STUDIES ON THE STRUCTURE OF POLY-L-PROLINE IN SOLUTION

WILLIAM F. HARRINGTON and MICHAEL SELA*

Laboratory of Cellular Physiology and Metabolism, National Heart Institute, National Institutes of Health, Bethesda, Md. (U.S.A.)

INTRODUCTION

The imino acid proline differs from an amino acid in a polypeptide chain in that the imide link which it forms is devoid of a hydrogen atom and thus hydrogen bonding to a neighboring group is prevented. Moreover, because of the rigidity and geometrical form of the pyrrolidine ring, the proline residue cannot fit into an undistorted α-helix except at the ends of such a helix[1]. If the proline imide bond is in the *cis*-configuration, a sharp bend can be produced in a helix at the proline position thus allowing a reversal of direction[2,3]. The known compact geometry of many globular proteins suggests that such a configuration may, indeed, exist.

Studies on the synthetic polymers and copolymers of proline may be expected to help in clarifying the role of proline in the structure of proteins, particularly those rich in proline such as collagen, gelatin, casein and zein. Polymers of L-proline have been synthesized by BERGER, KURTZ and KATCHALSKI through polymerization of N-carboxy-L-proline anhydride[4,5]. Poly-L-proline exists in two forms, one exhibiting a specific rotation $[\alpha]_D^{25} = +40°$ in water (which we will designate polyproline I) and the other exhibiting a specific rotation, $[\alpha]_D^{25} = -540°$ (polyproline II)[6]. Polyproline I mutarotates in water to polyproline II over a period of several days. COWAN AND MCGAVIN have concluded from an analysis of the X-ray powder patterns as well as X-ray diffraction patterns of oriented films that polyproline II in the solid state exists in a helical configuration, the helix having the absolute left-handed sense.[7] The helix has 3 residues per complete turn and a residue repeat distance along the longitudinal axis of 3.12 A. The structure of polyproline I in the solid state has not as yet been elucidated although it has been demonstrated that polyproline I has a distinctly different X-ray diffraction powder pattern from that produced by polyproline II[8]. This suggests that the fundamental molecular configurations of these two forms are different. Evidence to be presented below supports this conclusion.

The COWAN-MCGAVIN helix has a rather high degree of steric rigidity and it seems probable that the steric restraints would be great enough to maintain the molecule in its helical configuration in solution[6,7]. The optical rotatory properties of such a system should consequently be a reflection of the rotatory characteristics of a helix superimposed on the intrinsic residue rotation[9].

In recent years a number of investigations of both a theoretical[9,10,11,12,13] and experimental[14,15,16,17,18,19] nature have clarified considerably the complex relation between optical activity and the configurational pattern of the back-bone chain in

* On leave of absence from the Weizmann Institute of Science, Rehovot, Israel.

References p. 40/41.

polypeptides and proteins. LINDERSTRØM-LANG AND SCHELLMAN[14] proposed that changes in the rotatory dispersion constant λ_c, could be related to changes in the amount of the folded configuration in the polypeptide chain. COHEN[15] suggested that the observed optical rotations of globular proteins could result from the configurational pattern of the chain superimposed on the optical rotation of the asymmetric centers. On the theoretical side FITTS AND KIRKWOOD[10] have applied the KIRKWOOD polarizability theory[20] to a calculation of the optical rotation of helical molecules, while MOFFITT[11, 13] has examined the effect of the α-helical configuration on optical rotation and its dispersion.

The application of these ideas to studies of polypeptides and proteins in solution has been very fruitful, leading to evidence from optical rotation that the α-helical pattern exists in proteins[17], and to the suggestion that many globular proteins may be only partially folded[17, 18]. A more refined viewpoint is now possible for the well-known optical rotation changes on denaturation of proteins, and the stabilizing forces which maintain the configurational patterns of proteins in solution can be more deeply explored.

Optical rotatory studies are of special significance in aqueous solutions, the natural environment of most proteins, since the hydrogen bonds which are of major importance in maintaining the helical polypeptide patterns in proteins are weakened significantly in aqueous systems. From thermodynamic data on the association of urea, SCHELLMAN[21, 22] estimates a value of 1.5 kcal/mole per peptide hydrogen bond in aqueous solution which should be compared to a value of 7–8 kcal/mole in non-aqueous media. The implications of these calculations have been explored in a study on the configurational properties in water of a polypeptide chain, oxidized ribonuclease, devoid of cross-linking disulphide bonds[18]. In a more recent study, the effect of lowering the activity of solvent water on the configurational pattern of polypeptides and proteins in aqueous solution has been examined[23]. It was found that concentrated aqueous solutions of lithium bromide and similar substances (possessing high activity coefficients, and therefore, according to the Gibbs-Duhem equation, low water activity) bring about the formation of maximum intramolecular hydrogen bonding in proteins and polypeptides. Two polypeptide chains, clupein and oxidized ribonuclease, known to be unfolded in aqueous solution, were examined. In water, clupein exhibits a specific rotation $[\alpha]_D^{20} = -104.5°$ and a rotatory dispersion constant, $\lambda_c = 200$ mμ, whereas in 6 M lithium bromide, $[\alpha]_D^{20} = -48.9°$ and $\lambda_c = 230$ mμ. Similarly oxidized ribonuclease in water gives $[\alpha]_D^{20} = -91.1°$ and $\lambda_c = 225$ mμ whereas in 9.9 M lithium bromide $[\alpha]_D^{20} = -49.5°$ and $\lambda_c = 250$ mμ. The changes in the optical rotatory properties of the above two materials in lithium bromide solutions are consistent with those expected from the formation of a folded, hydrogen-bonded configuration. On the other hand, concentrated aqueous lithium bromide solutions had no significant effect on the specific rotation or dispersion constant of the extensively hydrogen bonded protein serum albumin, even at temperatures up to 95° C[23]. The action of lithium bromide, then, is opposite to that of the usual denaturing agents such as urea, guanidinium salts and formamide whose normal action is to break peptide hydrogen bonds.

In the light of these investigations it seemed worth-while to examine the effect of denaturing and "contra-denaturing" substances on the properties of the poly-L-proline molecule in solution. Since poly-L-proline is devoid of peptide hydrogen bonds

References p. 40/41.

any configurational effects of the substances mentioned above must be attributed to causes other than the breaking or forming of such hydrogen bonds. Much of the work to be reported here deals with polyproline II. Some studies have been carried out on polyproline I.

METHODS AND MATERIALS

Optical rotations and rotatory dispersions were measured with a Rudolph precision ultraviolet polarimeter, model 80, equipped with the Rudolph photoelectric polarimeter attachment and an oscillating polarizer prism. Dispersion studies included the spectral range from 400 to 750 mμ, and were obtained through the use of a zirconium arc lamp (Sylvania, type K100). The temperature dependence of rotation of the sodium D line was measured with a Model D Keston Polarimeter unit (Standard Polarimeter Company) in conjunction with the Beckmann DU spectrophotometer. Water from a circulating waterbath served to control the temperature over the range 3° to 90° C. In these studies a 0.5 dm water-jacketed cell was employed.

Many of the optical rotatory measurements to be reported here were made on concentrated aqueous solutions. Since the indices of refraction of these solvents differ from that of water, there is a small but significant effect on the measured rotation[24]. Specific rotations have therefore been corrected to equivalent values in water (unless otherwise noted) by the equation

$$[\alpha]_S^{20} = [\alpha]^{20} \times \frac{n_{D,W}^2 + 2}{n_{D,S}^2 + 2}$$

where $n_{D,W}$ is the index of refraction of water at 20° C and $n_{D,S}$ is the index of refraction of solution at 20° C. The indices of refraction of the solutions of interest have been determined in an Abbe refractometer and are given in Table I.

Viscosity measurements were made at 25 ± 0.01° C in an Ostwald type viscometer having an average shear gradient of 300 sec^{-1}. The outflow time for water in this viscometer (2 cc volume) was 80 seconds.

Sedimentation was observed by means of a Spinco Model E ultracentrifuge operating at 59,780 r.p.m. either at room temperature or at 5° C as noted. The synthetic boundary cell was used in all experiments in view of the low molecular weight of the proline polymers employed. Photographic plates were measured with a Gaertner microcomparator in conjunction with a movable stage designed to give a two-way movement.

Poly-L-proline II samples of number average degrees of polymerization, DP, 30 and 50 (calculated from end-group analysis according to SELA AND BERGER[25]), as well as poly-L-proline I (DP 50) were obtained through the courtesy of Dr. E. KATCHALSKI of the Weizmann Institute of Science. These materials were stored in a desiccator over phosphorus pentoxide and all solutions of the polymers were made up by weight, assuming them to have a negligible water content.

Lithium bromide, calcium chloride, urea and pyridine were reagent grade, and were used without further purification. Lithium perchlorate was prepared by neutralizing 25% aqueous perchloric acid with an equivalent amount of lithium carbonate. The salt was recrystallized from water. Reagent grade guanidine·HCl was recrystallized once from methanol and stored in a desiccator over phosphorus pentoxide.

TABLE I

INDICES OF REFRACTION

Substance	*Concentration*	n_D^{20}
Guanidine·HCl	6 M	1.4353
Urea	8 M	1.4009
Lithium bromide*	6.5 M	1.4100
Lithium bromide*	12.9 M	1.4783
Lithium perchlorate	Saturated soln.	1.3608
Calcium chloride	Saturated soln.	1.4624
Pyridine (saturated with lithium bromide)		1.5270

* Values for other lithium bromide concentrations were obtained from Landolt-Börnstein Tabellen E.I., 534, 5th Edition, Berlin, 1931 .

References p. 40/41.

RESULTS AND DISCUSSION

The relatively rigid helical configuration of polyproline II in the solid state may also hold in aqueous solution. Assuming that such is the case, the contribution of the helix to the rotation may be estimated from the theory of FITTS AND KIRKWOOD for the optical rotation of helical molecules[9]. According to the polarizability theory of KIRKWOOD[20], the rotation of the sodium-D line of an optically active molecule in solution is given by

$$[\alpha]_D = 4.930 \times 10^5 \frac{(n^2 + 2)\, g}{3\, M} \tag{1}$$

where n is the index of refraction of the solution, M the molecular weight of the compound and g is the molecular rotatory parameter. For infinitely long helical molecules in which the polarizability is distributed in a continuous fashion over the length of the helix, FITTS AND KIRKWOOD have shown that the most general expression for g is given by:

$$g^\circ = \frac{\alpha_1^2 \beta^2 \gamma\, \nu}{12\pi\, a^2\, (1 + \gamma^2)^2} = \left[\int_0^\varepsilon f(\theta)\mathrm{d}\theta + \int_\varepsilon^\infty F(\theta)\mathrm{d}\theta\right] \tag{2}$$

where

$$f(\theta) = \frac{\theta \sin\theta - 2\,(1 - \cos\theta)}{[2(1 - \cos\theta) + \gamma^2\,\theta^2]^{3/2}} \left[\cos\theta + \gamma^2 - \frac{3\,(\sin\theta + \gamma^2\,\theta)^2}{2\,(1 - \cos\theta) + \gamma^2\,\theta^2}\right] \tag{3}$$

and

$$F(\theta) = \frac{[\theta \sin\theta - 2\,(1 - \cos\theta)]\,(\cos\theta - 2\gamma^2)}{\gamma^3\,\theta^3} \tag{4}$$

Here α_1 is the polarizability per turn of the helix, β_1 is the anisotropy ratio[20], $\gamma = b/a$ and ν is the number of turns of the helix. a is the average radius of the *main chain* atoms of the helix and b is the translational distance of the helix per radian.

In the calculation to follow we assume

(1) that the mean polarizabilities are additive

(2) that the anisotropy factor is $1/3$[20,10].

According to COWAN AND MCGAVIN[7] the polar coordinates (in A) of atoms forming the *main structural chain* in polyproline are O_1, 1.14; N_1, 1.01; αC_1, 1.30; and C_1, 0.32. The average radius of the atoms in the main chain is thus $a = 0.943$ A. Since the translational distance per turn of the helix is 9.36 A, $b = 1.49$ A. The value of γ is therefore 1.58.

The mean polarizability of the substituent groups in the main chain was determined from their atomic refractions according to the equation

$$\alpha_i = \frac{3}{4\pi N} \sum A_s$$

The values of α_i are 1.39 for C–H, 1.82 for C=O, and 1.13 for —N<. The mean polarizability per turn, α_1, is $4.34 \cdot 3$ A^3. In this calculation we have neglected the vicinal interactions of the side-chain methylene groups.

The integral $\int_0^\varepsilon f(\theta)\mathrm{d}\theta$ was evaluated by numerical integration between o and ε, letting $\varepsilon = -6\pi$. This integral has a value of —0.490. The second integral, $\int_\varepsilon^\infty F(\theta)\mathrm{d}\theta$, can be integrated by parts to yield the integral cosine

$$C_i(\varepsilon) = -\int_\varepsilon^\infty \frac{\cos x}{x}\,\mathrm{d}x$$

References p. 40/41.

which can be evaluated between limits from tables*. Integrating between -6π and -12π, the integral $[C_i(-12\pi) - C_i(-6\pi)]/\gamma 3$ is found to be insignificant ($-5 \cdot 10^{-4}$) compared to -0.490, and therefore for a left-handed *trans*-proline helix the rotatory parameter, $g^\circ = -1.88 \cdot 10^{-3}\ a^2{}_1\beta^2\nu$. The contribution of the helix to the rotation of the sodium-D line by polyproline II would be

$$[\alpha]_D = \frac{927\, a_1{}^2\beta^2(n^2 + 2)}{3\, m} \tag{5}$$

where $m = M/\nu$ is the molecular weight per turn. The estimated contribution of the helix in water is therefore -230°.

According to the calculation made above, if poly-L-proline in aqueous solution exists as a left-handed helix with $[\alpha]_D = -540^\circ$, then the intrinsic residue rotation of L-proline in the peptide chain should be in the neighborhood of -300°. Evidence in this direction is forthcoming from the specific rotations of a series of glycine-proline copolymers which have recently been measured by KURTZ, BERGER AND KATCHALSKI[26]. If it is assumed that poly-L-proline possesses a specific homogeneous configuration in solution, then the introduction of glycine residues into the poly-proline chain should result in a gradual elimination of the regularity of this structure. In the limit of copolymers with very high glycine to proline ratios, the initial proline configuration would be completely eliminated. Moreover, since glycine is optically inactive, the optical rotation observed under such conditions should result entirely from the intrinsic residue rotation of proline. This argument neglects the possibility that extensive sequences of glycine residues along the chain could form α-helices. However, the glycine residue is non-asymmetric, and helical segments, if they form at all, should be composed of a racemic mixture of left- and right-handed helices.

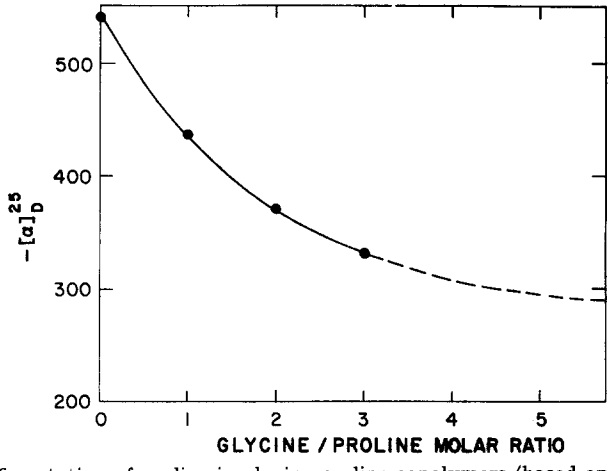

Fig. 1. Specific rotation of proline in glycine-proline copolymers (based on the weight fraction of proline).

Furthermore, recent work[27,28,29] has shown that four or more amino acids in a sequence are required to form the α-helix. Thus copolymers of low glycine proline ratios would not be expected to form α-helical segments. In Fig. 1 the specific rotation of the proline

* See Table of Sine and Cosine Integrals for Argument from 10 to 100. National Bureau of Standards, Applied Mathematics Series 32.

References p. 40/41.

residue in glycine-proline copolymers is plotted as a function of the molar ratio of glycine to proline. Rotations have been calculated from the specific rotations observed for these polymers by KURTZ, BERGER AND KATCHALSKI[26] by multiplying by the reciprocal of the molecular weight fraction of proline. Extrapolating from this plot it appears that the specific rotation of the proline residue is somewhere between —250° and —300°, which is in reasonably good agreement with the value predicted for the rotation of the proline residue from an estimation of the helical contribution as detailed above.

Properties of polyproline II in solution

Polyproline II is soluble in water[5] and dilute salt solutions. It is insoluble in concentrated solutions of sodium chloride[5], though readily soluble in aqueous solutions of lithium bromide, lithium perchlorate, calcium chloride, guanidine·HCl and urea. It is insoluble in pyridine, but dissolves readily in pyridine saturated with lithium bromide. The optical rotatory characteristics of polyproline II in these solutions exhibit profound differences. Fig. 2 gives the rotatory dispersion plotted according to the suggestion of YANG AND DOTY[17], while Table II gives values of the dispersion constant, λ_c, evaluated from the slope of these plots as well as the rotation of the D line, $[\alpha]_D^{25}$, in the various solvents. In all of the systems for which dispersion data are recorded in the present, paper plots of $\lambda^2[\alpha]$ *vs.* $[\alpha]$ were linear over the spectral range 400 to 750 mμ. Consequently the specific rotation at various wavelengths, λ, follows a one-term Drude equation,

$$[\alpha] = \frac{k}{\lambda^2 - \lambda_c^2}$$

where λ_c is the rotatory dispersion constant, and k is a parameter which is independent of wavelength but is, in general, a function of temperature, pH, etc.

Anomalous dispersion has been observed recently for high molecular weight polypeptides which exist in the α-helical pattern[16,17]. However, we do not feel that the observations summarized above are inconsistent with the view that poly-L-proline exists in a helical configuration, since the two polymer samples investigated

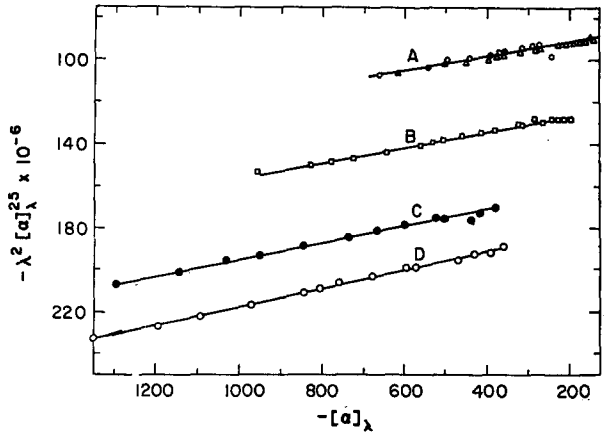

Fig. 2. Modified dispersion plot for polyproline II in (A) ▲ – 12.9 *M* lithium bromide; ○ – pyridine-LiBr; (B) □ – 6.5 *M* lithium bromide; (C) ● – 0.1 *M* potassium chloride and (D) ○ 8 *M* urea. Specific rotations have not been corrected for the index of refraction of the solvent.

References p. 40/41.

TABLE II

OPTICAL ROTATORY PROPERTIES OF L-PROLINE AND POLY-L-PROLINE

Material	*Solvent*	$[\alpha]_D^{25}$	$\lambda_c(m\mu)$
I. L-Proline*	H_2O	—86.8	126
L-Proline (hydrochloride)*	H_2O	—58.0	170
L-Proline (sodium salt)*	H_2O	—99.1	200
L-Proline	12.9 *M* LiBr	—58.5	184
L-Proline	95% ethyl alcohol	—67	—
II. Poly-L-proline II	0.1 *M* KCl	—540	202
	8 *M* urea	—546	210
	6 *M* Guanidine·HCl	—516	206
Poly-L-proline II	6.45 *M* LiBr	—364	192
	12.9 *M* LiBr	—243	185
	$CaCl_2$(sat'd)	—233	—
	$LiClO_4$ (sat'd)	—215	—
	Pyridine (sat'd) with LiBr)	—237	185
III. Poly-L-proline I	H_2O	+ 33	100

* Data taken from reference[30].

have relatively low molecular weights. Anomalous dispersion has not yet been observed in the proteins in aqueous systems, where presumably shorter helical segments exist than in the synthetic polypeptides which have been studied.

The solvents listed in Table II fall into two general classes in their effect on the rotation of polyproline II. In water, 8 *M* urea and 6 *M* guanidine·HCl the value of the specific rotation $[\alpha]_D^{25}$ is about —540°, and the dispersion constant, λ_c, falls between 202–210 mμ. In view of the fact that polyproline is devoid of peptide hydrogen bonds, the lack of a significant alteration in rotatory properties in urea or guanidine·HCl is not surprising.

In the presence of lithium bromide, lithium perchlorate, calcium chloride or pyridine (saturated with lithium bromide), the absolute value of $[\alpha]_D^{25}$ decreases to a value around —250°, while λ_c undergoes a relatively small change to 185 mμ. The magnitude of the change in the optical rotation is such as to suggest that it cannot be due to a solvent effect on the asymmetric centers. Moreover, the rotational change is similar throughout a variety of solvents. It seems possible that a profound configurational disorientation in the structural regularity of the molecule is responsible for the changes observed.

It is of interest that the dispersion constant of poly-L-proline II, λ_c, has a value of 202 mμ in water. This value is well below that observed for other polypeptides and proteins in the folded configuration[14, 16, 17, 18]. In fact, polypeptides in the random chain configuration and denatured proteins devoid of extensive secondary structure exhibit a dispersion constant of about this magnitude. Nevertheless, it cannot be presumed that this comparison affords evidence for the lack of a configurational pattern in polyproline II. Support for this viewpoint comes from the very extensive optical rotation studies on collagen and gelatin. Ichthyocol collagen in aqueous solutions at low temperatures possesses structural properties consistent with the

References p. 40/41.

properties of collagen in the solid state[31,32,33,34], where the evidence is strong that the individual chains of the structure have a left-handed helical configuration[35,36,37]. On heating collagen to 40° C, the structural properties are lost and the absolute value of the optical rotation, $[\alpha]_D$, decreases from —300° to —100°[33,31]. On the other hand λ_c remains at 205 ± 15 mμ throughout these changes[31].

Temperature effects

Poly-L-proline II in water undergoes particularly striking visual changes in solubility with temperature[6]. A clear solution of polyproline II in water develops a sudden turbidity at around 63° when heated. On cooling, the solution remains turbid down to a temperature of about 25° when the solution becomes suddenly clear. This cycle can be repeated indefinitely. Fig. 3 presents the changes in rotation observed during one of the heating cycles. There is a relatively small decrease in rotation as the temperature is raised until the solution reaches a temperature of about 50°, when the rotation drops very rapidly, reaching its maximum rate of change between 60 and 65°

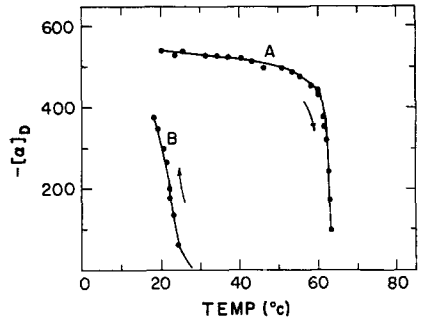

Fig. 3. The temperature dependence of the specific rotation of polyproline II in 0.1 *M* potassium chloride.

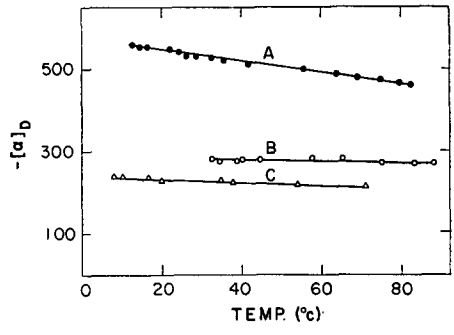

Fig. 4. The temperature dependence of the specific rotation of polyproline II in (A) 8 *M* urea; (B) 12.9 *M* lithium bromide, and (C) pyridine (saturated with lithium bromide).

at the onset of turbidity. On cooling, the solution remains turbid down to 30°. Below 30° a very rapid increase in rotation occurs as the temperature is lowered. Thus the measurement of rotation in the transition regions of both high and low temperatures is a measure of the turbidity of the solution.

The striking temperature transitions in solubility demonstrated for polyproline II in water are entirely absent when this material is heated and cooled in 8 *M* urea, or in saturated lithium bromide solutions in water or pyridine (see Fig. 4). Studies of the temperature effect in these solvents reveal that the solutions remain perfectly clear up to the boiling point. In 8 *M* urea there is a small but significant decrease in rotation with increasing temperature, the rate of change being about the same as that in water over the range from 20° to 50° C. The observed decrease is consistent with that expected for a structure in which the groups surrounding the asymmetric centers have some freedom of movement[24,38]. Temperature coefficients in aqueous lithium bromide and in pyridine–lithium bromide show similar though decreased effects above 30° C. All the above changes are reversible.

The effect of lithium bromide

We turn now to a more detailed inquiry into the effect of lithium bromide on the

References p. 40/41.

rotatory properties of polyproline II. In Fig. 5 are plotted the D line rotations of a series of polyproline II solutions in which the concentration of lithium bromide has been varied from 0 to 12.9 *M*. Essentially the same effect on rotation is observed for materials of number average degrees of polymerization 30 and 50. The absolute value of the rotation decreases steadily with increasing salt concentration levelling off in the neighborhood of —250° at the highest lithium bromide concentration examined. The effect is completely reversible. A solution of polyproline II in 8 *M* lithium bromide gave a specific rotation of —287°. On dilution to 4 *M*, $[\alpha]_D$ increased to —370°.

In seeking an explanation of the above effect several possibilities must be considered. It is conceivable that the solvent brings about a direct alteration in the wavelengths of the optically active absorption bands of the proline residues in the polypeptide. If this were true, the solvent might possibly exhibit a similar effect on the rotational characteristics of the free L-proline in solution. The rotatory dispersion of L-proline has been measured in 12.9 *M* lithium bromide. A plot of λ^2 $[\alpha]$ *vs.* $[\alpha]$ over the visible range is linear and yields a dispersion constant, $\lambda_c = 184$ mμ and $[\alpha]_D^{25} =$ —58.5°, corrected for index of refraction of the solvent. Thus the rotation characteristics change markedly from those observed in water (see Table II). Here it is well to consider the effect of the charges on the rotatory properties of the free acid (Table II). Measurements in acid and base suggest that elimination of the charge effect would result in rotatory properties similar to those observed for the free acid in the lithium bromide medium. Reference has already been made to the very high activity coefficient of lithium bromide in concentrated aqueous solution. The activity of water, calculated from the data of ROBINSON AND STOKES[39], in 12.9 *M* lithium bromide is about $3 \cdot 10^{-4} M$. Such a low water activity undoubtedly represses ionization of the carboxyl group of L-proline and also acts to damp out the effect of the imino nitrogen charge. The argument is given additional weight by a measurement of the rotation of L-proline in 95% ethanol where $[\alpha]_D^{25} = -67°$. This is a substantial decrease from the rotation in water, and must be attributed to a damping out of the charge effects. The action of the lithium bromide solvent then would appear to have little direct effect on the optically active absorption bands of the proline residues in the polymer where charge effects are essentially eliminated.

It is unlikely that simple aggregation phenomena could lead to rotational changes

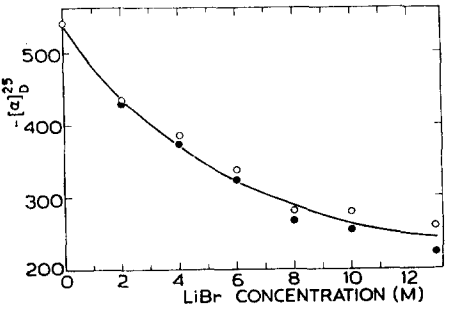

Fig. 5. Specific rotation of polyproline II as a function of lithium bromide concentration. ○ DP = 50; ● DP = 30.

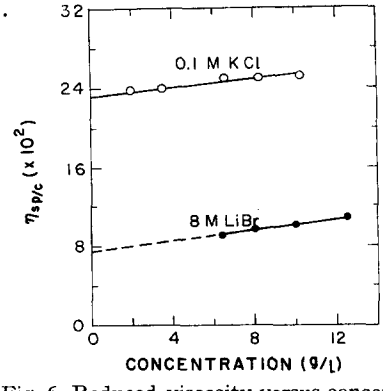

Fig. 6. Reduced viscosity versus concentration of polyproline II in: ○ —0.1 *M* potassium chloride; and ● —8 *M* lithium bromide.

References p. 40/41.

of the magnitude observed unless a regular configurational pattern is established on aggregation which is nonexistent in the non-aggregated structure. COHEN has demonstrated that hot evaporated gelatin films give very low specific rotations in contradistinction to cold evaporated films which have large *laevo*rotations and other optical properties very similar to those of collagen[31]. Evidently, the close approach of molecules in the hot evaporated gelatin has little influence on rotation, whereas the development of a collagen-like structure, which occurs on cold evaporation, leads to the observed rotational changes.

The possibility of aggregation of poly-L-proline II in lithium bromide has been examined in a study of the sedimentation and viscosity properties of the polymer in the absence and presence of this salt. (See Table III and Figure 6). Sedimentation coefficients of polyproline II in 0.1 *M* potassium chloride and in 8 *M* lithium bromide, when corrected for the density and viscosity of the solvent, are essentially identical.

TABLE III

SEDIMENTATION OF POLY-L-PROLINE

Material	*Concentration (%)*	*Solvent*	*Time (hours)*	S^{w}_{20} *(fast)*	S^{w}_{20} *(slow)*
Polyproline I	1.0	H_2O	0.5	13.5	0.54
	0.64	H_2O	27	14.9	0.54
	0.47	H_2O	170 (heated)	Absent	0.57
Polyproline II	1.0	0.1 *M* KCl	—	Absent	0.5_4
	1.0	8 *M* LiBr	—	Absent	0.5_4

On the other hand, a plot of the reduced viscosity versus concentration of polyproline II shows a striking decrease in the viscosity contribution of the polymer when it is transferred to the high salt medium (Fig. 6). The sedimentation and viscosity data, when taken together*, are therefore unambiguous in ruling out aggregation in the lithium bromide solvent. Moreover, the marked decrease in reduced viscosity must be attributed to a configurational change in the polyproline molecule resulting in a lowering of the asymmetry of this molecule. The value of the reduced viscosity, η_{sp}/c, for the polymer in 0.1 *M* potassium chloride at infinite dilution is 0.235 $(g/ml)^{-1}$. The osmotic pressure, sedimentation and diffusion experiments of KURTZ, BERGER AND KATCHALSKI[26] have shown that polyproline II exists in a non-aggregated form in water and dilute salt solution. Our sedimentation and viscosity experiments confirm this observation. The relatively high value of intrinsic viscosity (Fig. 6) must consequently result from the asymmetry of the polyproline molecules.

Using values of 3.17 A for the radius (the radius of the C atom of the ring) and 3.12 A for the translational distance per residue[7], we have calculated the axial ratios to be expected for poly-L-proline in the COWAN-MCGAVIN helix for polymers containing on the average 30, 50, 90 and 100 residues per molecule as judged by end-group

* The invariance of the sedimentation coefficient in the two solvents remains somewhat anomalous since a reduction in viscosity increment should increase the sedimentation coefficient. It seems possible that errors inherent in the measurement of the sedimentation coefficient in concentrated lithium bromide may explain this discrepancy.

References p. 40/41.

analysis. The axial ratio has also been estimated from the reduced viscosity data, summarized in Table IV, using the SIMHA equation for prolate ellipsoids[40]. It will be seen from this table that the axial ratio calculated in this way is in reasonable agreement with that expected for the COWAN-MCGAVIN helix.

TABLE IV

AXIAL RATIO OF POLYPROLINE II IN SOLUTION

*Average number of residues in polymer**	*Axial ratio expected***	*Axial ratio found from viscosity****
30	15	17
50	26	19
90§	43	49
100§	49	36

* End group analysis[25].
** Based on coordinates of COWAN-MCGAVIN helix[7].
*** SIMHA equation[40]; density assumed to be 1.32 g/ml[7].
§ From data of KURTZ, BERGER AND KATCHALSKI[26].

Configuration of polyproline II in aqueous solution

It is now apparent that both the rotatory and hydrodynamic properties of polyproline II in water and dilute salt solutions are entirely consistent with a left-handed *trans*-helical configuration. On the other hand, in the solvents of low water activity, there is a decrease in the absolute magnitude of rotation which is, within experimental error, that expected for the elimination of the helical pattern. Moreover, the rotation change is accompanied by a significant decrease in the value of λ_c from 200 to 185 mμ as the water activity of the surrounding medium is lowered towards zero. It should be noted that the final value of λ_c observed is identical to that of L-proline in saturated lithium bromide. The consequence of this development is that elimination of the helix results in a decrease of only 15–20 mμ in the dispersion constant. This value is much lower than the change in λ_c observed on elimination of the α-helix in proteins and polypeptides[14,16,18,23,41]. It must be remembered, however, that in polyproline II we are dealing with a helix on which the main chain atoms of the screw have a much smaller radius[7] (average $r = 0.94$ A), and which has a much steeper spiral than has the α-helix. In the next section where we will discuss polyproline I, we will present evidence that a polyproline helix corresponding more closely in radius and pitch to the α-helix exhibits a much greater change in λ_c on destruction.

In considering the configurational changes in poly-L-proline II in the presence of lithium bromide, changes leading to a destruction of the helical pattern, we are faced at once with a rather remarkable fact: the steric restraints imposed by the ring structure of the proline residues makes a random chain impossible. From an examination of models of polyproline II, it seems clear that the destruction of such a helical configuration can only be accomplished through a *trans-cis*-isomerization at the peptide bonds. Such a mechanism would allow the collapse of the helical pattern and inversion in direction of the chain segments necessary to produce the striking drop in viscosity which is observed (Fig. 6). Why this situation is brought about in systems of low water activity, cannot be fruitfully discussed at this time. On the other hand, if the above interpretation is correct, it is of great interest in the problem of protein

References p. 40/41.

structure, and particularly in the elucidation of the structure of globular proteins where the *cis*-proline configuration has been suggested[2,3] as a possible mechanism for changing the direction of the chain by 180°. Thus any *trans-cis*-isomerization at the peptide bond would change in a striking manner the over-all dimensions of the molecule.

Studies on poly-L-proline I

Poly-L-proline I is prepared by precipitating the polymer with ether from a pyridine polymerization mixture[6,26]. It has a slight positive rotation (*ca.* +40°) in water when measured immediately after dissolution. On standing in water, the system undergoes mutarotation over a period of several days at room temperature to polyproline II[6,26]. Polyproline I is only partially soluble in water at room temperature[6,26], but is readily soluble at 2° C giving a water-clear solution. It is insoluble in concentrated aqueous or pyridine solutions of lithium bromide. In fact, traces of salt (NaCl or LiBr) are sufficient to precipitate the polymer from water.

The changes in the optical rotation of the D line during mutarotation of polyproline I have been reported by KURTZ, BERGER AND KATCHALSKI[6]. The changes in rotatory dispersion of polyproline I in water, as it mutarotates to polyproline II at 25° C, have now been measured and are presented in Fig. 7.

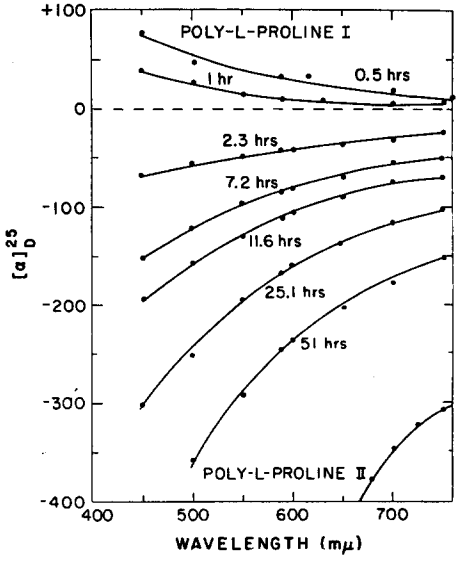

Fig. 7. Rotatory dispersion during the transformation of polyproline I to polyproline II in water.

In the first hour after the polymer is dissolved, positive rotations are observed at all wavelengths, the rotation increasing with diminishing wavelength. As mutarotation proceeds, the specific rotation soon becomes negative at all wavelengths, the rotation now becoming more *laevo*-rotatory with diminishing wavelength. Thus the form of the dispersion curve is typical of a *dextro*-rotatory material in the early stages of the transition, changing over to that characteristic of *laevo*-rotatory substances within two hours of dissolution. Rotatory dispersion data presented in Fig. 7 exhibit simple linear dispersion throughout all stages of the mutarotation, when plotted as $\lambda^2[\alpha]$ *versus* $[\alpha]$. The rotatory dispersion constants, λ_c, calculated from these slopes have been plotted in Fig. 8 as a function of time. Here it will be seen that λ_c of polyproline I in water is of the order of 100 mμ and that it increases in a continuous fashion as polyproline I is transformed into polyproline II. In the first two or three hours the rate of change of rotation is so great that dispersion measurements are not very accurate. Nevertheless the true dispersion constant is probably within $\pm$10 mμ of that calculated, and we are thus confronted with the following question: if the polyproline molecule, devoid of configurational contributions of the chain to the rotatory parameters, has a dispersion constant, λ_c = 185 mμ and a specific rotation of —250°

References p. 40/41.

to —300°, as was proposed above, what is the structure of polyproline I? The possibility that the chemical structure of the proline residue differs in the two polymers can be immediately discarded. Both polymers yield L-proline on acid hydrolysis[6]. It seems reasonable to assume then, that the changes in rotation are entirely due to the destruction of a unique spatial configuration of the L-proline residues in polyproline I, followed by a transition to the more stable configuration in water characteristic of polyproline II.

KURTZ, BERGER AND KATCHALSKI have suggested that the observed mutarotation may result from a *cis-trans*-isomerization[6]. In considering this proposal it is pertinent to inquire if the spatial geometry of the proline residues would permit the formation of a structure linked only by *cis*-peptide bonds. A poly-L-proline model in which the peptide bonds are exclusively in the *cis*-configuration has been constructed recently by CRICK AND RICH[42]. As in the *trans*-left-handed helix, the *cis*-chain configuration is restrained considerably by the proximal steric effects of the pyrrolidine rings. The resulting structure is a right-handed helix with approximately 3-1/8 residues per complete turn, and a unit residue translation near 1.85 A. The diameter of the helix is significantly larger than that of polyproline II. Confirmation of the existence of this structure in solid polyproline I must await an analysis of the X-ray diffraction patterns. Such a structure would appear, however, to be compatible with the rotatory characteristics observed for polyproline I in water. The destruction of this helical pattern should result in significant changes both in the absolute value of rotation and in the magnitude of the dispersion constant. Of great importance is the fact that a *cis-trans*-isomerization in the imide linkage is possible. Steric restraints though present are not sufficient to hinder complete rotation of the pyrrolidine ring about the peptide linkage.

Accepting the provisional dimensions of CRICK AND RICH[42] for polyproline with all peptide bonds in the *cis*-configuration, we may now estimate the contribution of

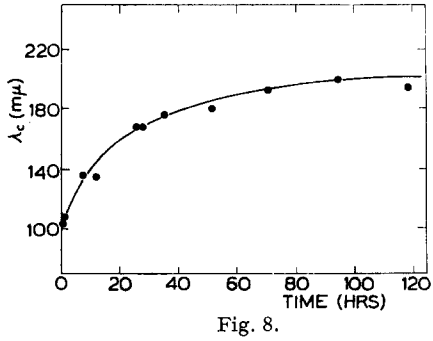

Fig. 8.

Fig. 8. The rotatory dispersion constant, λ_c, as a function of time during the transformation of polyproline I to polyproline II in water.

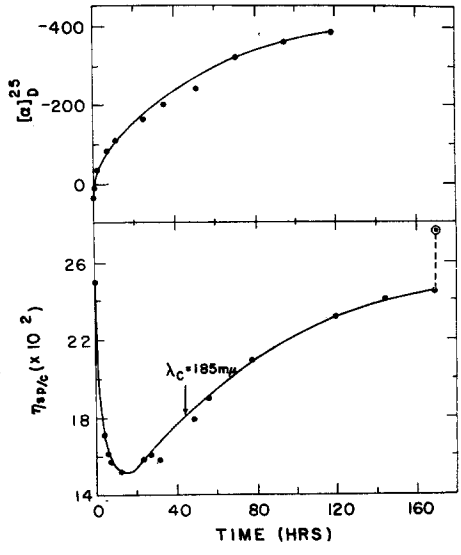

Fig. 9.

Fig. 9. The reduced viscosity (lower plot) and specific rotation (upper plot) as a function of time during the transformation of polyproline I to polyproline II in water.

References p. 40/41.

this right-handed helix to the specific rotation of the D line. We proceed as in the case of polyproline II. The average radius of the main chain atoms is near 1.70 A which is approximately double that of polyproline II. The value of b is $5.78/2\pi$, and therefore $\gamma = 0.538$. This value of γ lies sufficiently close to that calculated for an α helix by FITTS AND KIRKWOOD[9], so that we have accepted their value of 0.467 for the integrated function given in equation 3. As shown earlier, the value of this integral changes from 0.467 to 0.490, *i.e.* only about 5 %, with a change in γ from 0.472 to 1.58. Tabular evaluation of the 2nd integral gives $\int_{+6}^{12\pi} F(\theta)\mathrm{d}\theta = +0.0127$. Thus

$$g_o = \frac{a_1^2\beta^2\gamma\nu}{12\pi a^2(1+\gamma^2)^2}(0.467 + 0.0127) \qquad (6)$$

and $[\alpha]_D = 53(n^2 + 2)$. Thus the approximate helical contribution in water is $[\alpha]_D = +204°$ and assuming a value of $-250°$ for the proline residue, the estimated value of $[\alpha]_D$ for the rotation of polyproline I in a *cis*-right-handed helix in water is $-50°$.

A study of the changes in the viscosity of a solution of polyproline I in water with time would afford a critical test of the assumptions made above. A right-handed *cis*-helix should exhibit the hydrodynamic properties of a rigid rod in solution. As the *cis*-linkages are destroyed during mutarotation, the rod would be expected to collapse, resulting in a decrease in viscosity. In time, a transition to the completely *trans*-structure of polyproline II (exhibiting again the hydrodynamic properties of a rigid rod in solution) would occur, accompanied by an increase in viscosity. The viscosity plot should therefore go through a minimum, eventually reaching a final value of reduced viscosity greater than the initial value of polyproline I, since the axial ratio of the left-handed helix is nearly twice that of the right-handed helix. Fig. 9 presents the results of such a study at 25° C. The reduced viscosity of a solution of polyproline II in water is plotted as a function of time and exhibits the predicted minimum. On the other hand, η_{sp}/c of polyproline I at zero time is much greater than we would expect from the polyproline I model. The polymer contained on the average 50 residues per molecule. Assuming a translational distance of 1.85 A per residue and an approximate radius of 3.70 A[42], the right-handed polyproline I helix would have an axial ratio of 12.5. The intrinsic viscosity expected is therefore of the order of 0.14 $(g/ml)^{-1}$, whereas the observed value is 0.25 $(g/ml)^{-1}$.

The foregoing calculation assumed that polyproline I is dispersed in aqueous solution as monomolecular units. To test this assumption, we have made a number of sedimentation studies on the polyproline I–water system. Since mutarotation is relatively rapid during the first few hours at room temperature, a sedimentation run at 5° C was carried out on the polymer immediately after dissolution in water. Later runs were made at room temperature. Sedimentation studies do indeed show the presence of an aggregate in the polyproline I–water system. Two discrete peaks, with sedimentation coefficients given in Table III, are observed in the sedimentation patterns soon after reaching operating speed, with the faster peak comprising about 25% of the total schlieren area. A run made in the ultracentrifuge 27 hours later yielded similar results (see Table III) in that the relative amount of the faster sedimenting peak remained unchanged. A final sedimentation run after 170 hours exhibited a single schlieren peak. (This solution was heated to 80° C prior to study in the ultracentrifuge, in order to complete the conversion of polyproline I into polyproline II). Consideration of the sedimentation results in Table III leads immediately to

References p. 40/41.

several conclusions: (1) The aggregate cannot be in equilibrium with the non-aggregated polyproline I, since the relative amount of the aggregate does not decrease significantly over a period of time in which large rotatory changes occur; (2) One cannot assume that the aggregate represents polyproline I, while the material of lower molecular weight is polyproline II, since we would obtain a highly improbable value of $[\alpha]_D$ (*ca.* $+1800°$) for the aggregate. Moreover, as in (1), we should expect to find a continuous decrease in the amount of aggregate with time. This was not observed. For these reasons we feel that the most reasonable assumption is that polyproline I can exist in two forms in water. The aggregate, however, would appear to have a much slower rate of mutarotation than the low molecular weight polymer. In fact, to disperse the aggregate completely, it was necessary to heat a solution of polyproline I (80° C), even though it had been in the presence of water at 25° C for over a week's time. This is illustrated graphically in Fig. 9, which demonstrates an abrupt viscosity rise on heating a polyproline I solution which has been standing for 170 hours. The specific rotation was also observed to increase from $-430°$ to $-540°$ on heating.

It seems possible that the presence of the aggregate of polyproline I accounts for the relatively high viscosity which is observed before mutarotation begins. The observed minimum in the plot of η_{sp}/c as a function of time may be ascribed primarily, however, to configurational changes in the low molecular weight polymer, and are consequently in qualitative agreement with the prediction.

CONCLUSION

The experimental data presented in this paper are in accord with the following interpretation: (1) Poly-L-proline II in water has the same configuration as in the solid state[7], *i.e.*, it is a left-handed helix in which the peptide bonds are in the *trans*-configuration. (2) Poly-L-proline I in water is a right-handed helix in which the peptide bonds are in the *cis*-configuration. (3) The mutarotation of poly-L-proline I in water into poly-L-proline II consists of a series of *cis-trans* isomerizations at the peptide bonds.

From the work of YANG AND DOTY[17] it appears that changes in the α-helical content of polypeptides and proteins can be correlated directly with a change in the optical rotation of the D line, as well as with a change in the rotatory dispersion constant, λ_c. Fig. 10 shows a plot correlating the change in $[\alpha]_D^{25}$ and the change in λ_c, as poly-L-proline I is converted into poly-L-proline II. The optical rotatory properties of poly-L-proline II in aqueous lithium bromide are also plotted.

If we assume that changes in the helical content of polyproline II and of polyproline I are proportional, as in the case of the α-helix, to changes in the optical rotatory properties, then it would appear that two steps are involved in the transition, polyproline I → polyproline II. The change in λ_c as a function of $[\alpha]_D^{25}$ is linear in each of the two steps, but the slope and magnitude of the changes involved are not identical. From the results and discussion which have been presented above, it seems possible that these two steps are (a) destruction of the right-handed helix of polyproline I and (b) formation of the left-handed helix of polyproline II. It is interesting to observe that the transition point corresponding to the disappearance of the helical configuration of polyproline I has the same rotatory parameters ($[\alpha]_D^{25} = -230°$; $\lambda_c = 185$ mμ) as those observed for polyproline II in saturated aqueous solutions of

References p. 40/41.

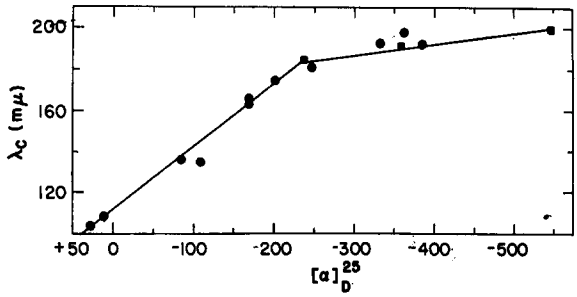

Fig. 10. The rotatory dispersion constant of poly-L-proline as a function of the specific rotation; ● Data taken during mutarotation of polyproline I; ■ Data taken from dispersion studies of polyproline II in varying concentrations of lithium bromide.

lithium bromide, lithium perchlorate and calcium chloride. The value of $[\alpha]_D^{25}$ for this transition point also corresponds closely to the limiting specific rotation observed on destruction of the configurational pattern of polyproline II through the introduction of glycine residues into the polymer (see Fig. 1). Moreover, it agrees reasonably well with the intrinsic residue rotation of poly-L-proline estimated from the theory of FITTS AND KIRKWOOD. It is pertinent to keep in mind that this transition point cannot correspond to a true "random coil", as this is not possible in the case of a polymer of proline. Thus the complete loss of regularity can be attributed only to a distribution of *cis*- and *trans*-forms of the peptide bonds along the main structural chain.

In an earlier paper[23] it was suggested that a lowering of the water activity in concentrated aqueous solutions of lithium bromide, and other salts exhibiting high activity coefficients, results in the formation of maximum intramolecular hydrogen bonding in proteins and polypeptides. This conclusion must now be supplemented to include the possibility of another effect, namely, a *trans-cis* isomerization at the proline* peptide bond.

It appears from the study of the optical rotatory and viscometric properties of polyproline that the *trans*-configuration of the proline peptide bond is the more stable one in water. However, a *trans-cis* isomerization of the peptide bond would seem possible under certain conditions such as (1) a specific amino acid sequence in the peptide chain, (2) a characteristic geometric configuration in the vicinity of the proline residue, or (3) some special property of the solvent. The role of *cis* proline bonds in the structure of proteins has been discussed by PAULING[2] and EDSALL[3]. The possibility of *trans-cis* isomerizations could be of considerable importance in the biological activity of proteins.

ACKNOWLEDGEMENT

We wish to thank Professor EPHRAIM KATCHALSKI (Weizmann Institute of Science) for the samples of polyproline, as well as for the opportunity of reading a manuscript of his, prior to publication.

* Though only proline is mentioned specifically in the above discussion, most of the conclusions of this paper would be applicable also to hydroxyproline. The recent synthesis of poly-L-hydroxyproline[43] will permit a detailed study.

References p. 40/41.

SUMMARY

1. The specific rotation of poly-L-proline II ($[\alpha]_D^{25} = -540°$) in water has been estimated making use of the theory of FITTS AND KIRKWOOD for the optical rotation of helical molecules and the reported specific rotations of glycine-proline copolymers.

2. The optical rotation, temperature dependence of rotation and rotatory dispersion of polyproline II in various solvents is reported. In those solvents of low water activity, the specific rotation changes to a limiting value of around $-250°$ while sedimentation and viscosity studies demonstrate a marked decrease in the asymmetry of the molecule.

3. The mutarotation of poly-L-proline I ($[\alpha]_D = +40°$) in water has been followed by rotatory dispersion, viscosity and sedimentation studies.

4. The above results are shown to be consistent with the proposal that polyproline I exists in aqueous solution as a right-handed helix with peptide bonds in the *cis*-configuration whereas polyproline II exists in aqueous solutions as a left-handed helix with peptide bonds in the *trans*-configuration.

REFERENCES

1 H. LINDLEY, *Biochem. Biophys. Acta*, 18 (1955) 197.
2 L. PAULING, *J. Am. Chem. Soc.*, 62 (1940) 2643.
3 J. T. EDSALL, *J. Polymer Sci.*, 12 (1954) 253.
4 A. BERGER, J. KURTZ AND E. KATCHALSKI, *Bull. Research Council Israel*, 4 (1954) 113.
5 A. BERGER, J. KURTZ AND E. KATCHALSKI, *J. Am. Chem. Soc.*, 76 (1954) 5552.
6 J. KURTZ, A. BERGER AND E. KATCHALSKI, *Nature*, 178 (1956) 1066.
7 P. COWAN AND S. MCGAVIN, *Nature*, 176 (1955) 501.
8 A. RICH, private communication.
9 D. D. FITTS AND J. G. KIRKWOOD, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 33.
10 D. D. FITTS AND J. G. KIRKWOOD, *J. Am. Chem. Soc.*, 78 (1956) 2650.
11 W. MOFFITT, *J. Chem. Phys.*, 25 (1956) 467.
12 W. MOFFITT AND J. T. YANG, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 596.
13 W. MOFFITT, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 736.
14 K. U. LINDERSTROM-LANG AND J. A. SCHELLMAN, *Biochim. Biophys. Acta*, 15 (1954) 156.
15 C. COHEN, *Nature*, 175 (1955) 129.
16 P. DOTY AND J. T. YANG, *J. Am. Chem. Soc.*, 78 (1956) 498.
17 J. T. YANG AND P. DOTY, *J. Am. Chem. Soc.*, 79 (1957) 761.
18 W. F. HARRINGTON AND J. A. SCHELLMAN, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 30 (1956) 21.
19 W. KAUZMANN, in W. D. MCELROY AND B. GLASS, *The Mechanism of Enzyme Action*, Johns Hopkins Press, Baltimore, 1954.
20 J. G. KIRKWOOD, *J. Chem. Phys.*, 5 (1937) 479.
21 J. A. SCHELLMAN, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 29 (1955) 223.
22 J. A. SCHELLMAN, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 29 (1955) 230.
23 W. F. HARRINGTON AND J. A. SCHELLMAN, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, (in the press).
24 W. KAUZMANN, J. E. WALTER AND H. EYRING, *Chem. Revs.*, 26 (1940) 339.
25 M. SELA AND A. BERGER, *J. Am. Chem. Soc.*, 77 (1955) 1893.
26 J. KURTZ, A. BERGER AND E. KATCHALSKI, in *Recent Advances in Gelatin and Glue Research*, in the press.
27 P. DOTY AND R. D. LUNDBERG, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 213.
28 A. ELLIOTT, W. E. HANBY AND B. R. MALCOLM, *Nature*, 178 (1956) 1170.
29 E. R. BLOUT, P. DOTY AND J. T. YANG, *J. Am. Chem. Soc.*, 79 (1957) 749.
30 J. W. PATTERSON AND W. R. BRODE, *Arch. Biochem.*, 2 (1943) 247.
31 C. COHEN, *J. Biophys. Biochem. Cytol.*, 1 (1955) 203.
32 C. R. ROBINSON AND M. J. BOTT, *Nature*, 168 (1951) 325.
33 P. M. GALLOP, *Arch. Biochem. Biophys.*, 54 (1955) 486, 501.
34 H. BOEDTKER AND P. DOTY, *J. Am. Chem. Soc.*, 77 (1955) 248.
35 G. N. RAMACHANDRAN AND G. KARTHA, *Nature*, 174 (1954) 269.
36 A. RICH AND F. H. C. CRICK, *Nature*, 176 (1955) 915.
37 P. COWAN, S. MCGAVIN AND A. C. T. NORTH, *Nature*, 176 (1955) 1062.
38 W. J. KAUZMANN AND H. EYRING, *J. Chem. Phys.*, 9 (1941) 41.
39 R. A. ROBINSON AND R. H. STOKES, *Electrolyte Solutions*, Academic Press. Inc., New York, 1955.
40 R. SIMHA, *J. Phys. Chem.*, 44 (1940) 25.
41 M. SELA, C. B. ANFINSEN AND W. F. HARRINGTON, *Biochim. Biophys. Acta*, in the press.
42 A. RICH, private communication.

[43] E. KATCHALSKI, J. KURTZ, G. FASMAN AND A. BERGER, *Bull. Research Council Israel*, 5A (1956) 264;
J. KURTZ, G. D. FASMAN, A. BERGER AND E. KATCHALSKI, *J. Am. Chem. Soc.*, in the press.

Received July 22nd, 1957

Note added in proof: Drs. P. M. COWAN AND R. E. BURGE have kindly pointed out an error in the assumed value of m, the molecular weight per turn of the proline helix (eqn. 5). Based on the coordinates of main chain atoms *alone* the helix contributions of Polyproline I and II should be $[\alpha]_D = +68°$ and $-77°$, respectively. If side chain atoms are included, $[\alpha]_D = +160°$ (I) and $[\alpha]_D = -310°$ (II).

STUDIES ON THE STRUCTURE OF KERATIN

III. THE REACTION OF WOOL AND HORN KERATINS WITH SOLUTIONS OF SODIUM HYPOCHLORITE

C. EARLAND AND D. J. RAVEN
Department of Textile Industries, The Technical College, Bradford (England)

The reactivity of the combined cystine in keratin has been the subject of considerable study, and from the behaviour of these residues towards a number of reagents it has been deduced that the cystine in wool may be divided into a number of fractions. PHILLIPS *et al.*[1] have divided the cystine of wool into two main fractions, one of which reacts more readily with reagents such as sodium bisulphite, alkalis, formaldehyde and thioglycollic acid, although BLACKBURN AND LEE[2] consider that in their reaction with alkalis, the cystine residues of wool show a gradual gradation in reactivity rather than a definite break. ALEXANDER *et al.*[3] also have divided the cystine residues of wool and horn into two fractions, which are unrelated to those of PHILLIPS. These workers consider that only 25 % of the cystine in wool and horn is capable of oxidation by solutions of sodium hypochlorite and potassium permanganate, whereas it may all be oxidized by peracetic acid and chlorine in acid solution. Contrary to the conclusions of ALEXANDER *et al.*, ELLIOT AND ROBERTS[4], from the microscopical examination of oxidized wools, consider that the oxidation of only a portion of the cystine by permanganate is due to the morphology of the fibre. In view of the importance which is attached to the reactivity of the cystine residues of keratin a further investigation as to the nature of the fractions described by ALEXANDER *et al.* has been made.

The reaction between permanganate and wool is very complex since it is accompanied by the deposition of manganese dioxide at the sites of reaction, and the present paper has therefore been restricted to a reassessment of the reaction between solutions of sodium hypochlorite and the keratins of wool and horn.

EXPERIMENTAL

Materials

Virgin Australian wool of three qualities was purified as described in Part I[5]. Cow's horn was reduced in a Sturtevant 8" Laboratory Disintegrator, and the ground material separated into

References p. 45.