thermal equilibration was attained at the given temperature of the calorimeter, current (supplied from an external 1.5-V battery, with a resistor in series) was passed through the heater for a known interval of time (say, 15 or 30 min). The current was measured with a Rubicon Instrument Model No. 2781 potentiometer and a 1-ohm standard resistor. Simultaneously, the difference in emf output, $\Delta E'$, of the detectors was recorded as a function of time (see Figure 2a). The curve of Figure 2b (base line) was obtained by thermally equilibrating both cells (each containing water), and recording $\Delta E''$. When both cells contain water, the base line is perfectly straight (Figure 2b) because the calorimeter and cells are in thermal equilibrium, *i.e.*, we are not scanning. However, when scanning (*i.e.*, while putting in heat) the usual base line is curved (see above). As shown in Appendix A, the area between the heating curve and the base line (Figure 2c) is $K_1\Delta Q$. Since ΔQ was computed from the known input of electrical energy, K_1 was thereby determined as 441 (mV sec)/cal; this value was constant (within ±1%) over the temperature range from 10 to 60°.

With K_1 thus determined, the heat of fusion of *n*-hexadecane was then measured as a test of the accuracy of the calorimeter. The sample cell contained the solid hydrocarbon, and the reference cell contained air; the base line was obtained with air in both cells. The average of more than ten experiments (with different amounts of hydrocarbon in each run) gave a value for the

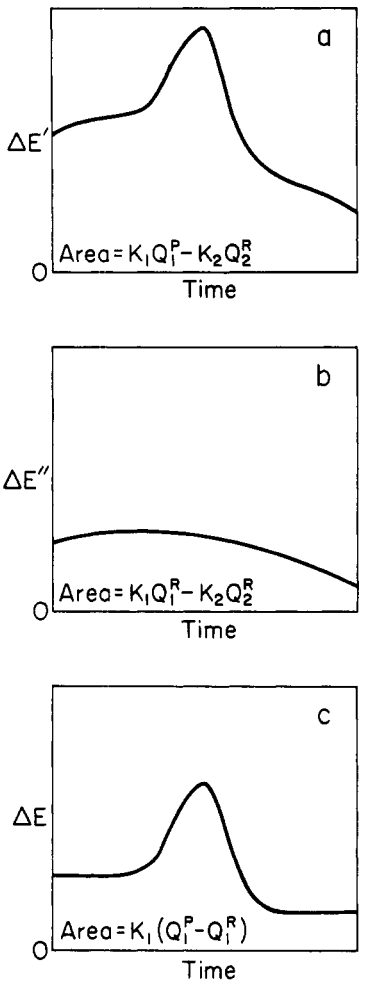

Figure 3. Schematic ΔE *vs.* t curves for a typical run: (a) with polymer solution in the sample cell and solvent in the reference cell ($\Delta E'$) during heating; (b) with solvent in both cells ($\Delta E''$) during heating of the "sample" cell at the same rate; (c) the difference between curves (a) and (b); $\Delta E = \Delta E' - \Delta E''$.

heat of fusion which agreed to within 1.5% with the literature value[18] of 12,750 cal/mol.

Additional tests of the accuracy of the calorimeter were carried out by measuring the enthalpy changes of water[19] and $HCCl_2COOH$[20] over a 50° range of temperature. In these experiments, the sample cell contained a known amount of solvent (ranging from 0.1 to 1.54 ml) and the reference cell contained air; in order to obtain the base line, both cells contained air. Again, the results were within 1.5% of published values.[19,20]

The following characteristics of the calorimeter are of interest. The rms noise level, excluding slow drifts, of the heat detecting system was found to be 4×10^{-9} cal/sec, when the calorimeter was at thermal equilibrium with its surroundings. The reproducibility of the instrument base line was found to be better than 1×10^{-5} cal/sec. The calorimeter was found to respond to fast heat changes (using a heating rate of 0.7°/min) with a relaxation time of about 2 min.[21]

(18) (a) "Selected Values of Physical and Thermodynamic Properties of Hydrocarbons and Related Compounds," Carnegie Press, Carnegie, Pa., 1953, p 769; (b) H. L. Finke, M. E. Gross, G. Waddington, and H. M. Huffman, *J. Amer. Chem. Soc.*, **76**, 336 (1954).

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(21) The existence of this relaxation time means that the temperature of the calorimeter cells lags behind that of the copper block (where it is measured) by about 1.5°, at any given time, when the higher heating rate (0.7°/min) is used and by about 0.4° when the lower heating rate (0.175°/min) is used. While this would cause an error in the determination of the transition temperature, T_{tr}, as the midpoint of the transition, negligible error is made in the value of $\Delta H°$ because of this temperature lag (see below).

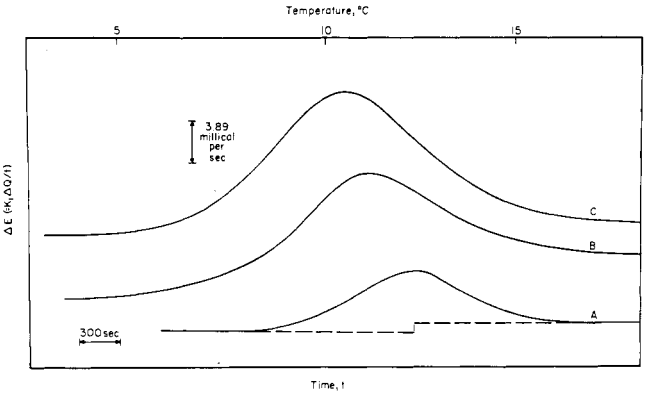

Figure 4. ΔE *vs.* t curves (corrected for instrument base line) for PBLG in 70 wt % $HCCl_2COOH$ solution. The concentrations of PBLG used are: A, 0.075 *M*; B, 0.150 *M*; C, 0.200 *M*. The dotted lines shown in curve A indicate the extrapolation procedure used for calculating the area under the peak.

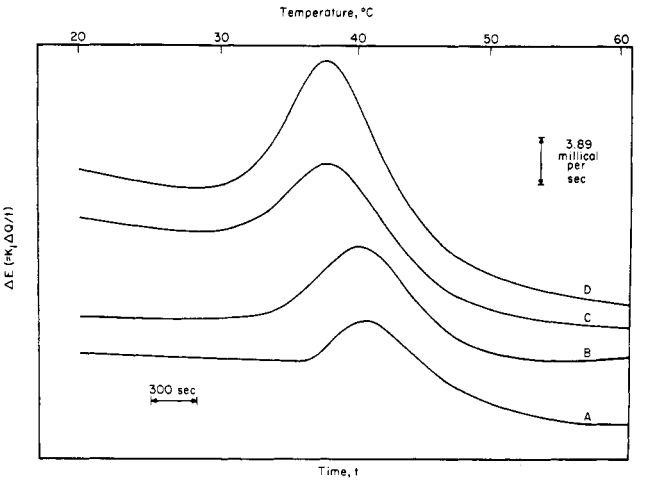

Figure 5. ΔE *vs.* t curves (corrected for instrument base line) for PBLG in 82 wt % $HCCl_2COOH$ solution. The PBLG concentrations are: A, 0.075 *M*; B, 0.125 *M*; C, 0.150 *M*; D, 0.200 *M*. The curve at 0.100 *M* is not shown.

A measurement of ΔQ of 0.025 cal/g of solution over a temperature range of 30° can be made with a precision of ±5%; this compares favorably with a similar precision for 0.05 cal/g over the same temperature range, reported[16] for an adiabatic calorimeter.

Analysis of Data. As shown in Appendix A and Figure 3, two tracings are obtained, one ($\Delta E'$ *vs.* t) with polymer solution in C_1 and solvent in C_2, and the other ($\Delta E''$ *vs.* t) with solvent in both cells. The difference between these two tracings is ΔE *vs.* t, the area under which is $K_1\Delta Q$; division by K_1 gives ΔQ. As described below, ΔQ can then be converted to $\Delta H°$ [which can be expressed on a molar basis by taking account of the helix content θ_h, obtained in the previous paper;[3] the value of θ_h was taken from the high-temperature side of the transition where the *maximum* amount of helix was obtained[3] (0.80 and 0.75 in 70 and 82% wt $HCCl_2COOH$, respectively)]. Figures 4 and 5 show curves of ΔE *vs.* t, *i.e.*, after the above subtraction has been carried out (see Results). Since the extreme portions of the curves, below and above the transition temperature, are not colinear, the following procedure[14] is used to compute the area between the curve and the dashed lines in Figure 4. The extreme portions are extrapolated linearly into the transition region up to a time t_x (determined as described below), and connected by a vertical line as shown[22] in curve A of Figure 4. The enclosed area is then measured, and is interpreted as $K_1\Delta H°$.

Two procedures were used to obtain t_x, both of which gave the

(22) In Figure 4, the extrapolation is carried out on a plot of ΔE *vs.* time instead of one of ΔE *vs.* temperature. However, the shapes of these two plots are identical if the heating rate is constant. Since the variation of heating rate with temperature is small,[17] we assume that the data obtained from a ΔE *vs.* time plot are the same as those obtained from a ΔE *vs.* temperature plot.

same results within the experimental error. First, t_x was taken as the time corresponding to the peak of the ΔE *vs.* t curve, even though the corresponding temperature T_p does not correspond to the transition temperature T_{tr} because of the relatively fast heating rates used.[21] Second, t_x was taken as the time corresponding to T_{tr}, *i.e.*, with the vertical line at t_x bisecting the area into two equal parts.

Results

The values of $\Delta H°$ were determined, as outlined above, for PBLG in 70 and 82 wt % $HCCl_2COOH$ (in Cl_2Et) solutions at several polymer concentrations (see Figures 4 and 5). The lower limit of the concentration was dictated by the inability to measure heat changes accurately below 0.075 *M*, and the upper limit by the high viscosity (with attendant cell-filling problems) above 0.2 *M*. Many more experiments were carried out in 82% $HCCl_2COOH$ than in 70% $HCCl_2COOH$ solutions to check the concentration dependence of $\Delta H°$, since the dependence was expected to be greater in 82% $HCCl_2COOH$ solutions according to the results of Ackermann and Neumann.[7]

Several observations pertain to the experiments carried out here. (1) The value of $\Delta H°$ is independent of chain length since the fractions used here are of large enough $\overline{DP}$ to approach the infinite-chain limit.[3] This is in agreement with the results of Kagemoto and Karasz,[13] who found $\Delta H°$ to be independent of chain length beyond $\overline{DP}$ = 500. (2) While T_p increased with increasing heating rate, the values of $\Delta H°$ were essentially independent of heating rate between 0.175 and 0.7° per min.[21] The faster heating rate was used primarily with the 82% $HCCl_2COOH$ solutions to avoid prolonged exposure of the solutions to the higher temperature conditions required for the transition at this concentration of $HCCl_2COOH$. (3) The solutions could be heated and cooled several times, each time yielding the same value of $\Delta H°$; *i.e.*, the transitions are reversible, and no significant evaporation of solvent occurred during heating. (4) Two successive experiments (involving refilling the cell) with the *same* polymer solution gave heating curves which were reproducible to 1×10^{-5} cal/sec along the whole curve. Repetition of the experiment with *different* solutions gave values of $\Delta H°$ which agreed with each other within the experimental error reported below. (5) The values of T_p decrease with increasing concentrations of PBLG (see Figures 4 and 5). This shift probably arises from the depletion of $HCCl_2COOH$ from the bulk solvent because of increased binding to the polymer at higher concentrations of PBLG.[23]

The values of $\Delta H°$ are summarized in Table I. The data represent average values from several experiments at each concentration of PBLG (at least three in 82% $HCCl_2COOH$ and two in 70% $HCCl_2COOH$).

Table I
Enthalpy Change ($\Delta H°$) for the Helix-Coil Transition of PBLG in $HCCl_2COOH$-Cl_2Et Mixtures

PBLG Concn (*M*)	$\Delta H°_{obsd}$[a] ((cal/mol)/Residue) 70 wt % $HCCl_2COOH$	82 wt % $HCCl_2COOH$
0.075	430 ± 45	285 ± 40
0.100	475 ± 40	260 ± 25
0.125		290 ± 15
0.150		330 ± 15
0.200	435 ± 35	303 ± 15
	Mean 444 ± 45	Mean 295 ± 25
	$\Delta H°$[b,c] = 555 ± 55	$\Delta H°$ = 390 ± 35

[a] As observed. [b] Computed by dividing $\Delta H°_{obsd}$ by θ_h. [c] The error limits given are values of the standard deviation from the mean over all PBLG concentrations.

Discussion

As indicated by the accuracy of the measured heat of fusion of *n*-hexadecane, and of the enthalpy changes accompanying the heating of water and $HCCl_2COOH$, it appears that the DTA apparatus described here will be useful for determining enthalpy changes in the helix-coil transition in biopolymers. The advantages cited earlier and the precision and accuracy of the instrument make it comparible to currently used adiabatic calorimeters.[16]

The lower limit in concentration used here (0.075 *M*) was dictated by the fact that the observed heat change in 1.54 ml of solution (at this concentration) was only 0.05 cal. While a heat change of 0.025 cal can be measured with a precision of ±5%, the problem of extrapolation (indicated in Figure 4, curve A) leads to a practical lower limit of 0.05 cal. This extrapolation problem, which introduces an undeterminable error in $\Delta H°$, is present not only in DTA measurements but also in those from adiabatic calorimetry. The standard deviations in $\Delta H°$, reported in Table I, presumably arise from errors in (a) the concentrations of PBLG and $HCCl_2COOH$ (±3%[3]), (b) filling of cells C_1 and C_2 to equal volumes, and (c) the area of curves in Figures 4 and 5 (±3%).

The values of $\Delta H°$ obtained here are 555 ± 55 and 390 ± 35 cal/mol for 70 and 82% $HCCl_2COOH$, respectively. In contrast to Ackermann and Neumann,[7] the data of Table I show no dependence of $\Delta H°$ on the concentration of PBLG.[24,25]

Our values of $\Delta H°_{obsd}$ for the PBLG-$HCCl_2COOH$-Cl_2Et system are compared with those from other laboratories in Table II. (These are values of $\Delta H°_{obsd}$, and not of $\Delta H°$, which is obtained by dividing $\Delta H°_{obsd}$ by θ_h). Our values of $\Delta H°_{obsd}$ are smaller than the others, especially at the higher $HCCl_2COOH$ concentrations. However, comparison of our values of $\Delta H°$ (*i.e.*, $\Delta H°_{obsd}/\theta_h$) given in Table I with those obtained from the ORD experiments[3] indicates good agreement for 82 wt % $HCCl_2COOH$ (390 ± 35 cal/mol by calorimetry and 360 ± 15 cal/mol by ORD). The agreement is less satisfactory for 70 wt % $HCCl_2COOH$ (555 ± 55 cal/mol by calorimetry and 770 ± 50 cal/mol by ORD). We believe that the discrepancy here arises primarily from the strong temperature dependence of $\Delta H°$ at 70 wt % $HCCl_2COOH$, as observed in the ORD study.[3]

With the determination of the thermodynamic parameters for the PBLG-$HCCl_2COOH$-Cl_2Et system, reported here, and in the previous paper,[3] we may next consider the influence of a guest residue on the helix-coil transition of PBLG in this solvent mixture.

Appendix

Basis of Operation of Calorimeter. A. By passing current through the heaters H during a time t, a quantity of heat Q flows from the copper block S through the detector D to the calorimeter cell C. The resulting emf E is given by[15]

$$E = KQ/t \quad (1)$$

where K is a constant characteristic of the detector. In a

(23) D. Puett and A. Ciferri, *Biopolymers*, 10, 547 (1971).

(24) After completion of this manuscript, our attention was drawn to the recent paper of Teramoto and Norisuye[25] who also find no concentration dependence of $\Delta H°$.

(25) A. Teramoto and T. Norisuye, *Biopolymers*, 11, 1693 (1972).

Table II
Reported Calorimetric Values of $\Delta H°$ for the Helix–Coil Transition of PBLG in $HCCl_2COOH$–Cl_2Et Mixtures

PBLG Concn (M)	$HCCl_2COOH$ Concn (wt %)	$\Delta H°_{obsd}$ (cal/mol)	Ref
0.075–0.20	70	444 ± 45	This work
0.06	79	525 ± 80	8
0.09	79	550 ± 60	13
0.075–0.20	82	295 ± 25	This work
0.07–0.25	81, 82, 83, 85, 88	950 ± 20	5–7
0.006	0–100[a]	650 ± 30	10
0.076	0–100[a]	750 ± 100	11

[a] From heat of solution measurements at fixed temperature.

run in which the sample cell C_1 contains the polymer solution (P) and the reference cell C_2 contains the reference solvent (R), the difference, $\Delta E'$, in the outputs of the two detectors (which is recorded as a function of t) is given by an equation of the form of eq 1, *viz.*

$$\Delta E' = (K_1 Q_1^{P} - K_2 Q_2^{R})/t \quad (2)$$

where K_1 and K_2 are the constants for the two detectors, and Q_1^{P} and Q_2^{R} are the quantities of heat passing to C_1 and C_2, respectively (see Figure 3a). The non-zero value of $\Delta E'$ at the beginning and end of the run arises from the differences in heat capacity of the contents of C_1 and C_2.

If the run is repeated with the reference solvent in *both* cells (to obtain the instrument base line), the difference, $\Delta E''$, in the outputs of the two detectors is

$$\Delta E'' = (K_1 Q_1^{R} - K_2 Q_2^{R})/t \quad (3)$$

(see Figure 3b). Subtraction of eq 3 from eq 2 gives

$$\Delta E = \Delta E' - \Delta E'' = K_1(Q_1^{P} - Q_1^{R})/t = K_1 \Delta Q/t \quad (4)$$

which is independent of the constant K_2 of the reference detector. Thus, a plot of ΔE *vs.* t is one of $K_1 \Delta Q/t$ *vs.* t, and the area under the curve gives $K_1 \Delta Q$ (see Figure 3c). If K_1 is known (see text), ΔQ is thus determined.

B. The temperature rise, ΔT, in a cell when a quantity of heat, Q, flows through it is given by

$$\Delta T = Q/C_p \quad (5)$$

where C_p is the constant pressure heat capacity of the cell (including its contents). Thus, ΔQ of eq 4 is given by

$$\Delta Q = C_p^{P} \Delta T^{P} - C_p^{R} \Delta T^{R} \quad (6)$$

If we assume that $\Delta T^{P} = \Delta T^{R}$, *i.e.*, that the temperature rise is the same when the sample cell contains the polymer solution as when it contains the reference solvent, then

$$\Delta Q = \Delta C_p \Delta T \quad (7)$$

where

$$\Delta C_p = C_p^{P} - C_p^{R} \quad (8)$$

and corresponds to the difference in heat capacity of the solution and the solvent since the heat capacities of the cell and related parts of the calorimeter cancel out. Substitution of eq 7 in eq 4 gives

$$\Delta E = K_1 \Delta C_p \Delta T/t \quad (9)$$

Thus, a plot of E *vs.* t is equivalent to a plot of $K_1 \Delta C_p \Delta T/t$ *vs.* t, and the area under the curve gives $K_1 \Delta C_p \Delta T$.

The assumption that $\Delta T^{P} = \Delta T^{R}$ (and that Q_2^{R}/t in eq 2 is the same as Q_2^{R}/t in eq 3) is equivalent to the assumption that the heating rate is the same in both runs, *i.e.*, when the sample cell contains polymer solution and solvent, respectively. The slight variation in heating rate with temperature, mentioned in footnote 17, does not affect ΔQ, since the heating rate is assumed to be the same in both runs *at a given temperature*