EFFECT OF MOLECULAR AGGREGATION ON CIRCULAR DICHROISM AND OPTICAL ROTATORY DISPERSION OF HELICAL POLY-L-GLUTAMIC ACID IN SOLUTION

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Circular dichroism (CD) and optical rotatory dispersion (ORD) have been shown to be useful techniques in studies of the secondary structure of polypeptides and proteins. As yet, their application to the investigation of tertiary and quaternary structures has been limited. Such studies may become exceedingly useful for our understanding of molecular interactions *in vivo*.

Recently several laboratories have reported studies of aggregation in a model polypeptide, poly-L-glutamic acid (PGA) in solution (Jennings *et al*., 1965; Schuster, 1965; Zezin *et al*., 1965; Tomimatsu *et al*., 1966).

Evidence from light scattering, titration, and hydrodynamic measurements have indicated that PGA in water or very dilute salt solutions strongly aggregates below pH 4.5 (Wada, 1960;

Jennings et al., 1965; Schuster, 1965; Tomimatsu et al., 1966). This polypeptide is in the coiled form above pH 6 and is essentially unaggregated (Yang and McCabe, 1965; Tomimatsu et al., 1966) but not necessarily completely helical (Cassim and Taylor, 1965b) at pH 4.5. These optical rotation measurements have been limited to the visible and near ultraviolet regions, thus providing little information about the electronic transitions responsible for the optical properties. We have now extended the ORD measurements to 186 mμ and studied the CD spectra under similar conditions. The advantage of CD as a means of better resolving rotatory bands has been discussed by Holzwarth (1964).

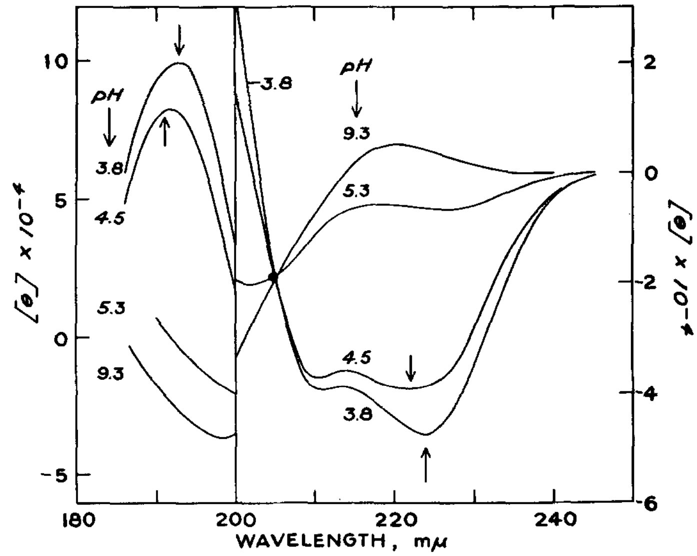

Fig. 1 Mean residue ellipticity of poly-L-glutamic acid in 0.1 M $NaClO_4$ at 25.0°C. Concentration, 0.08%; path lengths, 0.5 and 0.1 mm. Signal-to-noise ratios: 80 at 222.5 mμ, 25 at 210 mμ, and 5 at 191 mμ. The dot indicates the isosbestic point in the helix-coil transition region.

Our results are summarized in Figs. 1 and 2. At pH 3.8 all three dichroic bands previously observed by Holzwarth and Doty (1965) and Velluz and Legrand (1965) show distinctive, albeit small, red shifts (Fig. 1): 222.5 to 224 mμ; 210 to 211 mμ; 191 to 193 mμ. Marked increases in band strength accompany these shifts. The relative intensities of the two negative bands are approximately equal in the unaggregated state but the band at 224 mμ is relatively stronger than that at 211 mμ. The reversibility of these spectral shifts was established by adjusting the pH of the solution first to 3.80 and then back to 4.51. The results were identical to the ones obtained by lowering the pH from 6.00 to 4.51. The relatively low signal-to-noise ratio below 200 mμ somewhat obscured the shift of the 191 mμ band. The direction of the shift, if not the magnitude, of this band was ascertained by repeated measurements.

Similarly, aggregation of helical PGA causes small red shifts of the trough and peak of the Cotton effects (from 232.5 to 233.5 mμ and from 198.5 to 199.5 mμ), and significant increases in their intensities (Fig. 2). This change in trough intensity has been observed by Tomimatsu _et al_. (1966). The shoulder near 210 mμ does not shift noticeably, but its intensity does change.

A second feature in the figures is that the ORD curves (Fig. 2) of the helical (unaggregated) and coiled forms intersect at two wave-lengths ("isosbestic" points),

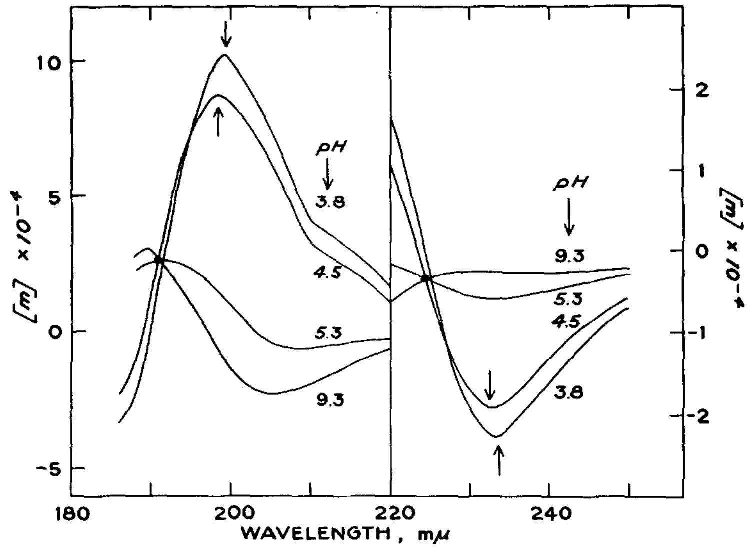

Fig. 2 Mean residue rotation of poly-L-glutamic acid in 0.1 M $NaClO_4$ at 25.0°C. Concentration, 0.08%; path lengths, 5 and 0.1 mm. Signal-to-noise ratios: 200 at 232.5 mμ and 25 at 198.5 mμ. The dots indicate the isosbestic points in the helix-coil transition region.

through which all other curves in the helix-coil transition region pass. Once aggregation occurs, the curve at pH 3.8, for instance, no longer intersect at these two "isosbestic" points. The same phenomenon is of course also present in the CD spectra (Fig. 1). However, the rapid change of ellipticity with wavelength makes it less obvious.

We have also observed, as did Tomimatsu *et al.* (1966), that in dilute salt solutions the CD and ORD spectra of PGA remain essentially constant in the pH range of 4.5 to 4.8, indicating that the coil-to-helix transition has reached its plateau. The simplest conclusion to be reached from these observations is that the observed changes in spectra below pH 4.5 cannot be due to the same factors perturbing the spectra at higher pH regions. Therefore, it is more reasonable to correlate these spectral changes with molecular aggregation than with increase in helical content or decrease in ionization _per se_.

Since end effects are negligible in long polypeptides, end-to-end aggregation cannot effect the spectra significantly. A side-to-side model would be in better agreement with hydrodynamic as well as optical measurements (Jennings _et al._, 1966). It has been suggested that changes in the local dielectric constant may perturb the ORD spectra (Shechter and Blout, 1964; Herskovits _et al._, 1964; Schuster, 1965). Cassim and Taylor (1965a) could not find any such correlations with poly-γ-benzyl-L-glutamate in a large variety of solvents. However, for small molecules, π-π^* transitions undergo a characteristic blue shift and n-π^* transitions red shift as the polarity of the solvent is decreased (Bayliss, 1950; Kasha, 1961). Our CD study has shown an apparent red shift of all three bands. The two bands at 191 and 210 mμ have been assigned to the split π-π^* transition and the band at 222.5 mμ to the n-π^* transition (Schellman and Oriel, 1962; Holzwarth and Doty, 1965). Whether the local dielectric constant decreases or increases with aggregation, these band shifts cannot be simply reconciled with characteristic shifts noted for small molecules.

Another possibility is that aggregation may result in the formation of an asymmetrical structure similar to a model proposed by Robinson (1961) for polypeptide liquid crystals. The molecules may aggregate at a slight angle and form a helical twist instead of having their helical axes aligned parallelly. Each split π-π* transition in the helix would split again. The bands due to the macrostructure would superimpose on those of the helix resulting in an apparent shift of helix bands. The validity of such model is being investigated at the present time.

Experimental. - PGA, purchased from the Pilot Chemicals, Inc. (lot G-80), was dialyzed exhaustively against double-distilled water, and its concentration was determined by micro-Kjeldahl analysis.

The CD was measured with a Jasco ORD/CD/UV-5 and ORD with a Cary 60 spectropolarimeter. Since aggregation is sensitive to changes in temperature, a constant temperature of 25.0 °C. was maintained throughout all the measurements. The pH of the solutions, determined with a Radiometer pH-meter 25 with a scale expander, was adjusted by adding dilute hydrochloric acid or sodium hydroxide. The CD and ORD were measured simultaneously with the same solutions.

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References

Bayliss, N. S., J. Chem. Phys. 18, 292 (1950).
Cassim, J. Y., and Taylor, E. W., Biophys. J. 5, 553 (1965a).
Cassim, J. Y., and Taylor, E. W., Biophys. J. 5, 573 (1965b).
Herskovits, T. T., Townend, R. and Timasheff, S. N., J. Am. Chem. Soc. 86, 445 (1964).
Holzwarth, G. M., Dissertation, Harvard University, Cambridge, Mass., (1964).
Holzwarth, G. M. and Doty, P., J. Amer. Chem. Soc. 87, 218 (1965).
Jennings, B., Spach, G., Benoit, H., and Schuster, T. M., preprint No. 562, International Symposium on Macromolecular Chemistry, Prague (1965).
Kasha, M. in "Light and Life", W. D. McElroy and B. Glass (Eds.), Johns Hopkins Press, Baltimore, Md., 1961; p. 31.
Robinson, C., Tetrachedron 13, 219 (1961).
Schellman, J. A. and Oriel, P., J. Chem. Phys. 37, 2114 (1962).
Schuster, T. M., Biopolymers 3, 681 (1965).
Shechter, E., and Blout, E. R., Proc. Natl. Acad. Sci. U.S. 51, 794 (1964).
Tomimatsu, Y., Vitello, L., and Gaffield, W., Biopolymers 4, 653 (1966).
Velluz, L., and Legrand, M., Angew. Chem. Intern. Ed. Engl. 4, 838 (1965).
Wada, A., Mol. Phys. 3, 409 (1960).
Yang, J. T., and McCabe, W. J., Biopolymers 3, 209 (1965).
Zezin, A. B., Bakeyev, N. F., Merzlov, V. P., Shaldina, L. A., and Kozlov, P. V., Biofizika 10, 207 (1965).