COMMUNICATIONS TO THE EDITOR

New Chain Conformations of Poly(glutamic Acid) and Polylysine

A voluminous literature exists[1] on the structure of poly(L-glutamic acid) (PGA) in solution, particularly as determined by optical rotatory dispersion (ORD) and circular dichroism (CD). These studies have led to the conclusion that PGA is α-helical at low pH and that it has a random conformation at a pH of 7 or above. CD spectra in the high pH region have been given[2-6] and are indicated to have bands near 238 mμ (negative), 218 mμ (positive), and 198 mμ (negative). Such CD curves have been used[7] as representative of the random conformation of polypeptide chains which are present in globular proteins.

We wish to note that the CD spectrum of PGA referred to above is that of an intermediate structural state of the polypeptide chain. Furthermore, the conformational state to which the chain is tending in the high pH region is not a random one, but is an ordered structure not heretofore considered in interpreting the CD spectra.

In curve *a* of Figure 1 we show the CD spectrum of PGA at pH 7–12 (in NaOH) in the absence of additional salt. It will be seen that there are only two bands, a positive band at about 218 mμ and a negative band near 198 mμ. (We have observed that $\Delta\epsilon$ for the 218 mμ band is larger at lower concentration of PGA.) A careful search at the highest sensitivity of the instrument (Durrum-Jasco ORD/CD/UV-5) failed to reveal the negative band at 238 mμ (which should have been seen if its intensity ratio with respect to the 218 mμ band were the same as ratios previously reported[4-6]). On the other hand, by lowering the pH (cf. curves *b* and *c* of Fig. 1), or by adding salt, a minimum near 238 mμ is introduced into the spectrum. It is seen, therefore, that the three-band CD spectrum is that of an intermediate structural state of PGA. It should be noted that organic solvents (dioxane, chloroethanol, ethylene glycol, and methanol) have a similar effect. For example, the effect of increasing methanol concentration on the CD spectrum of PGA at pH 8 (no salt) is shown in Figure 2. It would appear, therefore, that the previously reported CD spectra of PGA at high pH[2-6] were obtained in the presence of sufficient salt to prevent the development of the simple two-band spectrum; the observed variability[2-6] in the intensity ratio of the bands at 218 and 238 mμ is consistent with this supposition.

Various arguments suggest that the two-band CD spectrum of PGA is not that of a random chain conformation but rather corresponds to a relatively ordered structure over significant portions of the molecule. First, CD spectra of the random forms of collagen[8] and polyproline[9] exhibit no positive band at long wavelengths; rather the curve decreases uniformly from 250 mμ to a negative band near 200 mμ. Although the latter band may well be of variable intensity from one random polypeptide to another and for different degrees of randomness, the qualitative shape of this curve seems to be characteristic of the unordered chain. (It should be noted that a CD spectrum similar to this is obtained in the range of 75–80% methanol (cf. Fig. 2), indicating that the PGA chain is largely random under these conditions.) Thus, the significantly different form of the two-band PGA spectrum is indicative of the presence of regularity of some kind in the chain. Second, the two-band PGA spectrum has strong resemblances to the two-band CD spectrum of polyproline II.[9,10] (The small differences in the frequencies of the bands could well be due to the different electronic structures and spectra of the component monomer units.) Since the CD spectrum can reflect the local conformation of adjacent peptide groups,[11] this suggests the possible similarity in structure between

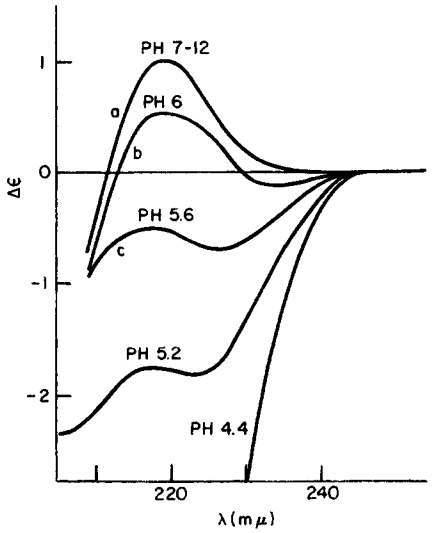

Fig. 1. Circular dichroism spectra of poly(L-glutamic acid) (Pilot) as a function of pH. Concentration, 1.29 g/l; sample dissolved in NaOH and dialyzed, HCl added to change pH; path length, 1 mm.

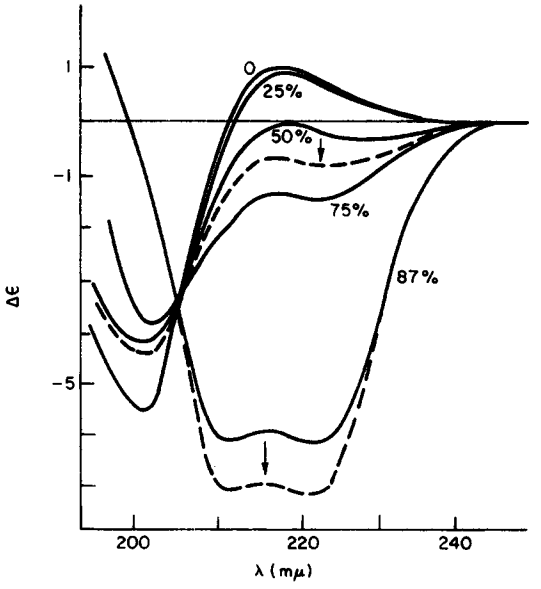

Fig. 2. Circular dichroism spectra of poly(L-glutamic acid) (Pilot) as a function of methanol concentration.: (——) initial readings; (- - -) readings 6 days later. Concentration, 0.182 g/l; initial pH, 7; path length, 1 mm.

high pH PGA and polyproline II, viz., that the former contains chain conformations close to that of a threefold helix. Finally, it is not reasonable to assume that the structure of a polyelectrolyte chain (viz., that of PGA at high pH in the absence of salt) will be truly random. If anything, the electrostatic interactions along the chain will favor an extended chain in which the charged side chains take up a regular helical arrangement. A simple calculation shows that the rotation angle of the helix for which the electrostatic energy is a minimum is determined by the ratio of the radius of the charge from the axis to the vertical rise between charges. For the PGA structure the calculation shows that this angle is near 120°. Since such a threefold helix is in an energy minimum with respect to steric interactions,[11] the enthalpy contribution to the determination of the structure will be influenced predominantly by the electrostatic energy component. We therefore propose that the structure of PGA at high pH and low salt concentration is one in which significant portions of the chain have a conformation close to that of the threefold helix of polyproline II.

It is important to observe that the presence of an ordered structure of the kind which we propose for PGA at high pH can explain many heretofore puzzling observations. For example, the viscosity of PGA goes through a minimum near pH 6, rising at lower and higher pH.[12] The increased viscosity at low pH is clearly due to the formation of α-helix structure. The increase at high pH we believe to be associated with the formation of the regular threefold helical structure. In this region the viscosity increases with decreasing salt concentration,[13] paralleling the structural changes which are indicated by the observed variations in the CD spectrum. The random conformation predominates near pH 6. The existence of an ordered conformation at high pH also allows a reasonable interpretation of the presence of an optically active complex which PGA forms with acridine orange at pH 8,[14] for we no longer need to regard this state of PGA as random. It is especially interesting that the CD bands of this dye complex are different from those obtained when the PGA is in the α-helical state (pH 4.5), and that it is impossible to form any complex in the intermediate pH range (where the chain is indeed predominantly random). The plateau in the titration data[1] can also be understood in terms of the presence of more than just two structures.

We find that the CD spectrum of poly-L-lysine in the low pH region, in the absence of salts, contains two bands similar to those of PGA. We believe that in this case also the structure cannot be considered random but in fact consists of a significant component of a regularly ordered conformation.

Further details of these studies will be published subsequently.

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