Calorimetric Heat of the Helix–Coil Transition of Poly-L-glutamic Acid[1a]

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Abstract: When solutions of sodium poly-L-glutamate are acidified, the polymer undergoes a transition from random coil to helix. The changes in enthalpy associated with these pH changes have been measured using a Calvet microcalorimeter. The enthalpy was found to change abruptly in the range where the transition is detected from a change in optical rotation but is otherwise independent of pH. The enthalpy of the conformational change is determined to be -1100 cal/mole of monomer unit, and is found to be in agreement with the value obtained earlier from potentiometric titration curves at various temperatures. This large negative value is considered to be due to a perhaps even larger negative heat of formation of the peptide hydrogen bond.

As was pointed out by Zimm and Rice,[2] potentiometric titration of ionizable synthetic polypeptides is a powerful tool for obtaining the free energy of formation of the poly-L-glutamic acid helix (*i.e.*, the form present at low pH) from the random coil (also at low pH), expressed per mole of monomer unit, in a variety of solvents. This method has been applied successfully to study quantitatively the effect of ionic strength,[3a] polymer composition,[3b] temperature,[4] and urea concentration[5] on the stability of the helix. Since the α helix is a structural feature of many globular proteins, the results obtained by these authors have a great deal of bearing on the problem of understanding the stability of proteins under various conditions. Because of the usefulness of this type of experimental data, it is of interest to perform independent measurements giving the same information by some other technique, as follows.

Molar free energies can also be obtained by measuring enthalpies of denaturation as a function of temperature in a range including the point where helix and coil are in equilibrium[6] (*i.e.*, where $\Delta F^\circ_{hel} = 0$[7]). In principle, one can achieve this by measuring the heat capacity of the polymer solution near the "melting" temperature of the helix.[8,9] However, the type of equipment needed for this type of study was not available to us. Instead of with a change of temperature, one can obtain the conversion from helix to coil with a pH change. The accompanying heat effect should be measurable with techniques which

have already been applied successfully to study the ionization and denaturation reactions of myoglobin.[10] The limitations of the instrument used restricted the measurements to a temperature of 30°. The single enthalpy value obtainable in this way will therefore have to be compared with the enthalpy calculated from the temperature dependence of the molar free energies established before.[4]

Experimental Section

Various lots of poly-L-glutamic acid from Sigma (Type III) were used in this study. Calorimetric measurements and pH measurements were carried out as described for sperm whale myoglobin. About 3 mg of polyglutamate in 6 ml of 0.1 N KCl was mixed with a varying amount of dilute HCl in each experiment. Measurements of optical rotation were carried out with a Cary Model 60 spectropolarimeter, with a cell thermostated at 30°, on solutions containing $\sim$0.2 mg of polyglutamate/ml. The accuracy of both sets of data is largely determined by the reliability of a pH measurement on an unbuffered solution taken approximately 1 hr (calorimetry) or 10 min (polarimetry) after the measurement and is difficult to assess.

The potentiometric titration curve was obtained as described before[4] on a solution containing approximately 40 mg of polymer.

Results and Discussion

The results obtained are shown in Figure 1. This includes enthalpy values calculated per mole of monomer unit in the solutions, ΔH°, the specific rotation at 233 mμ, $[\alpha]_{233}$, and the degree of ionization, α. The scales have been adjusted so that the upper and lower limits of the three parameters coincide. It should especially be noted that the values of the enthalpy are uncorrected for instrumental heat effects. Thus upon mixing a polymer solution of pH 7 with a small volume of salt solution of pH 7, a heat effect is noted which is about the same as that noted when the final pH is 6.

It is apparent from these data that the enthalpy does not follow the degree of ionization, but changes abruptly in the pH range where the coil to helix transition occurs. It would be tempting to equate the enthalpy change of -1100 cal/mole in this narrow range with the value -1120 cal/mole of ΔH°_{hel}, the enthalpy of helix formation, for the uncharged helix which we obtained from the temperature dependence of the free energy of helix formation of the polyacid.[4] However, it

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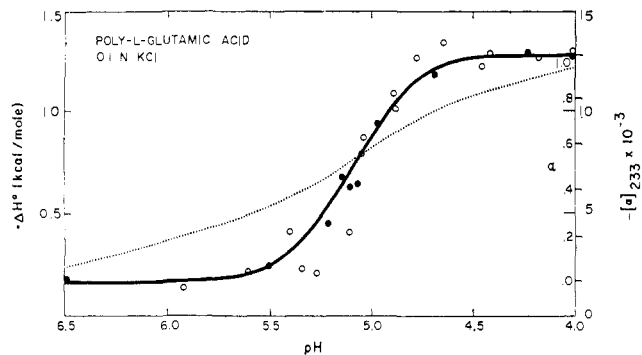(10) J. Hermans and G. Rialdi, *Biochemistry*, 4, 1277 (1965).

Figure 1. Molar heat content, ΔH° (open circles, left-hand scale), specific rotation at 233 mμ $[\alpha]_{233}$ (closed circles and drawn curve, outer right-hand numbers), and degree of ionization, α (dashed curve, inner right-hand numbers), of poly-L-glutamic acid in 0.1 N KCl as a function of pH at 30°.

should be realized that the enthalpy change observed here is given by

$$\Delta H^\circ_{\mathrm{obsd}} \sim \Delta H^\circ_{\mathrm{hel}} - 0.7(\Delta H_i{}^\circ)_{\mathrm{coil}} + 0.3(\Delta H_i{}^\circ)_{\mathrm{helix}} \quad (1)$$

where 0.7 and 0.3 represent the degree of ionization of the carboxyl groups just below and just above the transition region (see Figure 1), and the two $\Delta H_i{}^\circ$ represent the heat of ionization of the carboxyl groups in the coiled and in the helical molecule. (These are assumed to be independent of α.)

In general, heats of ionization of carboxyl groups are in the range 0–2 kcal/mole[11]; furthermore, the intrinsic pK of the carboxyl group in helical and randomly coiled poly-L-glutamic acid is independent of temperature within experimental error, and thus the values $\Delta H_i{}^\circ$ of eq 1 fall within this range.[4] However, if we wish to apply eq 1 to our data, the accuracy provided by the quoted range is insufficient.

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The only information which will allow us a sufficiently definite estimate of the values of $\Delta H_i{}^\circ$ applicable *in this case* is provided by the enthalpy measurements of Figure 1 at pH above 5.5 and below 4.5. Here the degree of ionization varies, but the degree of helicity does not. Therefore, any observed enthalpy changes must be due to ionization. This observation allows us to estimate both enthalpies as 0 ± 200 cal/mole. Therefore one can conclude on the basis of the enthalpy measurements that

$$\Delta H_{\mathrm{hel}}{}^\circ = -1100 \pm 200 \text{ cal/mole} \quad (2)$$

and that the value obtained previously using an entirely different method is confirmed. (The exact agreement would indicate that $(\Delta H_i{}^\circ)_{\mathrm{helix}} \sim 2(\Delta H_i{}^\circ)_{\mathrm{coil}}$, or simply that both heats of ionization are close to zero.)

Thus our calorimetric observations bear out the validity and usefulness of the method of Zimm and Rice.[2] Furthermore, these results allow one to again point out the large negative value of the heat of helix formation for poly-L-glutamic acid in aqueous solution, which one can only attribute to the formation of the peptide hydrogen bond.[4] The heat of formation of this bond would then fall in the range of the estimate which Schellman derived from a consideration of the thermodynamics of urea solutions.[12] Also, it would appear that in those cases where lower enthalpies for breaking hydrogen bonds between NH and CO groups were postulated on the basis of experimental evidence,[13,14] compensating effects may be present which lower the net value of the enthalpy for these reactions. However, we prefer not to speculate about the nature of these effects[15] in this article.

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