

determined, but within the large experimental uncertainty they are in the expected locations in the plane of the ring.

The iron atom is almost equidistant from the carbon atoms in the ring and the five neighbors in the icosahedron. The configuration is almost exactly eclipsed rather than staggered as in the ferrocene crystal.<sup>4</sup> We tested the staggered structure in the least-squares calculations but got significantly worse agreement. Because of the large twisting motion, the configuration in the crystal may have little chemical significance.<sup>5</sup>

(5) This work was done under the auspices of the U. S. Atomic Energy Commission.

Allan Zalkin, David H. Templeton, Ted E. Hopkins  
Lawrence Radiation Laboratory and Department of Chemistry  
University of California, Berkeley, California 94720

Received May 20, 1965

### Sense of Helix of Poly-L-histidine<sup>1</sup>

Sir:

Current interest in the optical rotatory properties of synthetic polypeptides centers largely on detection of ordered structures, such as the  $\alpha$ -helix, in these molecules. Information about the screw sense of helix and estimates of helix content may be obtained from analysis of optical rotatory dispersion (ORD) curves and circular dichroism (CD) spectra.<sup>2-9</sup> Until quite re-

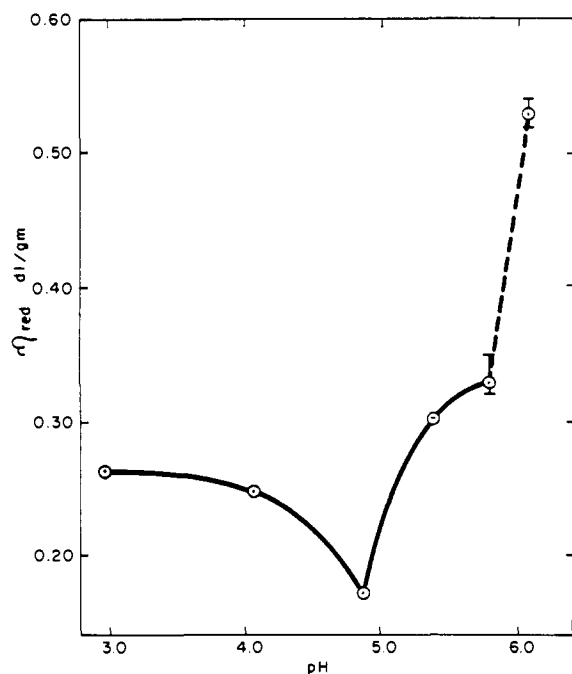


Figure 1. Reduced viscosity of poly-L-histidine at 0.5% in 0.10 M acetate buffer. Viscosities were measured in Cannon-Fenske viscometers with water flow times in excess of 100 sec. at 25°; the temperature was 25 ± 0.02 °.

(1) This work was supported in part by grants from the United States Public Health Service (GM 10576) and from the National Science Foundation (GB 1913).

(2) E. Schechter and E. R. Blout, *Proc. Natl. Acad. Sci. U. S.*, **51**, 794 (1964).

(3) G. Fasman, *Methods Enzymol.*, **6**, 928 (1963).

(4) J. Schellman and C. G. Schellman in "The Proteins," Vol 2, H. Neurath, Ed., Academic Press Inc., New York, N. Y., 1964, Chapter 7.

(5) E. R. Blout, I. Schmier, and N. S. Simmons, *J. Am. Chem. Soc.*, **84**, 3193 (1962).

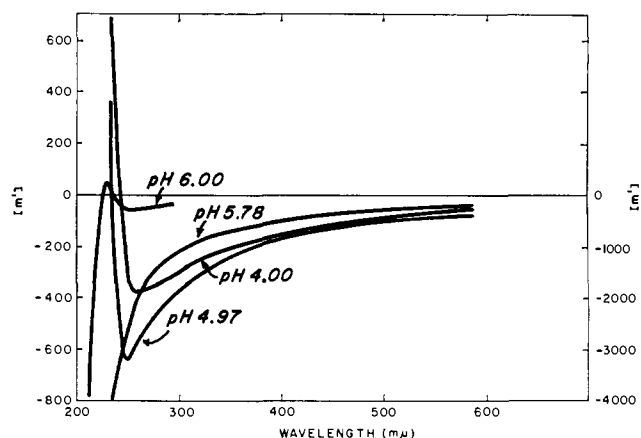


Figure 2. Optical rotatory dispersion spectra of poly-L-histidine in acetate buffer at ionic strength 0.10. The ordinate on the right is for the curve marked pH 5.78.

cently, little was known about side-chain chromophore Cotton effects and their influence on estimates of helix content and helix sense. Studies on poly-L-tyrosine<sup>7,8</sup> and poly-L-tryptophan<sup>9</sup> show, however, that in these polymers side-chain Cotton effects influence the rotatory dispersion sufficiently that not even the presence of helix is certainly established from ORD measurements in the *visible* spectrum, much less the helical sense.

Norland, *et al.*,<sup>10</sup> have investigated the ORD of poly-L-histidine. These authors noted a marked increase in levorotation at the mercury green line associated with neutralization of the imidazolium groups. On the basis of this and other evidence, they suggested that poly-L-histidine may exist in aqueous solution as a left-handed  $\alpha$ -helix over a narrow range of pH.

In this communication we report the CD spectra of poly-L-histidine solutions at pH values between 3.0 and 6.0. Circular dichroism spectra present narrow bands and are thus useful in resolving optically active absorption bands.<sup>11</sup> The CD spectra of poly-L-histidine and the viscosities indicate that in this pH interval poly-L-histidine undergoes a transition from random coil to right-handed  $\alpha$ -helix.

Figure 1 shows the reduced viscosity at 0.5% poly-L-histidine as a function of pH. Between pH 4 and 4.9 the viscosity decreases, as expected for the random coil which is being discharged. As the pH is raised above 4.9, there is an abrupt rise in viscosity. The value at pH 6.0 was time dependent, with clearly discernible loss of solubility within a few minutes after preparation of the solution. Between pH 5.0 and 5.8, however, solutions were stable for at least 24 hr.

Figure 2 shows the ORD spectra of poly-L-histidine at the pH values indicated. There is a large change in the ORD spectrum between pH 4.97 and 5.78 both in quality and quantity. We do not believe that the minimum at 240 m $\mu$  exhibited by the solutions at pH 4 and 5 is the trough of a negative Cotton effect but

(6) G. Holzwarth, W. Gratzler, and P. Doty, *ibid.*, **84**, 3194 (1962).

(7) G. D. Fasman, E. Bodenheimer, and C. Lindblow, *Biochemistry*, **3**, 1665 (1964).

(8) S. Beychok and G. D. Fasman, *ibid.*, **3**, 1675 (1964).

(9) G. D. Fasman, M. Landsberg, and M. Buchwald, *Can. J. Chem.*, **43**, 1588 (1965).

(10) K. Norland, G. D. Fasman, E. Katchalski, and E. R. Blout, *Biopolymers*, **1**, 277 (1963).

(11) S. Beychok, *Proc. Natl. Acad. Sci. U. S.*, **53**, 999 (1965).

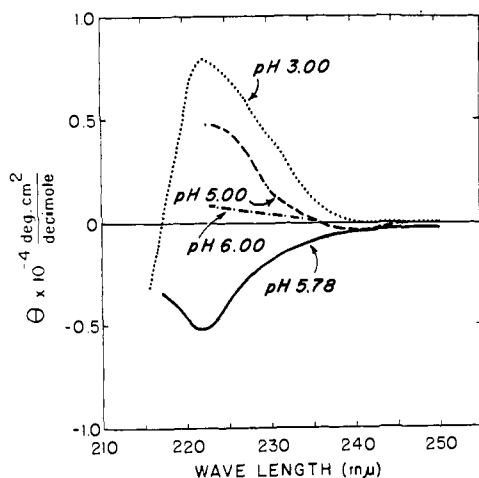


Figure 3. Circular dichroism spectra of poly-L-histidine in acetate buffer at ionic strength 0.10. Measurements were performed on a Jouan Dichrographe modified for 10-fold increased sensitivity. Formulas for calculating  $\theta$  are given elsewhere<sup>8</sup>; 1-mm. and 0.5-mm. cell paths were employed. The same curve was obtained at pH 5.78 in unbuffered solution at ionic strength 0.10; the temperature was  $25 \pm 0.1^\circ$ .

rather that it reflects the increasing dominance of a positive Cotton effect at shorter wave lengths.

In Figure 3 are shown the CD spectra at several pH values. As the pH is raised through the transition interval a negative band appears, the wave length maximum and sign of which are characteristic of the right-handed  $\alpha$ -helix.<sup>6</sup> The magnitude of the maximum ellipticity is, however, only about  $1/6$  of that observed for helical poly- $\alpha$ -L-glutamic acid.<sup>12</sup> There are at least two possible explanations for this. The first is that the molecule is only partially helical. The second is that the side chains contribute an oppositely signed band which effectively diminishes the ellipticity band arising from the peptide bonds in  $\alpha$ -helical segments. At pH 3.0, the large positive band occurs at wave lengths corresponding to a known absorption band of the imidazolium ring in histidine.<sup>13</sup> The magnitude of the positive ellipticity band in this spectral interval which is associated with random coil polypeptides,<sup>14,15</sup> and the ORD at pH 4.0 shown in Figure 2 seems entirely incompatible with random coil optical activity uncomplicated by substantial side-chain contributions.

Urry and Eyring have recently reported the ORD spectra of the free amino acid.<sup>16</sup> They infer that the imidazolium ring contributes no Cotton effect in the 220-m $\mu$  region of the spectrum. Indeed, circular dichroism measurements of histidine performed in this

(12) E. Breslow, S. Beychok, K. Hardman, and F. R. N. Gurd, *J. Biol. Chem.*, **240**, 304 (1965).

(13) L. J. Sidel, A. R. Goldfarb, and S. Waldman, *ibid.*, **197**, 285 (1952).

(14) G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).

(15) We have observed that, at least in the case of poly- $\alpha$ -L-glutamate at pH 7, the magnitude of the positive ellipticity band centered near 218 m $\mu$  depends on the salt concentration, the greatest magnitude occurring in the absence of added electrolyte. However, even in the absence of added salt, the magnitude of the band does not equal what is observed with poly-L-histidine at pH 3 and at an ionic strength of 0.15. The positive band observed in random coil poly- $\alpha$ -L-glutamate and poly-L-lysine is doubtless due to restricted rotation about single bonds and elements of local order. To what extent these factors may be influenced by the nature of the side chain is, at present, unknown.

(16) D. W. Urry and H. Eyring, *J. Am. Chem. Soc.*, **86**, 4574 (1964).

laboratory reveal no band other than that due to the carboxyl, at wave lengths longer than 208 m $\mu$ . It does not necessarily follow, of course, that the 215-m $\mu$  imidazole absorption band is optically inactive in the random coil polymer at pH 3.0. We are currently examining the CD spectra of histidine-containing peptides. These results, along with the results of an investigation of the interaction of helical poly-L-histidine with hemin, will be reported in due course.

(17) Research Career Development Awardee of the United States Public Health Service.

S. Beychok,<sup>17</sup> M. N. Pflumm, J. E. Lehmann

Departments of Biochemistry and Neurology  
Columbia University, College of Physicians and Surgeons  
New York, New York 10032

Received June 23, 1965

### The Cotton Effect Associated with Certain Tyrosine Residues in Ribonuclease<sup>1</sup>

Sir:

Conformation-dependent Cotton effects associated with the aromatic absorption bands of tryptophan and tyrosine have recently been reported in several proteins<sup>2-4</sup> and in poly-L-tyrosine.<sup>5</sup> Little information is

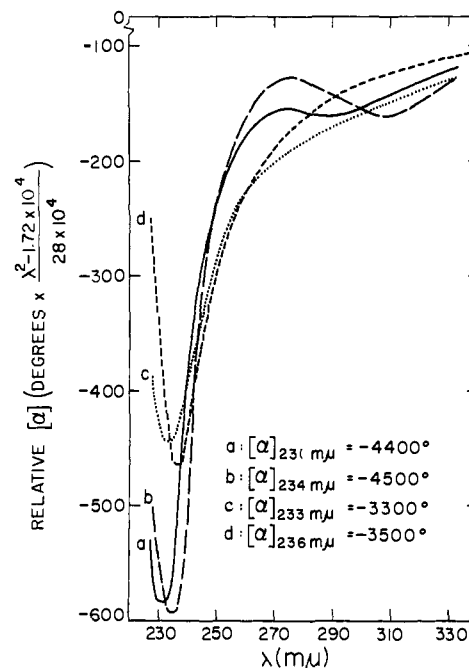


Figure 1. The ultraviolet optical rotatory dispersion of 0.48% pancreatic ribonuclease, in a 1-mm. cell, in (a) 0.15 M phosphate buffer at pH 6.2; (b) 0.15 M glycine-NaOH buffer at pH 11.5; (c) 0.1 N HCl; (d) 1.5% sodium dodecyl sulfate (recrystallized from isopropyl alcohol).

(1) This work was supported in part by Grant No. GM 11061, Career Award No. 1-K6-DE-1094-01 from the National Institutes of Health, U. S. Public Health Service, and Contract AT(04-1)GEN-12 between the U. S. Atomic Energy Commission and the University of California.

(2) N. S. Simmons and E. R. Blout, *Biophys. J.*, **1**, 55 (1960).

(3) D. V. Myers and J. T. Edsall, *Proc. Natl. Acad. Sci. U. S.*, **53**, 169 (1965).

(4) A. N. Glazer and N. S. Simmons, *J. Am. Chem. Soc.*, **87**, 2287 (1965).

(5) G. D. Fasman, E. Bodenheimer, and C. Lindblow, *Biochemistry*, **3**, 1665 (1964).