[2] Fraenkel-Conrat, H., *Trans. Faraday Soc.*, **55**, 494 (1959).
[3] Hausen, P., and S. W. Schafer, *Z. Naturforsch.*, **17b**, 15 (1962).
[4] Schaffer, F. L., in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 27 (1962), p. 89.
[5] Fraenkel-Conrat, H., and B. Singer, in *Proc. Intern. Congr. Biochem., 5th, Moscow, 1961* (1962).
[6] Montagnier, L., and F. K. Sanders, *Nature*, **199**, 664 (1963).
[7] Baltimore, D., *Science*, **143**, 1034 (1964).
[8] Weissmann, C., P. Borst, R. H. Burdon, M. A. Billeter, and S. Ochoa, these PROCEEDINGS, **51**, 682 (1964).
[9] Ralph, R. K., R. E. F. Mathews, A. I. Matus, and H. G. Mandel, *J. Mol. Biol.*, **11**, 202 (1965).
[10] Kaerner, H. C., and H. Hoffman-Berling, *Nature*, **202**, 1012 (1964).
[11] Shipp, W., and R. Haselkorn, these PROCEEDINGS, **52**, 401 (1964).
[12] Dulbecco, R., and M. Vogt, *J. Exptl. Med.*, **99**, 193 (1964).
[13] Lockart, R. Z., Jr., *J. Bacteriol.*, **85**, 556 (1963).
[14] Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).
[15] Scherrer, K., and J. E. Darnell, *Biochem. Biophys. Res. Commun.*, **7**, 486 (1962).
[16] Lockart, R. Z., Jr., and T. Sreevalsan, in *Viruses, Nucleic Acids, and Cancer*, 17th Annual Symposium on Fundamental Cancer Research at the University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas (Baltimore; Williams and Wilkins, 1963), pp. 447–461.
[17] Sueoka, N., and T. Y. Cheng, *J. Mol. Biol.*, **4**, 161 (1962).
[18] Reich, E., and R. M. Franklin, these PROCEEDINGS, **47**, 1212 (1961).
[19] Franklin, R. M., and D. Baltimore, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 27 (1962), p. 175.
[20] Shatkin, A. J., *Biochim. Biophys. Acta*, **61**, 310 (1962).
[21] Lockart, R. Z., *Biochem. Biophys. Res. Commun.*, **15**, 513 (1964).
[22] Tobey, R. A., *Virology*, **23**, 10 (1964).
[23] Ochoa, S., in *Proc. Robert A. Welch Found. Conf. Chem. Res., 8th* (1964), p. 9.

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*THE OPTICAL ROTATORY PROPERTIES OF THE β-CONFIGURATION IN POLYPEPTIDES AND PROTEINS**

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While the rotatory properties of the α-helical and disordered (or randomly coiled) forms of the polypeptide chain are now rather well characterized and understood both in the ultraviolet and the visible spectral regions,[1–4] the next most frequently occurring form, the β-configuration, has been much more elusive. Moreover, the number of proteins whose optical behavior cannot be explained in terms of particular proportions of α-helical and disordered residues grows,[5, 6] and the possibility that these contain significant amounts of the β-configuration deserves careful evaluation. The recent X-ray structure determination of lysozyme[7] reveals the presence of all three configurations, and hence the need to deal with the rotatory characterization of the β-form is no longer academic.

Actually, attempts to define the optical properties of polypeptides in the β-form span a decade,[8–12] but they have been severely limited by being carried out only in organic solvents. This was necessitated by solubility considerations, but it re-

moved from view the behavior in the far ultraviolet, and in the visible it provided information of uncertain relevance to aqueous solutions.

The starting point of the work reported here has been the earlier observation[13] that poly-L-lysine in alkaline solution is converted from the α-helical form to the β-configuration by heating. It was first necessary to ensure that the conversion was complete and then to determine whether the new form consists of single molecules or aggregates before undertaking a complete rotatory and circular dichroic study. In addition, use was made of infrared spectra to help identify the β-form, a copolymer of lysine and tyrosine was examined, and the rather unusual properties of the β-configuration formed in neutral solution with sodium dodecyl sulfate were explored. Finally, it is shown that the optical rotatory dispersion (ORD) and the circular dichroism (CD) of several proteins that cannot be fitted by a combination of helical and disordered form characteristics can indeed be fitted by a combination of the contributions made by the β and the disordered forms.

Methods.—Materials: Poly-L-lysine hydrobromide was exhaustively dialyzed against 0.01 *N* HCl, then glass-distilled water, and finally lyophilized in order to remove the highly absorbing bromide ions. Most observations were made on a sample of 58,000 mol wt. An L-tyrosine containing copolymer $L_{96}T_4$, of 450,000 mol wt, prepared by Dr. T. Gill, was also used. Concentrations were determined by Kjeldahl nitrogen analyses. A portion of the 58,000 mol wt sample was tritiated to give a specific activity of 1 mc/mg. The absence of racemization was checked by trypsin digestion and paper chromatography. α_1-Acid glycoprotein was kindly supplied by Dr. K. Schmid. Its concentration was determined on a weight basis assuming 10% water content.[5] γ-Globulin from rabbit serum was kindly provided by Dr. T. Gill. Its concentration was based on $E_{280}^{1\ gm/dl} = 13.8$.

Preparation of the β-form in solution: The concentration of poly-L-lysine HCl had to be below that which yielded aggregation upon heating in alkaline solution and yet high enough to permit optical measurements. A concentration of 0.01 gm/dl (0.6 mM) fulfilled these conditions. The pH was adjusted to 11 by adding 1 *M* NaOH (several μl from a micrometer syringe) with stirring. This was then heated in a sealed container, often the optical cuvette, at 50°C for 10 min and cooled to 25° for measurements. Heating at higher temperatures or for longer times led to aggregation. For the copolymer the concentration had to be reduced 2–4 times further in order to avoid aggregation.

Infrared spectra: A Perkim-Elmer double beam spectrophotometer, model 21, was used. For solution work, samples were first dissolved in D_2O, lyophilized, dissolved again, diluted to the desired concentration, and brought to pD 12 with 1 *M* NaOD.[14] A concentration of 0.1 gm/dl (6 mM) proved to be a suitable compromise between moderate aggregation and path length, the latter being 0.2 mm. At this concentration, scattering by the light opalescence was negligible.

Sedimentation rates in sucrose gradient: Sucrose gradients of 5–20% were established in polypropylene tubes of 4-ml capacity. A 0.2-ml sample of a 0.01 gm/dl solution containing tritiated poly-L-lysine was layered on top. The tubes were then spun at 39,700 rpm for 50 hr at 20°. About 20 fractions of 16 drops each were then collected in vials containing Bray's scintillation fluid and counted.

Optical rotatory dispersion: A Cary recording spectropolarimeter, model 60, was used throughout. A 2-mm path length cell was employed in the far ultraviolet and a 10-cm cell in the visible. A 0.1-mm cell was used for the concentrated solutions required for the infrared spectra. When possible, solutions were measured in both the 2-mm and 10-cm cells. The mean residue rotations, $[m']$, have been corrected for the refractive index of water.

Circular dichroism: These measurements were made on an instrument described by Holzwarth[15] employing an electrooptic plate in a modified Beckman DK-2 spectrophotometer. Instrument performance was checked by reproducing reported circular dichroic spectra for α-helical poly-L-lysine[2] and camphor.[16]

Ultraviolet spectra: A Beckman DK-2 spectrophotometer was employed with 2-mm quartz cells. The additional precautions of Rosenheck and Doty were observed.[13]

Results.—Properties of β-form poly-L-lysine: In order to establish the ORD of the β-conformation in aqueous solution, it is essential that the sample under examination be free of other conformations. Two lines of argument demonstrate that the conversion achieved in this work is practically complete. In the one case the far ultraviolet spectra were found to be of the same nature as those already reported:[13] none of the splitting apparent in the α-helix spectra could be seen in that of the β-form. Similarly, when the ORD and CD were determined, none of the characteristic features of the α-helical or disordered conformations could be found in these.

The other evidence came from infrared spectra in D_2O, shown in Figure 1. The low absorbances are due to the dilution at which it was necessary to work (see *Methods*). It is clear that the β-conformation displays a distinctive band at 1610 cm^{-1} uncontaminated by the amide I band which occurs at 1638 cm^{-1} for the disordered form and at 1628 cm^{-1} for the α-helical form. Since the minimum concentration at which these could be observed was ten times higher than that used for the other optical studies, it was necessary to establish that the ORD in the more concentrated solution remained essentially unchanged. Measurements of somewhat diminished precision showed that the location of the trough and peak remained the same (230 and 205 mμ) as those observed in dilute solution (see Fig. 3). However, the magnitudes were diminished by 20 and 40 per cent, respectively, presumably as a result of depolarization due to scattering.

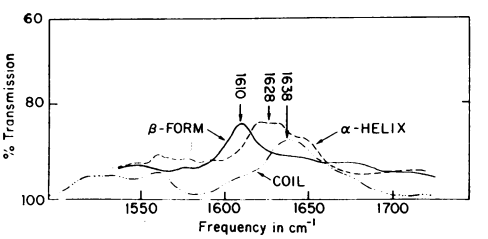

FIG. 1.—Infrared spectra of poly-L-lysine in D_2O: conc., ~6 mM; path length, 0.2 mm.

With the conformation established, the next step was to determine the molecular state of the β-form. Specifically, is the β-form a consequence of intra- or intermolecular hydrogen bonding? If it is the former, the sedimentaion constant should be much like that of the same sample in the other two forms. If it is the latter, the sedimentation should be much faster and reveal a wide distribution of sizes. To test this point, sedimentation had to be done at the working concentration of 0.01 gm/dl: this required the use of sucrose density gradient centrifugation with radioactive labeling. The sample was mixed with a tritiated sample of the same molecular weight in the proportion of 40:1, the three forms were produced, and the results obtained are shown in Figure 2. Assuming equal partial specific volumes, the relative sedimentation rates are found to be 1.0:0.7:0.5 for the coil, helix, and β-forms, respectively. Thus, the former case is clearly realized. This implies that the β-form results from an accordion-like folding of single chains, resulting in an antiparallel arrangement of chain segments all in the same plane.

The ORD of the three forms of poly-L-lysine: The mean residue rotations in the ultraviolet for the three conformations are plotted in Figure 3. In a very rough way the ORD for the β-form is seen to lie between that for the other two forms. Closer inspection shows, however, that it is distinctly different. The two dashed curves represent the ORD for mixtures of helix and coil rotations in 90:10 and 50:50 proportions. This illustrates that mixtures of these two forms cannot mimic the ORD for the β-form.

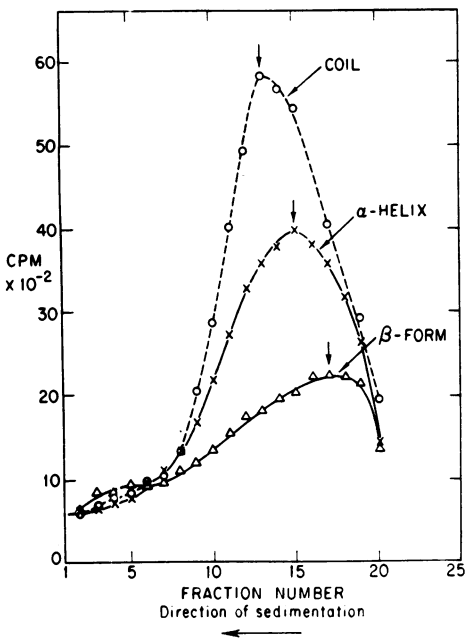

FIG. 2.—Sedimentation of the three forms of poly-L-lysine in sucrose (5–20%) gradient: speed, 39,700 rpm, temp., 20°; time, 50 hr; coil, pH 7 in 0.1 *M* sodium chloride; α-helix, pH 11 in 0.01 *M* phosphate plus 0.1 *M* sodium chloride; β-form, same as α-helix but heated at 50° for 10 min.

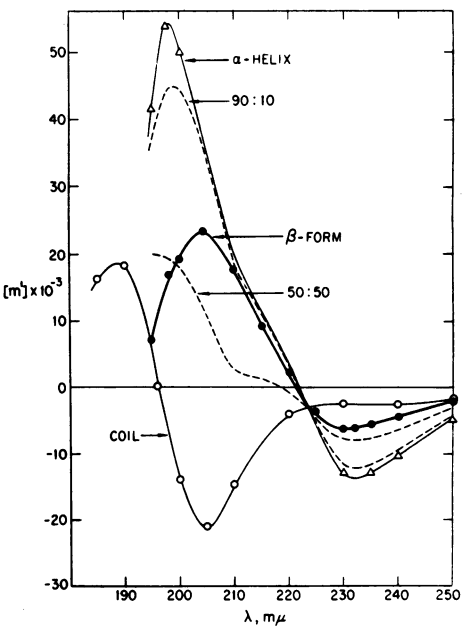

FIG. 3.—The ORD of poly-L-lysine in three different conformations in water: conc., 0.6 mM, path length, 2 mm; coil, pH 7; α-helix, pH 11; β-form, pH 11 after heating at 50° for 10 min. The two dashed lines represent calculated values for mixtures of 90% helix and 10% coil and 50% helix and 50% coil.

The unique characteristics of the β-form are the peak and trough at 205 and 230 mμ, respectively (as opposed to 198 and 232 mμ for the α-helix), the lower magnitude of the peak and trough (about half that of the α-helix), and the absence of the shoulder near 210 mμ which is so evident in the ORD of the α-helix.

The ORD data in the near-ultraviolet and the visible is very well fitted with the Moffitt equation. The values of the characteristic parameters of these plots and the main features of the far-ultraviolet region are summarized in Table 1 together with results obtained on a second sample, the L-tyrosine copolymer, $L_{96}T_4$, which absorbs significantly in the ultraviolet region. The values for poly-L-lysine are the average of four sets of measurements: it is seen that the β-form in alkaline solution displays a b_0 value of -152 and a λ_c value of 241 mμ.

The Moffitt parameters were evaluated by means of the statistical treatment of Sogami *et al.*[17] using a 1620 IBM computer. For the α-helical form the best values of λ_0 were 212 ± 4 mμ for the region above 290 mμ. For the same range in the case of the β form, the best value was about 240 mμ, a value at which b_0 is nearly zero. Thus, the ORD of the β-form can be fairly well fitted by a one-term equation, unlike the ORD of the α-helical form.

It is interesting to note that the Moffitt plots for the helix and β-form cross. As a result, the ORD for the β-form cannot be represented by a linear combination of the ORD of the other two forms. Hence, it has its own characteristic dispersion at higher wavelengths as well as in the absorption region.

The ORD of the β-form induced in neutral solution by sodium dodecyl sulfate: Sodium dodecyl sulfate evidently shifts the conformational stability of various proteins in different ways. In numerous instances it serves as a denaturing agent.[18] But recently there have been indications that it eliminates the β-form but not the α-helix in some proteins,[19] while in others it converts the random coil form to α-helix.[20] Our own experience has added another effect: at 0.06 *M* sodium dodecyl sulfate converts the disordered form of poly-L-lysine in neutral solution to an ordered form that seems to be the β-conformation. The ORD is considerably different from that shown above, but approaches that reported for the β-form in organic solvents. Thus the behavior with this detergent may provide a bridge between the two sets of results.

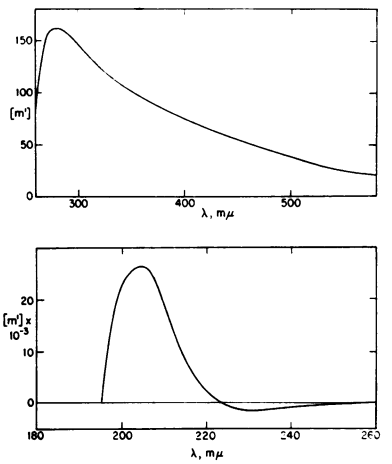

FIG. 4.—The ORD of poly-L-lysine in 0.06 *M* sodium dodecyl sulfate: conc., 4.59 × 10^{-4} *M*; path length, 10 cm for visible and 2 mm for the ultraviolet region.

The ORD in 0.06 *M* sodium dodecyl sulfate is shown in Figure 4. In the ultraviolet region the peak and trough occur at the same wavelengths as reported for the β-form, 205 and 230 mμ respectively, but the magnitude of the trough is greatly reduced. In the visible region the ORD falls continuously but remains positive.

This behavior is essentially unaffected by the concentration of sodium dodecyl sulfate (SDS) in the range of 0.06 *M* down to 0.005 *M* where precipitation occurs. Because of this the transition induced by the detergent could not be followed. Addition of SDS to the β-form produced in alkali gives essentially the same ORD profile as observed in SDS at neutral pH.

TABLE 1

COMPARISON OF THE IMPORTANT PARAMETERS OF ORD OF POLY-L-LYSINE AND A COPOLYMER OF LYSINE AND TYROSINE IN THREE DIFFERENT CONFORMATIONS

		UV Region				Visible Region		
		Trough (t), mμ	(m_t' × 10^{-3})	Peak (p), mμ	(m_p' × 10^{-3})	a_0*	b_0*	λ_c
Poly-L-lysine	Random coil (pH 7)	205	−21.5	190	18.5	−929	24	209
	α-Helix (pH 11)	232	−14.5	198	55.5	−65	−580	286
	β-Form, pH 11; heated 50° 10′	230	−6.3	204–205	23	−343	−147	241
Copoly-L-lysine L-tyrosine (96:4)	Random coil (pH 7)	205	−20.3	190	16.5	−883	44	206
	α-Helix (pH 11)	232	−14	198	59.5	27	−648	284
	β-Form, pH 11; heated 50° 10′	230	−6.5	205	21.5	−341	−156	241

* Calculated using λ_0 = 212 mμ.

Further evidence that the molecular conformation in sodium dodecyl sulfate solution is the β-form was obtained from infrared spectra, ultraviolet spectra, and circular dichroic spectra. The infrared spectra of poly-L-lysine at pH 7 shows the amide I band at 1638 cm^{-1} but in 0.03 *M* sodium dodecyl sulfate the band appears at

1610 cm^{-1}, the location characteristic of the β-form (see Fig. 1). The ultraviolet spectra of both poly-L-lysine and the L-tyrosine copolymer at pH 7 showed the peak to be at 192 mμ with molar extinction coefficients of 7870 and 8500, respectively, while in 0.01 M detergent the peak was shifted to 194 mμ and the extinction coefficients to 9870 and 9600, respectively. The corresponding values for the β-form prepared by heating alkaline solutions were 8800 and 8900 with the peak at 194 mμ. If the additional hyperchromicity in sodium dodecyl sulfate is viewed as a solvent effect in the presence of the detergent, all of these observations are consistent with the form in detergent being the β-conformation.

Circular dichroism: The circular dichroic spectra down to 200 mμ are shown in Figure 5 for the α-helical, and the β-form derived from heating the alkaline solution. In contrast to the two negative bands at 208 and 222 mμ for the α-helical form, the β-form displays a single well-defined minimum at 218 mμ. The dichroism becomes positive below 200 mμ and probably peaks near 195 mμ: however, the reliability was not sufficient to justify presentation. The CD for the β-form in sodium dodecyl sulfate had a shape very similar to that of the β-form shown in Figure 5, with a trough at 218 mμ, but with only about half the magnitude. The molar ellipticity at 218 mμ for the β-form in alkali was about 23,000 degree cm^2/decimole, while that in the detergent was 9,000. This is in contrast to values of 32,000 and 35,000 for the two bands of the α-helical form. These results are compatible with the observed

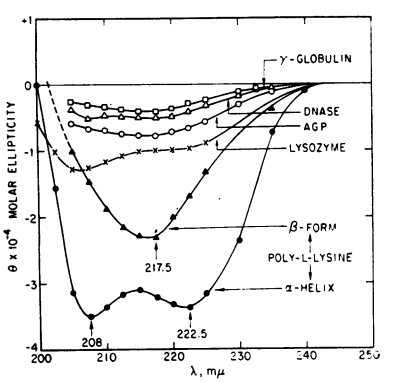

FIG. 5.—Circular dichroism of poly-L-lysine and some proteins. Poly-L-lysine (α-helix at pH 11 and β-form at pH 11 after heating at 50° for 10 min): conc., $1.24 \times 10^{-3} M$; path length, 2 mm; solvent, water. Lysozyme in 0.1 M potassium dihydrogen phosphate at pH 4.5: conc., $2.7 \times 10^{-3} M$, path length, 1 mm. α_1-Acid glycoprotein in water: conc., $2.174 \times 10^{-3} M$, path length, 2 mm. DNase in water: conc., $4.5 \times 10^{-3} M$; path length, 1 mm. γ-Globulin in 0.01 M phosphate buffer plus 0.1 M sodium chloride: conc., $2.23 \times 10^{-3} M$, path length, 2 mm. The signal-to-noise ratio for poly-L-lysine was about 8:1 and that for the proteins was about 4:1; the possible error in the value of $[\theta]$ in the latter may be as large as ±20%. An average residue weight of 115 was used in deriving the molar concentrations of proteins.

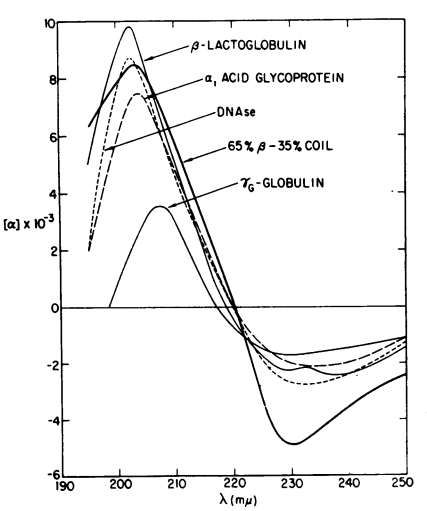

FIG. 6.—The ORD of γ-globulin, DNase, α_1-acid glycoprotein, and β-lactoglobulin in the far-ultraviolet region. γ-Globulin: conc., $1.9 \times 10^{-3} M$; path length, 1 mm. α_1-Acid glycoprotein: conc., $4.35 \times 10^{-4} M$; path length, 2 mm. DNase: conc., $4.37 \times 10^{-3} M$; path length, 0.13 mm. Solvents are same as in CD.

ORD which show the absence of any shoulder near 208 mμ for the β-form. This is a sensitive test for the absence of the helical form in both of the β-samples since even small amounts of the helical form display this band.[21]

Circular dichroism and optical rotatory dispersion of some proteins: The CD of four proteins are also shown in Figure 5. The three other than lysozyme were chosen because they were known to have very little α-helical content and were candidates for proteins having some β-structure.[5, 6] Keeping in mind that the CD of the disordered form is slightly positive from 225 to 210 mμ and then turns sharply negative, we see that the CD behavior of these three proteins is consistent with roughly 25–50 per cent β-form combined with disordered conformations for the remainder. The absence of the two-band feature would seem to eliminate the helical form.

Lysozyme, by contrast, shows the two-band pattern characteristic of the α-helix. The results shown are consistent with the X-ray structure assignment of about 10 per cent β, 35 per cent helix, and 55 per cent disordered. Since the helical regions are not all of the α-conformation,[7] a precise comparison seems unjustified.

The ORD in the far ultraviolet of the three proteins just discussed and β-lactoglobulin taken from a recently published report[22] are shown in Figure 6. They share the common features of a peak near 205 mμ and a trough near 230 mμ, features that are consistent with the β-form but not with the α-helix or coil alone or in any combination. It was therefore tempting to see if this behavior could be reproduced by a combination of an optimal proportion of β-form and disordered-form ORD. The result for a mixture of 65 per cent β and 35 per cent disordered form is shown as a heavier line. The agreement is reasonably good although the trough is deeper than that of the proteins and no adjustment is possible using the β-form data from the heated alkaline solution. However, the detergent form, with its much shallower trough, would permit a good fit in this region.

Discussion.—The most general conclusion to be drawn from this work is that the ORD and CD of the β-conformation are distinctly different from those of the α-helical and disordered conformations. As a consequence the β-conformation may be recognized when it occurs in significant amounts in globular proteins. Whereas the ultraviolet spectra of the disordered and the β-forms are not enough different to be of much analytical value even though that of the α-helical form is very different from the other two, each of the three forms gives a distinctive pattern in ORD and CD. The combined use of all three kinds of spectra in the ultraviolet together with the conventional use of ORD in the visible and near-ultraviolet range should provide an approximate breakdown of the conformational constitution of proteins and allow various transitions to be followed with considerable precision.

However, two problems—important in their own right—must be dealt with if such analyses are to be made with confidence. One of these is the rationalization of the quite different rotatory properties observed for the β-form in different media. The variations may be due to solvent effects; or two different β-forms, the parallel and the antiparallel arrangements, may be involved, each of which may have distinctly different rotatory properties.

The published ORD in the near-ultraviolet and visible[8–12] range all show positive values for a_0 and approximately zero values for b_0. Thus they do not differ significantly from our results in sodium dodecyl sulfate. In the far-ultraviolet the differ-

ence between this and the β-form in alkaline solution is concentrated in the band which has its minimum at 230 mμ in ORD and 218 mμ in CD. This is surely the n-π^* transition, and its contribution to the ORD at higher wavelengths is sufficient to produce the difference in a_0 and b_0 observed, if it is subject to solvent perturbation. This seems to be a reasonable explanation. The peptide bonds in the molecular configuration indicated for the β-form are much more exposed than in the α helix and because of their uniform orientation are particularly subject to identical interaction with solvent or detergent molecules. Moreover, the alternative explanation, that the molecular configuration is quite different in alkaline and detergent solution, seems to be ruled out by the experiment in which the ORD of poly-L-lysine in alkaline solution was found to shift to that characteristic of sodium dodecyl sulfate when the latter was added. This result is much more consistent with the detergent molecule solvating the pre-existing β-form than with an extensive intramolecular rearrangement that would convert an antiparallel to a parallel chain arrangement. Thus we tentatively conclude that the antiparallel β-form is present in both the alkaline and detergent solutions, that the n-π^* transition is sensitive to solvent and thereby exhibits behavior of one kind in water and another in organic solvents or with detergent, and that the π-π^* transitions are essentially unaffected by these different environments.

The shallow trough at 230 mμ found for the proteins is much more consistent with the trough found for the β-form in detergent solution. This is compatible with the above view that the environment of the regions of β-conformation of the proteins is probably closer to that of organic molecules than to water.

The other problem requiring attention is the theoretical analysis of the rotatory properties of the parallel and antiparallel forms of the β-configuration. This will be dealt with in a forthcoming paper.

One other observation with respect to the far-ultraviolet rotatory properties of the proteins seems justified. This is that the four proteins examined do have ORD and CD characteristics very close to that observed for what we believe to be the antiparallel β-form. If the rotatory properties of the parallel form are distinctly different, then it appears that the antiparallel form is of much more frequent occurrence, much as the right-handed α-helix is found almost to the exclusion of the left handed alternative.[23]

Near the end of this work we learned of the investigations of Iizuka and Yang[24] on the β-form of silk fibroin. The ORD and the CD are similar to that found here, but their data extend to lower wavelengths.

Summary.—Poly-L-lysine and an L-tyrosine copolymer have been converted to β-form by heating in alkaline solutions. Aggregation is suppressible, and the product appears to consist of single molecules in planar, folded configurations producing the antiparallel chain arrangments. This form has a trough at 230 mμ ($m' = -6400$), a peak at 205 mμ ($m' = +22{,}000$) in the ORD, and a trough at 218 mμ ($\theta = -23{,}000$) in the CD. A similar configuration is produced at neutral pH with the addition of sodium dodecyl sulfate. Here the trough is more shallow, presumably because of solvent perturbation on the n-π^* transition. Outside the absorption band the ORD is characterized by $a_0 = -340$, $b_0 = -152$ for the alkaline solution form, and by $a_0 = 174$ and $b_0 = -9$ for the form in detergent solution. Several proteins are shown to have ORD and CD spectra very similar to that expected

for certain proportions of β-form and disordered coil. Here the fit in the 230 region is better with the constants of the sodium dodecyl sulfate-produced β-form.

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[1] For a review, see Urnes, P., and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961).

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

[3] Yang, J. T., these PROCEEDINGS, **53**, 438 (1965).

[4] Carver, J. P., E. Shechter, and E. R. Blout, *J. Am. Chem. Soc.*, in press.

[5] Jirgensons, B., *J. Biol. Chem.*, **240**, 1064 (1965).

[6] *Ibid.*, **241**, 147 (1966).

[7] Phillips, D. C., lecture delivered at the 150th National Meeting of the American Chemical Society in Atlantic City, September 12–16, 1965.

[8] Yang, J. T., and P. Doty, *J. Am. Chem. Soc.*, **79**, 761 (1957).

[9] Wada, A., M. Tsuboi, and E. Konishi, *J. Phys. Chem.*, **65**, 1119 (1961).

[10] Fasman, G. D., and E. R. Blout, *J. Am. Chem. Soc.*, **82**, 2262 (1960).

[11] Imahori, K., and I. Yahara, *Biopolymers*, **1**, 421 (1964).

[12] Ikeda, S., A. Maeda, and T. Isemura, *J. Mol. Biol.*, **10**, 223 (1964).

[13] Rosenheck, K., and P. Doty, these PROCEEDINGS, **47**, 1775 (1961).

[14] Appel, P., and J. T. Yang, *Biochemistry*, **4**, 1244 (1965).

[15] Holzwarth, G., *Rev. Sci. Instr.*, **36**, 59 (1965).

[16] Velluz, L., M. Legrand, and M. Grosjean, in *Optical Circular Dichroism* (New York: Academic Press, 1965), p. 211.

[17] Sogami, M., W. J. Leonard, and J. F. Foster, *Arch. Biochem. Biophys.*, **100**, 260 (1963).

[18] Jirgensons, B., D. Yonezawa, and V. Gorguraki, *Makromol. Chem.*, **60**, 25 (1963).

[19] Troitsky, G. V., *Biofizika*, **10**, 895 (1965).

[20] Jirgensons, B., *J. Biol. Chem.*, **238**, 2716 (1963).

[21] Velluz, L., and M. Legrand, *Angew. Chem.*, **4**, 838 (1965).

[22] Timasheff, S. N., and R. Townsend, *Biochem. Biophys. Res. Commun.*, **20**, 360 (1965).

[23] There is no contradiction between this view and the observation by E. R. Blout and E. Shechter [*Biopolymers*, **1**, 565 (1963)] showing an ORD similar to ours for poly-L-isoleucine polymerized by initiation from a polymeric film, since there is no reason not to conclude that such polypeptide chains grown from a surface take up an antiparallel pleated configuration.

[24] Iizuka, E., and J. T. Yang, personal communications. (*Added in proof:* Likewise, we have learned that Drs. Fasman and Timasheffi have obtained ORD and CD data similar to some of those presented here.)

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*THE EFFECT OF HORMONES ON DNA**

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Gene activation and repression have been considerably elucidated in bacteria.[1, 2] Comparable mechanisms, although of greater complexity, probably exist in the cells of higher organisms. Hormones seem to be involved in the latter, for structural genes are in many cases activated by hormones.[3] Hormonal action may take