TABLE IIIa

EXCHANGE STUDIES OF $(CD_3)Hg-(CH_3)_2M$ SYSTEMS ANALYZED
BY MASS SPECTROMETRY

$$(CH_3)_2M + (CD_3)_2Hg \xrightarrow[X\ M \quad M\ X]{\text{THF} \atop 28°}$$

$(CH_3)_2M$	Concn., m./l.	Results
$(CH_3)_2Mg$	0.6	Exchange
$(CH_3)_2Zn$	2.0	Slight or no exch., 8 days
$(CH_3)_2Cd$	2.0	Slight or no exch., 8 days
$(CH_3)_2Hg$	2.0	Slight or no exch., 8 days

TABLE IIIb

$$(CH_3)_2Mg + (CD_3)_2Hg \xrightarrow{\text{THF}}$$
$$0.56\ M \qquad 0.87\ M$$

t, hours	Mole fraction $(CH_3)_2Hg$	CH_3HgCD_3	$(CH_3)_2Hg$	Exch., %
0	1.00	0	0	0
1	0.94	0.06	0	9.8
6	.77	.21	0.02	48
30	.44	.44	.12	91
175	.39	.46	.15	100

TABLE IIIc

$$\text{"CH}_3\text{MgBr"} + (CD_3)_2Hg \xrightarrow[0.59\ M \quad 28°]{\text{THF}}$$
$$0.64\ M$$

t, hours	Mole fraction $(CD_3)_2Hg$	CH_3HgCD_3	$(CH_3)_2Hg$	Exch., %
0	1.00	0	0	0
1	0.92	0.07	0.01	13
7	.68	.28	.04	55
31	.44	.44	.12	97
199	.42	.45	.13	100

pathways. If this conclusion can be extrapolated to the systems described above, which seems certain in the Hg–Hg case and likely in the Mg–Hg case, since R_3HgLi complexes are unknown[16] it would appear that the exchanges studied thus far occur by a mechanism which entails interchange of one group from each metal at a time. The present results suggest that an S_F2 exchange transition state is involved. It should be noted that an interchange involving migration of all four groups simultaneously is not strictly forbidden by this work; but it must be recognized that this type of exchange would not give rise to RHgR'. Reutov's surprising results[5] may possibly be explained by the thermodynamic instability of *p*-tolylphenylmercury under his isolation conditions or to distinct differences in the mechanistic pathways available to alkyl and arylmercury compounds in exchange reactions. The possibility of an ionic exchange mechanism in the group IIb–group IIa systems cannot be completely disregarded of course.

(16) G. Wittig. *Ann.*, **571**, 167 (1951).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY, CAMBRIDGE 38, MASS.]

A Conformation Examination of Poly-L-alanine and Poly-D,L-alanine in Aqueous Solution

BY WALTER B. GRATZER AND PAUL DOTY

A block polymer consisting of a central block of poly-L-alanine and two flanking blocks of poly-D,L-glutamic acid has been synthesized. Its water solubility permits the examination of the conformation of the poly-L-alanine in aqueous media: optical rotatory dispersion serves to establish the form of the poly-L-alanine since the glutamic acid blocks are optically inactive. It is found that this hydrophobic chain is in the form of an α-helix of extraordinary stability, although it lacks any basis of helix stabilization through interaction between side chains. Morever, the helical form is stable at 95°, and in detergent or 8 M urea solution. A random copolymer of similar composition is shown to have no helical conformation. Low molecular weight water-soluble poly-D,L-alanine was also examined. The far ultraviolet spectrum revealed a substantial hypochromicity in the band associated with the peptide chromophore, indicating the presence of some helical form. This too was resistant to heat, organic solvents and detergent. The relation of these results to the sources of the α-helix stability in proteins is discussed.

If synthetic polypeptides are to serve as valid models for understanding the complex structures of proteins, it is necessary to establish the conformational preferences of their chains in neutral aqueous solution, which approximates the native environment of most proteins. The fact is, however, that only a few polymers of the naturally occurring amino acids are soluble under these conditions; those that are, *viz.*, poly-L-glutamic acid,[1] poly-L-lysine[2] and poly-L-serine,[3] exhibit the randomly coiled, or disordered, form. The instability of the helical

conformation in these cases may be explicable in terms of electrostatic repulsions between charged groups or the presence of bulky or polar groups in the immediate proximity of the peptide bond. The question of the inherent stability in aqueous solution of the α-helix *per se* is thus left unanswered. The possibility must be considered then that other polyamino acids, particularly those made up of uncharged residues with small β-carbon substituents, might normally exist in the helical state in water solution. We have approached this problem, which is essentially one of bringing such insoluble polymers into solution, by synthesizing a three-component block copolymer in which the middle block is poly-L-alanine and the two flanking blocks poly-D,L-glutamic acid. Since the

(1) M. Idelson and E. R. Blout. *J. Am. Chem. Soc.*, **80**, 4631 (1958).

(2) J. Applequist and P. Doty. "Polyamino Acids, Polypeptides and Proteins," 1st International Symposium, Madison, 1961, Univ. of Wisconsin Press, Madison, Wis., 1962, p. 161.

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

two polar segments are optically inactive, the conformation of the poly-L-alanine block in aqueous solution can be established by optical rotatory dispersion methods.

In the light of the results obtained with the poly-L alanine blocks it became of interest to re-examine poly-D,L-alanine. At low degrees of polymerization this polypeptide is water soluble, but its conformation has not been clearly established.

A preliminary report of some of the results presented here has already been made.[4]

Experimental

Preparation of Block Polymers.—Polymers of the type poly-D,L-glutamic acid:poly-L-alanine:poly-D,L-glutamic acid were prepared as follows. n-Hexylamine was used to initiate the polymerization of the N-carboxyanhydride of γ-benzyl-D,L-glutamate; L-alanine N-carboxyanhydride was then added and this was followed finally by more γ-benzyl D,L-glutamate N-carboxyanhydride. The solvent was benzene, previously refluxed over sodium and distilled, and the initial N-carboxyanhydride concentration was 2%. The progress of the reaction was followed in trial runs by measuring the evolution of carbon dioxide.[5] In the preparative experiments each polymerization was allowed to proceed essentially to completion before the next anhydride was added in benzene solution.

The lengths of the blocks depended on the mole ratio of anhydride to initiator under the assumption that the polymer chains present at the second and third stages each initiated the polymerization of the new anhydride. It could not be assumed, however, that the chain lengths were defined by anhydride–initiator ratio alone, the relationship being complicated by a break in the kinetics of each polymerization stage,[6] and perhaps the presence of some inactive anhydride.

The final product was debenzylated by hydrolysis with gaseous HBr. Excess HBr was removed by prolonged passage of dry nitrogen and the benzyl bromide by Soxhlet extraction with acetone.[7]

The solubility of the resulting polymer in water demonstrates the absence of poly-L-alanine itself. Some purification was achieved by dissolving the polymer in 0.2 M disodium phosphate, dialyzing it against the same solvent, and acidifying the solution carefully to the point of precipitation (about pH 4.0). The precipitate was isolated and the supernatant discarded. The polymer was redissolved, dialyzed exhaustively against NaCl solution followed by water, and lyophilized.

A true copolymer of similar composition to the block polymer was prepared by n-hexylamine initiation of a mixture of the two N-carboxyanhydrides and debenzylation as described.

Several low molecular weight samples of poly-D,L-alanine were kindly provided by Dr. E. R. Blout and Mr. R. Karlson. These too were dialyzed against distilled water before use.

Copolymer Composition.—The amino acid compositions of the block polymers were determined with the Spinco model 120 amino acid analyzer. Acid hydrolyses of 48 and 72 hours gave concordant results. Moreover, the proportion of glutamic acid could also be determined by pH titration of solutions of known concentrations.

Hydrodynamic Measurements.—Sedimentation coefficients were determined in a standard manner with a Spinco model E ultracentrifuge, using Schlieren optics and a synthetic boundary cell. Sedimentation constants were determined over a concentration range of 0.33 to 1.5 g./100 ml. and extrapolated to zero concentration.

Intrinsic viscosities were determined from specific viscosity measurements made with an Ubbelohde viscometer at 25°. As in all other cases, concentrations were determined by Kjeldahl nitrogen analysis.

Molecular weights were estimated from sedimentation constants and intrinsic viscosities by the Scheraga–Mandelkern equation.[8]

Optical Rotatory Dispersion.—Optical rotations were measured in a Rudolph 200S photoelectric spectropolarimeter, using a General Electric AH-6 high pressure mercury arc as light source, and a 1P 28 photomultiplier as detector. In the heating experiments a 10-cm. jacketed polarimeter tube was used. Blank determinations to take account of strain in the cell windows and impurities in reagents were performed in all cases. Refractive indices were measured with an Abbé refractometer. For 8 M urea solutions only, a correction was made for wave length dispersion of refractive index.

Ultraviolet and Infrared Spectra.—Ultraviolet spectra in the range 185–250 mμ were obtained with a far ultraviolet Beckman DK-2 recording spectrophotometer, using 2-mm. path length cells with rapid nitrogen flushing below 200 mμ. The instrument was equipped with a hollow brass cell block through which thermostat fluid could be circulated.

A Perkin–Elmer model 21 spectrophotometer was used to observe the infrared spectra. All measurements were made in D_2O solution in 0.025-mm. path length cells with calcium fluoride windows.

Results

Block Polymers.—A number of block polymers were prepared before optimal results were obtained. It was first found that a single poly-D,L-glutamic acid block was inadequate to solubilize a poly-L-alanine block of comparable length in water. However, when a second poly-D,L-glutamic acid block was added a soluble product ensued. This observation is a good indication of the block-like nature of the product. It was noted also that the theoretical quantity of carbon dioxide was liberated at each polymerization stage, ensuring the absence of anhydride from the previous addition. Moreover, the absence of insoluble residue after polymerization of the L-alanine block indicated that the polymerization had in fact occurred at the N-terminal ends of the D,L-glutamic acid chains, poly-L-alanine itself being insoluble in benzene. The issue was placed beyond reasonable doubt by the results obtained on the random copolymer as described below. Thus it appeared that each block served quantitatively as an initiator for the subsequent block in a manner to be expected, in view of the previous reports of the effectiveness of the free amino groups of preformed polymers, and indeed proteins, in initiating polymerization.[9,10]

The polymer selected for investigation behaved normally in sedimentation. Extrapolation of sedimentation coefficients yielded a value of 4.45 S. for $s^0_{20,w}$ in 0.2 M Na_2HPO_4. Viscosity measurements in the same solvent gave $[\eta] = 19.6$ cc./g. The concentration dependence of the reduced specific viscosity was normal (Huggins' constant of 0.8) indicating that the molecules were completely dispersed since no dissociating effects were evident upon dilution. On the basis of the composition, the partial specific volume was computed to be 0.611. Using these results in the Scheraga–Mandelkern relation with a value of 2.5 × 10⁶ for $β$ led to a molecular weight estimate of 62,000.

The amino acid analysis showed that the mole ratio of glutamic acid to alanine was 1.54. Combining this with the molecular weight indicates that the average polymer molecule had a block of L-alanine consisting of about 175 residues connected to two blocks of D,L-glutamic acid composed of about 325 residues altogether.

Optical Rotation Measurements.—Most of the rotation data are shown in Fig. 1 in terms of Moffitt plots using the molar residue rotation, $[m'] = (3/100)M_0 \cdot [\alpha]/(n^2 + 2)$, M_0, the mean residue weight, is taken as 71, the residue weight of alanine, and the concentration used in determining the specific rotations is grams of L-alanine residues per 100 ml. Thus, the specific rotation applies directly to the L-alanine portion of the chain: it is assumed that no rotational effects arise from the D,L-glutamic acid regions.

The characteristic constants deduced from the plots in Fig. 1 are listed in Table I. A value of 212 mμ was used for λ_0 and helix estimates were based on a value of -630 for b_0.[11]

(4) P. Doty and W. B. Gratzer, ref. 2, p. 111.

(5) R. D. Lundberg and P. Doty. J. Am. Chem. Soc., **79**, 3961 (1957).

(6) P. Doty and R. D. Lundberg, ibid., **78**, 4810 (1956).

(7) E. R. Blout and M. Idelson, ibid., **78**, 497 (1956).

(8) H. A. Scheraga and L. Mandelkern, ibid., **75**, 179 (1953).

(9) A. Berger and A. Yaron, ref. 2, p. 13.

(10) H. Tsuyuki, H. Van Kley and M. A. Stahmann, J. Am. Chem. Soc., **78**, 764 (1956).

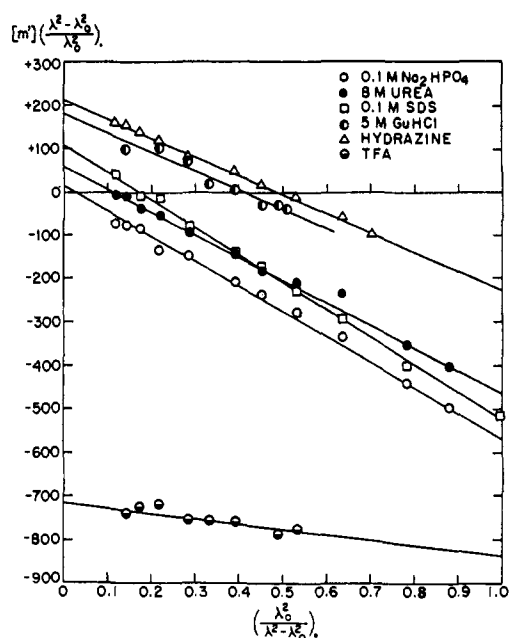(11) W. Moffitt and J. T. Yang, Proc. Natl. Acad. Sci. U. S., **42**, 596 (1956).

Fig. 1.—Moffitt plots for optical rotatory dispersion of a block polymer (poly-D,L-glutamic acid : poly-L-alanine : poly-D,L-glutamic acid) in various media. Molar residue rotations are computed on the basis of poly-L-alanine concentration, assuming no contribution from the racemic segments.

The results in neutral saline solution, i.e., 0.1 M Na_2HPO_4, show the alanine residues to be in essentially a completely helical conformation. The introduction of detergent, 0.1 M sodium dodecyl sulfate, does not reduce the helix content, and indeed leads to an increased b_0. This may reflect an environmental change in the optically active chromophore or increased helicity. In any case a similar phenomenon has been observed in proteins.[12] Urea (8 M) and 5 M guanidine hydrochloride, successively stronger denaturing agents, show only a modest denaturing effect. Thus one can conclude that the poly-L-alanine is quite stable in the α-helical form at room temperature; it is unaffected by detergent, and only slightly weakened by strong denaturing agents.

TABLE I

Solvent	a_0^H	$[\alpha]_D{}^a$	b_0	Per cent helix[b]
0.1 M Na_2HPO_4	+ 16	−15.1°	−581	92
0.1 M SDS + 0.1 M KCl^c	+110	+ 2.9	−634	101
8 M urea	+ 60	− 4.2	−523	83
5 M GuHCl	+182	+24.1	−442	70
Hydrazine	+212	+30.8	−383	61
TFA	−715	−153	−120	19

^{a} Interpolated from Moffitt plots. ^{b} Based on b_0 = −630 for 100% helix. ^{c} This solution was examined at 35° to prevent precipitation of the detergent.

To determine that the poly-L-alanine chain could be brought into the random solvated form under extreme conditions, the polymer was examined in hydrazine and trifluoroacetic acid solution. These are both highly polar, essentially randomizing solvents.[13,14] Here it is seen that the poly-L-alanine is only partly randomized in hydrazine but in trifluoroacetic acid the conversion is nearly complete. Indeed if the trifluoroacetic acid data are analyzed as a Drude plot, a value of 229 mμ is found for λ_c. This suggests that non-zero value of

(12) B. Jirgensons, *Arch. Biochem.*, **98**, 321 (1962).

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

(14) P. J. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961).
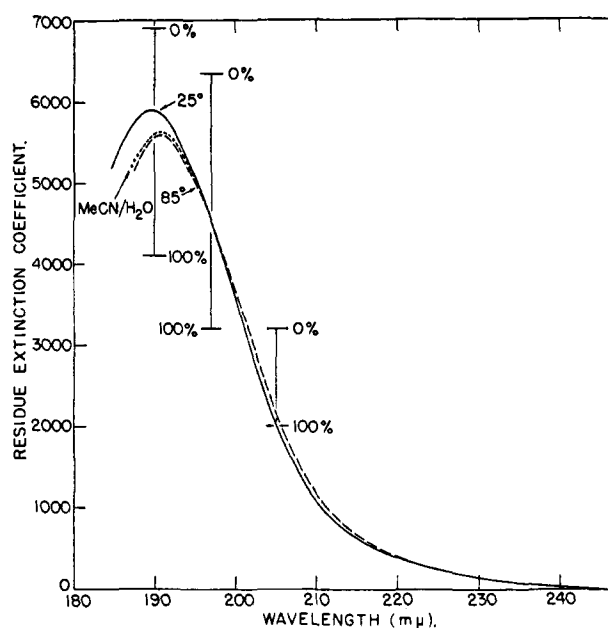
Fig. 2.—Ultraviolet absorption spectra of poly-D,L-alanine in water at 25° and 85°, and in an acetonitrile–water mixture (33 volume per cent acetonitrile). The bars show the extinction coefficients for polypeptide chains of 0 and 100% helicity. based on data of Rosenheck and Doty.[15]

b_0 in this case is probably an artifact[14] and the chain is completely disordered in this solvent.

The thermal stability of the helical conformation in Na_2HPO_4 solution was also examined. Using the wave length of 320 mμ, where the difference between the specific rotations of the helix and coil forms is great, the effect of raising the temperature was followed. Up to the highest temperature attainable, 95°, no significant change in rotation could be observed. Thus the stability of the helical form of poly-L-alanine over the entire temperature range is demonstrated.

Random Copolymer.—A true copolymer of L-alanine and D,L-glutamic acid was prepared and was shown by titration to have the composition L-ala:D,L-glu 1:2.5. The sedimentation constant, $s^0_{20,w}$, was 1.4 S. and the intrinsic viscosity 33.6 cc./g., with a Huggins constant of 0.36. Again taking β = 2.5 × 10^6 the Scheraga–Mandelkern equation gives a value of 7400 for the molecular weight.

In 0.2 M disodium phosphate solution, optical rotatory dispersion corresponded to b_0 = 0, indicating a wholly random structure, in complete contrast to the results with the block copolymer. Furthermore, it may be noted that at pH 4.13, where the glutamic acid side chains are almost completely protonated, the helix content, as judged by b_0, rose to 25% of net right-handed helix. This result appears to provide unambiguous proof of the block structure of the serially prepared polymer.

Poly-D,L-alanine.—This racemic polymer cannot profitably be studied by optical rotation because of the cancellation of contributions from left- and right-handed helices if these should be present. We therefore attempted to determine the helical content by measuring the degree of hypochromism associated with the peptide absorption band, centered at 190 mμ. The scope and reliability of this technique have been discussed elsewhere.[4,15]

Figure 2 shows the far ultraviolet absorption spectrum of poly-D,L-alanine in aqueous solution. Three

(15) K. Rosenheck and P. Doty. *Proc. Natl. Acad. Sci. U. S.*, **47**, 1775 (1961).

separate samples of the polymer all gave concordant results. On the scale set up in terms of poly-L-glutamic acid and poly-L-lysine in the helical and random states[14] (*i.e.*, $\epsilon_{max}^{helix} = 4100$; $\epsilon_{max}^{coil} = 6900$) the residue extinction coefficient at 190 mμ corresponds to a helix content of some 30–35%. However, it will be noted (Fig. 2) that the extinction coefficients at longer wave lengths give progressively higher values for the apparent helix content. The cause of this observation is not clear at this stage. However, the conclusion might be drawn that ordered structures are present which are not precisely α-helical.

In order to ascertain whether β-structures might be present, poly-D,L-alanine was dissolved in D_2O. The curious observation of Elliott[16] that the polymer dissolves with more difficulty in D_2O than in water was borne out. A part only of the polymer dissolved, the remainder forming a gel-like layer on centrifugation. This gel was examined by infrared spectroscopy and was found to exhibit a peak at 1620 cm.$^{-1}$, an amide frequency which has been associated with the β-form in aqueous systems.[17] The solution on the other hand showed only a peak at 1635 cm.$^{-1}$, and was therefore presumed to contain no appreciable concentration of β-structures.

The behavior of the ultraviolet absorption band on heating and in the presence of reagents was examined. Raising the temperature to 85° and the addition of either sodium dodecyl sulfate to 0.1 M or of acetonitrile to 33 volume per cent (these two reagents being among the few that are adequately transparent in this region of the spectrum) all gave rise to slightly decreased absorbance at 190 mμ. Isopropyl alcohol (30%) up to its cut-off point (*ca.* 205 mμ) also produced a small absorbance decrease. These observations would normally be taken to indicate a further slight increase in helix content, although in the case of heat some spreading of the absorption band is to be anticipated in any event.

Discussion

The principal conclusion of this investigation is that poly-L-alanine forms an α-helix of extraordinary stability in aqueous solution and that the helical conformation remains essentially unaltered by conventional denaturing agents or high temperatures. Naturally this poses the question of the origin and magnitude of the forces providing this stabilization.

The generally accepted view is that the free energy of formation of the α-helix in aqueous solution is slightly negative,[18,19] thereby providing a marginal stability which can be reinforced or eliminated by the net effect of side-chain interactions. On this basis one might at first thought assign to the methyl groups the source of interactions which would account for the substantial stabilization and accept this as an illustration of hydrophobic bonding.[19] However, the actual structure of poly-L-alanine does not permit this conclusion. In the α-helical form the methyl groups are separated by a distance of 5.7 Å. whereas the van der Waals radii are only 2.0 Å. Thus the methyl groups are too far apart to provide an attractive interaction.

It would appear that only two explanations are possible. One is that the α-helix, apart from side-chain interactions, is indeed considerably more stable than had been thought and that cases of instability are the consequence of adverse side-chain interactions. The other view is that the methyl groups do actually pro-

vide a source of stabilization, not through their own pairwise interaction, but rather through interactions with the two carbonyl groups that lie nearby in the next turn of the helix. The distance from the methyl carbon to each of the carbonyl oxygens is 3.0 Å. If a dispersive or dipole–induced dipole interaction were significant in this situation it would obtain in some degree for all amino acid residues except glycine and proline.

A simple calculation indicates that the polarizability-dipole interaction between the methyl group and a single carbonyl at the appropriate distance *in vacuo* would be about 0.3 kcal./mol. This, however, will be greatly diminished in a dielectric medium. The dispersion interaction energy was also calculated from the relation[20]

$$E = \frac{6mc^2}{Nr^6} \times \frac{\alpha_1\alpha_2}{\frac{\alpha_1}{x_1} + \frac{\alpha_2}{x_2}}$$

where α_1 and α_2 are the polarizabilities of the two groups, and x_1 and x_2 their diamagnetic susceptibilities; the other symbols have their usual meanings. This interaction energy for a pair of groups turns out to be about 0.2 kcal./mole. Thus a total of about 1 kcal./mole can be estimated as the maximum stabilization from combined dispersive and polarizability–dipole interactions between the methyl side chain and the two proximal backbone carbonyl groups.

If one adds this estimate to the estimated contribution from the hydrogen bond,[18] *viz.*, 1.5 kcal./mole, one obtains as a rough upper limit for the enthalpy of transfer of one residue from the random into the helical state a value of −2.5 kcal./mole. Assuming further an accompanying entropy change of −6 e.u./residue, as suggested by Peller,[21] a free energy of some −1.5 kcal./residue, at room temperature results. The corresponding equilibrium constant, which corresponds to the parameter s in the Zimm and Bragg theory,[22,23] is about 15, falling to about 1.5 at 90°.

We tentatively conclude that the side chain–backbone interactions are insufficient to stabilize the helix appreciably, and that the α-helix *per se* should in consequence be regarded as an innately stable structure in aqueous solution, stabilized by energy barriers associated with the peptide backbone.

It should be added, however, that the stabilization of the helical form could in principle arise from the entropy change being less positive and the enthalpy change being less negative. This situation could arise if the loss in entropy due to water being immobilized through solvation on the coil form compensated the configurational entropy gain, with the result that the observed result would be consistent with a small or even negligible enthalpy change accompanying the transition. This review seems rather unlikely to us and can only be decided by experiments directed to the origin of the stability that has been observed.

The considerable stability of the α-helix having been established for poly-L-alanine, the interpretation of the situation for poly-D,L-alanine should be somewhat simplified. This polymer was at one time supposed to be fully helical, and was indeed used as a model for the α-helix in studying deuterium exchange.[24] However, more recent examinations by the same technique[25] as well as by optical rotation studies on a series of D- and

(16) A. Elliott, *Nature*, **170**, 1066 (1952).

(17) P. Doty, K. Imahori and E. Klemperer, *Proc. Natl. Acad. Sci. U. S.*, **44**, 424 (1958).

(18) J. A. Schellman, *Compt. rend. trav. Lab. Carlsberg, Ser. Chim.*, **29**, 223 (1955).

(19) W. Kauzmann, *Advan. Protein Chem.*, **14**, 1 (1959).

(20) L. Salem, *Mol. Phys.*, **3**, 441 (1960).

(21) L. Peller, *J. Phys. Chem.*, **63**, 1149 (1959).

(22) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).

(23) B. H. Zimm, ref. 2, p. 229.

(24) A. Berger and K. U. Linderstrøm-Lang, *Arch. Biochem.*, **69**, 106 (1957).

(25) W. P. Bryan and S. O. Nielsen, *Biochim. Biophys. Acta*, **42**, 552 (1960).

L-alanine copolymers[26,27] have indicated that the *meso*-polymer is not predominantly helical. Nevertheless, the results with poly-L-alanine described here make it difficult to imagine that no helical structure would be present. Moreover, Wada[28] has recently shown that as a consequence of the greater affinity of a growing D,L-polymer chain for a monomer unit of the same configuration as the terminal group, poly-γ-benzyl-D,L-glutamate is substantially helical, being composed of many short regions of right- and left-handed helices. It is consistent with the observations made here that a similar situation holds for poly-D,L-alanine. The failure of the molecule to take up a completely helical conformation could be due to the fact that the closest methyl–methyl distance is such a helix, 3.3 Å., requires too much interpenetration of van der Waals radii, resulting thereby in steric hindrance rather than an attractive interaction.

The tentative conclusion that poly-D,L-alanine has perhaps one-third of its residues in the form of short helices carries with it the corollary that the helical form is indeed very stable. This follows from the fact that for a given polypeptide–solvent system, helix

(26) A. R. Downie, A. Elliott, W. E. Hanby and R. R. Malcolm, *Proc. Roy. Soc.* (London), **A242**, 325 (1957).

(27) A. Elliott, ref. 2, p. 119.

(28) A. Wada, *J. Mol. Biol.*, **3**, 507 (1961).

stability decreases with decreasing molecular weight.[22,23,29] Thus for quite short regions of the chain to form helices requires that longer chains of a pure enantiomorph will form very stable helices indeed.

The conclusions concerning the very considerable stability of the α-helical conformation in the two polymers of alanine have a direct bearing on the problem of the basis for the stability of this conformation in proteins. As previously remarked, the enthalpy of formation for the helix must be much greater than around 1–2 kcal./mol in order to account for the existence of short helical regions in proteins.[29] The present work suggests that the source of this greater stability resides in the polypeptide backbone.

If further experiments support the view developed here, the helical conformation will have to be considered as quite stable and the role of side chains as being primarily sources of weakness or instability.

(29) B. H. Zimm, P. Doty and K. Iso, *Proc. Natl. Acad. Sci. U. S.*, **45**, 1601 (1959).

The Elucidation of the Reaction of Benzohydroxamic Acid with Benzenesulfonyl Chloride and with Diisopropyl Phosphofluoridate Using Oxygen-18 as Tracer

BY DAVID SAMUEL AND B. L. SILVER

The reaction of benzohydroxamic acid with benzenesulfonyl chloride and with diisopropyl phosphofluoridate has been investigated using O^{18}-enriched water as solvent. The absence of oxygen-18 in any of the products confirms the mechanism in which the hydroxamic acid is first sulfonated or phosphorylated to form an unstable intermediate which breaks down by a Lossen type rearrangement to give the products.

A number of compounds have recently been studied in an attempt to counter the toxicity of certain organophosphorus compounds.[1] Among the most effective of these are the anions of hydroxamic acids first suggested by Wagner-Juaregg and co-workers.[2a,b]

It was found[2] that various substituted hydroxamic acids cause a rapid increase in the rate of breakdown of benzenesulfonyl chloride and of diisopropyl phosphofluoridate in slightly basic solutions. The increase in rate at room temperature and pH 7.6 was found[2b] to be many hundredfold. The products of the reaction were benzenesulfonic acid (or diisopropyl hydrogen phosphate) and the "dihydroxamic acid," namely, phenylcarbamoyl benzohydroxamate. On the basis of the earlier work of Hurd and Bauer on the Lossen rearrangement of hydroxamic acids[3] the following mechanism for this reaction in aqueous alkaline solution was suggested.[2]

$$\text{(A)} \quad C_6H_5CONHOH + ArSO_2Cl \xrightarrow{-HCl}$$

$$C_6H_5CONHOSO_2Ar \xrightarrow{-ArSO_3H} C_6H_5NCO \xrightarrow{PhCONHOH}$$

$$\text{I} \qquad\qquad\qquad\qquad C_6H_5CONHOCONHPh$$

(1) R. D. O'Brian, "Toxic Phosphorus Esters," Academic Press, Inc., New York, N. Y., 1960.

(2) (a) B. E. Hackley, Jr., R. Plapinger, M. Stolberg and T. Wagner-Juaregg, *J. Am. Chem. Soc.*, **77**, 3651 (1955); (b) M. A. Stolberg, R. C. Twelt, G. M. Steinberg and T. Wagner-Juaregg, *ibid.*, **77**, 765 (1955).

(3) C. D. Hurd and L. Bauer, *ibid.*, **76**, 2791 (1954).

$$\text{(B)} \quad C_6H_5CONHOH + (i\text{-PrO})_2P(O)F \xrightarrow{-HF}$$

$$C_6H_5CONHOP(O)(i\text{-PrO})_2 \xrightarrow{-(i\text{-PrO})_2PO_2H}$$

$$\text{II}$$

$$C_6H_5NCO \xrightarrow{PhCONHOH} C_6H_5CONHOCONHPh$$

The unstable condensation intermediates (I or II) were not isolated, since they immediately undergo the reaction found by Hurd and Bauer[3] giving the free oxy-acid and an aryl isocyanate which condenses with another molecule of hydroxamic acid.

Since in mildly basic solution this reaction competes with the hydrolysis of the acyl halides, it appears that hydroxamic acids can, in effect, catalyze the decomposition of arylsulfonates and of diisopropyl phosphofluoridate in aqueous solution. The kinetics of the reaction of analogous phosphofluoridates with benzohydroxamic acid were determined by Swidler and Steinberg,[4] who found that the stoichiometry and the rate of formation of fluoride and of acid were consistent with the scheme shown above. A more extensive kinetic investigation of the reaction related[5] the dissociation constant of various nucleophilic reagents including hydroxamic acids with their activity in the hydrolysis of toxic organophosphorus compounds.

(4) R. Swidler and G. M. Steinberg, *ibid.*, **78**, 3594 (1956).

(5) A. L. Green, G. L. Sainsbury, B. Saville and M. Stansfield, *J. Chem. Soc.*, 1583 (1958).