Circular Dichroism of Poly-L-tyrosine*

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Received April 1, 1964; revised August 14, 1964

Circular-dichroism measurements of solutions of poly-L-tyrosine in the helical conformation at *p*H 11.2 reveal three ellipticity bands at 270, 248, and 224 mμ. The band at 224 mμ, which is the n-π^* peptide transition characteristic of helical polypeptides, is negative in sign and indicates that the poly-L-tyrosine helix is right-handed. From the measured dichroism bands and an estimate of the contribution of an additional peptide transition at 190 mμ, an optical rotatory dispersion curve was computed and compared with the experimentally measured values in the wavelength interval 500–225 mμ. The agreement is sufficiently satisfactory to warrant the belief that the major transitions governing the optical rotation in the visible and near-ultraviolet spectrum have been considered. It is shown that the displacement of the negative trough of the rotation by several millimicrons from its usual position at 233 mμ in right-handed α-helical polypeptides is the result of an overlap of Cotton effects. The rotational strength, R_k, of the 224-mμ transition is only about one-third of the value for fully helical poly-α,L-glutamic acid, but it is likely that a side-chain-chromophore transition of opposite-sign rotational strength is partly responsible for the *apparently* low value of helix content. Measurements of the dichroism of a nonhelical form of poly-L-tyrosine at the same *p*H show a positive dichroism band at 225 mμ and another at about 245 mμ.

The difficulties which arise in the interpretation of the ORD curves of poly-L-tyrosine are due, in part, to the overlap of Cotton effects of several optically active absorption bands. Indeed, the sign and magnitude of the rotation due to the n-π^* peptide transition at 225 mμ which, in favorable circumstances, allow an assignment of helix sense and content (Simmons *et al.*, 1961; Blout *et al.*, 1962) cannot be gauged with certainty in poly-L-tyrosine solutions. As is made clear by Fasman and co-workers in the preceding paper (Fasman *et al.*, 1964), the very complex ORD behavior in the vicinity of 233 mμ is best interpreted on the basis of the presence of a right-handed α-helix, but other interpretations cannot be definitely excluded.

In this paper we present results of measurements of the circular dichroism associated with the absorption bands of poly-L-tyrosine. Circular dichroism possesses the inherent advantage of presenting discrete bands whose widths are comparable to the associated spectral bands (Moscowitz, 1960, 1961). Thus, while the limbs of a Cotton effect are of different sign on each side of the maximum of the absorption band and extend over hundreds of millimicrons, an ellipticity band has a single sign (which may be positive or negative) and a half-band width which may be no greater than 10 or 15 mμ.

Holzwarth *et al.* (1962) measured the ellipticity of the bands at 225 and 190 mμ associated with the peptide transitions of the poly-α,L-glutamic acid helix. Brahms and Spach (1963) showed that poly-L-aspartic acid has an ellipticity band of the same sign as poly-α,L-glutamic acid at 225 mμ, and is thus an α-helix of the same sense. In the present investigation we encounter, for the first time, optically active side-chain absorption bands at wavelengths longer than those of the prominent peptide transitions. It is the contention of the authors of the preceding paper (Fasman *et al.*, 1964) that the contribution of these absorption bands to the rotation in the visible causes the sign of b_o to be positive even though the helical sense would normally be associated with a negative b_o. Our analysis of the dichroism bands of poly-L-tyrosine supports this contention.

EXPERIMENTAL

Poly-L-tyrosine, sample GF 9-177-7, was prepared to yield a helical sample as described in the preceding paper (see experimental section). It is also pointed out in that paper that solutions of poly-L-tyrosine which are first brought to *p*H 12 or higher, then back-titrated to *p*H 11.2, appear to consist mainly of random-coil molecules as judged by ORD behavior, whereas poly-L-tyrosine, which is directly dissolved at *p*H 11.2 without exposure to higher *p*H, is a helical polymer. We have taken advantage of this to compare the circular dichroism of the two forms. In the text of the paper we refer to material which has been exposed to *p*H values higher than 12, then back-titrated, as random poly-L-tyrosine.

Circular dichroism measurements were performed on a Baird-Atomic/Jouan Dichrograph which was modified for 10-fold greater sensitivity. Focusing arrangements and source housing were modified as well. The instrument records, directly, the difference in absorbancy at any wavelength for left and right circularly polarized light. This difference is then converted to a difference in molecular extinction coefficient ($\epsilon_l - \epsilon_r$), and the molecular ellipticity obtained as (Moscowitz, 1961):

$$[\theta] = 2.303\left(\frac{4500}{\pi}\right)(\epsilon_l - \epsilon_r)$$

The units are deg cm²/decimole.

We have observed that to insure good spectral resolution the entrance-slit width, which is automatically regulated for constant energy at the phototube, should not exceed 1.5 mm. In practice this means maintaining optical densities below 1.5 even with maxi-

* This study was supported in part by research grants from the National Institutes of Health (GM 10576), the National Institute of Arthritis and Metabolic Diseases (A 5852), and the National Science Foundation (GB 1913 and GB 428).

† Research Career Development Awardee of the U. S. Public Health Service.

‡ This work was done during the tenure of an Established Investigatorship of the American Heart Association.

§ Contribution No. 316.

[1] Abbreviation used in this work: ORD, optical rotatory dispersion.

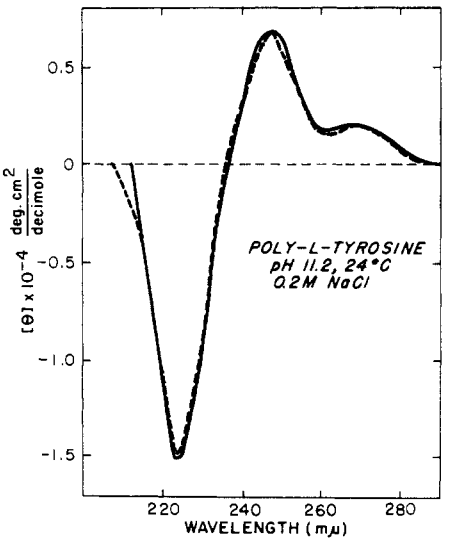

FIG. 1.—Circular dichroism of helical poly-L-tyrosine. The solid line is the experimentally measured curve. Measurements performed with 0.028 and 0.056% solutions in cells of 1.00 cm, 1.09 mm, and 0.167 mm path length. The dashed line is calculated from three Gaussian bands as described in the text.

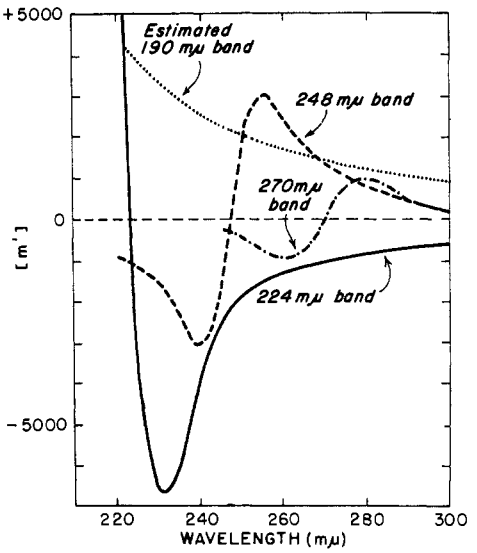

FIG. 2.—Calculated Cotton effects of helical poly-L-tyrosine. The three Cotton effects shown are calculated according to equations given in the text from the individual Gaussian ellipticity curves which are summed to give the dashed curve in Fig. 1. The estimated rotation for the 190-mμ band is explained in the text.

mum voltage across the phototube, and we have adhered to this requirement by proper dilution.

RESULTS

In Figure 1 is shown the circular dichroism curve of poly-L-tyrosine in the helical conformation in the wavelength interval 214–300 mμ. The curve comprises at least three dichroism bands and answers immediately one of the questions posed by the optical rotation data: The negative trough observed at 236–238 mμ is not the minimum of the negative limb of a Cotton effect at 248 mμ with a positive counterpart at 254 mμ (see Fig. 1 of preceding paper). In this region, there are two distinct Cotton effects, in addition to the one at 270 mμ. There is little doubt that the large negative ellipticity band observed at 224 mμ is the n-π^* peptide transition associated with the α-helical conformation of polypeptides.[2] In this case, the sign is the same as for poly-α,L-glutamic acid, which is a right-handed α-helix.

We have fitted the data with three Gaussian curves of the type

$$[\theta] = [\theta]^0_k e^{-(\lambda-\lambda^0_k)^2/\Delta^0_k{}^2} \qquad (1)$$

in which $[\theta]^0_k$ is the maximum ellipticity of the kth band occurring at λ^0_k mμ and Δ^0_k is the wavelength interval between $[\theta]^0_k$ and $(1/e)[\theta]^0_k$: $[\theta] = -1.51 \times 10^4 \exp\left(-\frac{(\lambda - 224)^2}{8^2}\right)$; $[\theta] = 6.70 \times 10^3 \exp\left(-\frac{(\lambda - 248)^2}{8^2}\right)$; $[\theta] = 2.00 \times 10^3 \exp\left(-\frac{(\lambda - 270)^2}{10^2}\right)$ for the 224, 248, and 270 mμ-bands, respectively. The composite of these three is indicated as a dashed curve in Figure 1, which is only occasionally noncoincident with the measured curve. From the individual Gaussian curves one can calculate rotational strengths, R_k, from equation (2) (Moscowitz, 1960). The rotational strengths thus

$$R_k \approx 0.696 \times 10^{-42}\sqrt{\pi}[\theta]^0_k \cdot \frac{\Delta^0_k}{\lambda^0_k} \qquad (2)$$

calculated from equation (2) are 0.654×10^{-39} cgs for the 224-mμ band; 0.266×10^{-39} cgs for the 248-mμ band, and 0.091×10^{-39} cgs for the 270-mμ band.

The band at 248 mμ clarifies another feature of the optical rotatory dispersion data. The maximum in the ORD curve at 254 mμ could not be identified with certainty as part of a positive Cotton effect. It is equally possible that the rotation is rapidly becoming positive in response to a band deeper in the ultraviolet and that a large negative n-π^* Cotton effect (due to the peptide transition) becomes dominant in the vicinity of its own minimum. This would then create a maximum where there is no positive Cotton effect. The significance of such an interpretation is that it implies a greater negative rotation near the trough at 236–238 mμ than is actually observed; and this, in turn, is important since the value of the negative rotation at the trough of this Cotton effect (233 mμ for several helical polypeptides) is directly related to the helix content of the polypeptide (Simmons *et al.*, 1961). The experimental dichroism curve with its discrete band at 248 mμ rules out this explanation for the failure of the observed rotation to exceed a value of about 50% of what is observed with fully helical poly-α,L-glutamic acid (Blout *et al.*, 1962). Furthermore, it follows from the presence of the ellipticity band that there must be an associated positive Cotton effect with an inflection point at 248 mμ and a negative limb at shorter wavelengths. Thus the negative rotation at 236–238 mμ is *in part* due to a band distinct from the n-π^* peptide transitions.

To further clarify the contribution of these partial rotations, we have plotted, in Figure 2, the partial rotations due to each of the three Gaussian ellipticity

[2] Holzwarth *et al.* (1964) have recently questioned whether the observed rotations and ellipticities in the vicinity of 233 mμ are entirely due to an n-π^* peptide transition or partly due to a π-π^* transition. The latter is one of a pair due to exciton-band splitting in the helix. The other of this pair gives rise to a large positive Cotton effect centered at about 190 mμ (Blout *et al.*, 1962) and theory predicts that a longer-wavelength negative Cotton effect should be observed (Tinoco *et al.*, 1963; Holzwarth *et al.*, 1964). The partial contributions due to one of the π-π^* transitions and to the n-π^* transition are not presently separable, but their sum is directly related to helix content and for the present empirical application they may be treated as one band, to which we refer in the text as n-π^*.

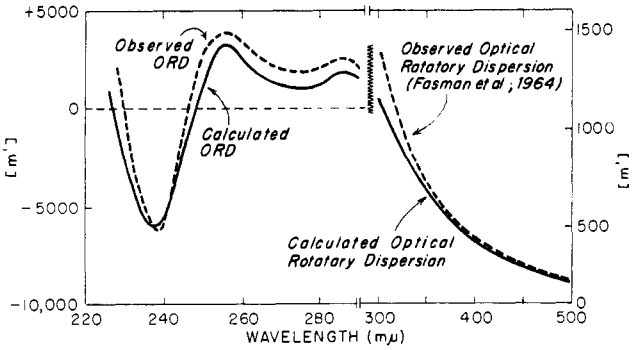

FIG. 3.—Comparison of experimental and calculated ORD curves for helical poly-L-tyrosine at *p*H 11.2 in 0.2 M NaCl. [*m'*] is the residue rotation in deg cm²/decimole.

bands. In order to calculate partial rotations associated with the observed dichroism, we make use of the Kronig-Kramers transforms relating ellipticity to rotation. The equations which follow have been extensively used by Moscowitz (1960, 1961).

$$m' = \frac{3}{n^2 + 2} \left\{ \frac{R_k}{0.696 \times 10^{-42}} \cdot \frac{\lambda^0_k}{\Delta^0_k} \cdot 2/\pi \left[e^{-x^2} \int_0^x e^{t^2} dt - \frac{\Delta^0_k}{2(\lambda + \lambda^0_k)} \right] \right\} \quad (3)$$

$$x = \frac{\lambda - \lambda^0_k}{\Delta^0_k}$$

In regions far from the band center equation (3) reduces to

$$m' = \frac{3}{n^2 + 2} \cdot \frac{R_k}{0.696 \times 10^{-42}} \cdot \frac{2}{\pi} \cdot \frac{(\lambda^0_k)^2}{\lambda^2 - (\lambda^0_k)^2} \quad (4)$$

The curves shown in Figure 2 are calculated from equation (3).[3]

The figure reveals that the 248-mμ band gives rise to a considerable negative rotation in the same spectral region where the peptide transition is contributing a partial negative rotation. It is also seen that the position of the minimum at 236–238 mμ in the experimental ORD curve *is not a reflection of a shift in the n-π* absorption maximum to a longer wavelength* but is the result of overlap of Cotton effects.

Before attempting to sum the partial-rotatory-dispersion curves in order to compare this with the experimentally obtained ORD curve, it is necessary to estimate the large positive contribution which arises from the peptide transition at 190 mμ. It has been shown that in helical polypeptides a very large positive Cotton effect centered at about 190 mμ is present (Blout *et al.*, 1962; Holzwarth *et al.*, 1962; Yang and Samejima, 1963). This Cotton effect is presumed to be partly responsible for the anomalous rotatory dispersion observed with helical polypeptides (Moffit, 1956; Schellman and Oriel, 1962). The magnitude of the maximum which occurs at 200 mμ has not yet been absolutely established but the residue rotation for 100% helix is somewhere between 60,000 and 80,000 degrees (Blout *et al.*, 1962; Holzwarth *et al.*, 1962; Beychok, 1964). We have measured, for poly-L-tyrosine, a residue rotation of about +40,000° at 204 mμ. Without considering for the moment whether this value results from less than 100% helix or represents a diminution owing to opposite-sign contributions from side-chain chromophores, we have estimated the contribution of the 190-mμ peptide transition by assigning

[3] Tables of e^{-x^2} and $\int_0^x e^{t^2}\,dt$ may be found in standard texts on statistics and probability.

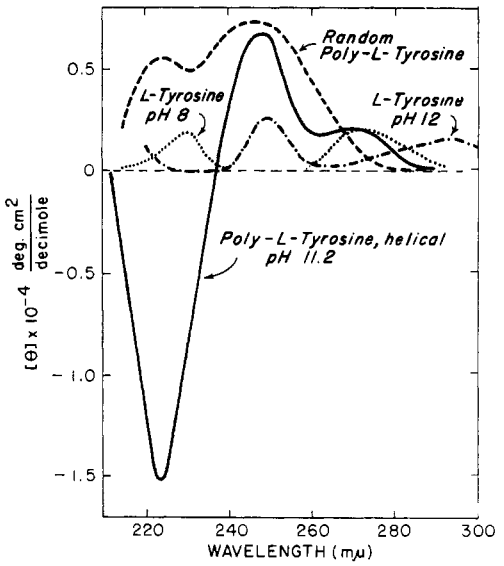

FIG. 4.—Circular dichroism of helical poly-L-tyrosine, random poly-L-tyrosine (both at *p*H 11.2), and L-tyrosine at the *p*H values indicated.

to it a rotational strength two-thirds of the published value for fully helical poly-α,L-glutamic acid (Holzwarth *et al.*, 1962). With this, and using equations (3) and (4), we can estimate its partial rotation at the wavelengths of interest. Summing this partial rotation with the three curves in Figure 2 gives the solid line in Figure 3 which is to be compared to the measured rotations taken from the preceding paper of Fasman *et al.* (1964). The agreement is quite satisfactory in the visible, less so in the immediate vicinity of the Cotton effects. However, the shape of the calculated curve follows the experimental curve well enough to support a belief that the major transitions dominating the ORD curve from 215 to 500 mμ are those which we have considered.

It was pointed out in the experimental section that it is possible to prepare poly-L-tyrosine of completely different rotatory properties than that which we have been discussing by exposing polytyrosine solutions to *p*H 12 or higher, then back-titrating quickly at room temperature (see preceding paper) to *p*H 11.2. The circular dichroism curve of such a solution is shown in Figure 4 along with the dichroism curves of the free amino acid, L-tyrosine, at *p*H 8 and 12. For the random polymer the negative ellipticity band at 225 mμ is completely missing as in the small positive band at 270 mμ. Instead two overlapping positive bands centered at approximately 225 and 245 mμ are observed. We believe these to be side-chain tyrosine bands. They have their counterparts in the free amino acids, although these are less intense. The amino acid bands at 276 and 294 mμ due to un-ionized and ionized (phenolic hydroxyl) L-tyrosine are absent in the random polymer. We have no explanation for this. Whereas the band at 225 mμ may be assigned to a transition in the un-ionized residue, that near 245 mμ cannot be due solely to an ionized residue transition because a Cotton effect which corresponds to a transition at 245 mμ occurs in poly-L-tyrosine films of the free acid. It occurs also in the helical polymer at *p*H 11.2, at which *p*H the fraction of ionized residues is small according to the spectra presented in the preceding paper. At *p*H 12.2 the polymer is random and highly ionized, but the Cotton effect near 248 mμ persists with only slightly diminished intensity. Thus any ionized residues make a contribution to the dichroism band at 248 mμ and the size of

this contribution (as well as the absorption intensity) may depend on the conformation of the polymer.

In view of the findings with the random poly-L-tyrosine, it seems reasonable that in the helical polymer a side-chain chromophore band at 225 mμ of positive sign is present but obscured by the larger n-π^* peptide transition at 225 mμ. (The other band at about 240 mμ in the random polymer is what we believe to be the 248-mμ band actually observed in the helical polymer.) Since the sign of the side-chain-chromophore band is opposite to the n-π^* band, we may infer that the latter is actually larger than observed. We may not, however, simply subtract the ellipticity found in the random polymer at 225 mμ from the negative band observed in the helical polymer, because in the helix the side chains are helically arrayed (as pointed out by Fasman *et al.*, 1964) with ensuing exciton-band splitting and alteration of magnitude and position of the absorption bands. A quantitative calculation of the magnitude of the n-π^* ellipticity band in helical poly-L-tyrosine thus is not possible.

Discussion

It is hardly surprising that a molecule with an absorption spectrum as complicated as that of poly-L-tyrosine exhibits highly complex ORD and circular-dichroism behavior. Among the puzzling features are the absence in the random polymer of dichroism bands corresponding to the high-wavelength amino acid bands and the absence of a Cotton effect at 294 mμ for the random-ionized polymer, as noted in the preceding paper. Added to these is our inability to decide from the dichroism data whether or not the helical polymer at *p*H 11.2 is *fully* helical. The rotational strength of the n-π^* transition in poly-L-tyrosine is only about one-third of the value for fully helical poly-α,L-glutamic acid but, as noted above, it is entirely likely that an opposite-sign side-chain-chromophore band is effectively reducing a larger rotational strength. Computations on the basis of 100% helix can be made which are compatible with the experimentally observed dichroism band, but these are *ad hoc* and unconvincing.

Finally, we may point out that an apparent paradox in the ORD paper, connected with the decreased value of the rotation at 238 mμ in water compared to 0.2 M salt, may be owing to a diminution of that contribution to the 248-mμ dichroism band which is due to the ionized tyrosine with a comparable increase in the (expected) opposite-sign 225-mμ un-ionized tyrosine, and it may not be necessary to invoke any large change in the n-π^* transition.

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A Deoxyribonuclease Reaction Requiring Nucleoside Di- or Triphosphates*

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Received August 2, 1963; revised July 23, 1964

An extract of *Micrococcus lysodeikticus* will split DNA in the presence of Mg^{2+} and a single nucleoside di- or triphosphate. The reaction requires native DNA. DNA is degraded from the ends of the molecule, since acid-soluble material is released before either the viscosity or the transforming activity of the DNA is appreciably reduced, and since compounds sensitive to *Escherichia coli* alkaline phosphatase and presumed to be mononucleotides are produced from the start of the reaction. The specific activity of the extract has been increased 25-fold by ammonium sulfate fractionation and DEAE-cellulose chromatography. This partially fractionated extract shows an absolute dependence on nucleoside triphosphate for the degradation of DNA; other chelating agents will not substitute. The amount of DNA degraded to acid-soluble material is strictly determined by the amount of nucleoside triphosphate in the reaction mixture.

We have recently been studying an enzyme prepared from *Micrococcus lysodeikticus* which preferentially inactivates transforming DNA that has been treated with either ultraviolet light or with monofunctional alkylating agents (Strauss, 1962; Strauss and Wahl, 1964). In the course of these experiments we noticed that a mixture of nucleoside triphosphates added to ^{32}P-labeled DNA promoted the liberation of acid-soluble radioactivity from the DNA upon addition of the *M. lysodeikticus* extract. Further investigation disclosed that the release of acid-soluble material from DNA was stimulated by the addition of a single nucleoside di- or triphosphate. We have fractionated the *M.*

* Supported in part by grants from the National Science Foundation and from the National Institutes of Health.

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‡ Public Health Service research career development program award (GM-K3-823) from the National Institute of General Medical Sciences.