mg. of the hexadeuterio ketone VII, which was used directly for mass spectrometry (shaded lines in Fig. 3), the introduction of six deuterium atoms having been established by the molecular ion at *m/e* 404. A silica gel thin-layer chromatogram (acetone–ethyl acetate 1:4) demonstrated the presence of some deacetylated material, which accounts for the peaks at *m/e* 359 (2,4,4-trideuteriodeacetyl derivative), 331 (loss of CO) and 302 (loss of CO and $C_2H_5$).

**Spegazzinidine Dimethyl Ether 3-Tosylate (VIII).**—Freshly recrystallized *p*-toluenesulfonyl chloride (150 mg.) was added to an ice-cold solution of 175 mg. of spegazzinidine dimethyl ether (IV) in 5 cc. of dry pyridine and the resulting pink solution was left standing at room temperature overnight. Dilution with water and extraction with methylene chloride produced a purplish residue (233 mg.) which was chromatographed on 10 g. of neutral alumina (activity IV) and eluted with benzene. Recrystallization from methylene dichloride–isopropyl ether afforded 140 mg. of colorless crystals, m.p. 158–160°, $[\alpha]^{22}D$ +42°; $\lambda_{max}^{CHCl_3}$ 6.02, 6.25 and 8.5 $\mu$.

*Anal.* Calcd. for $C_{30}H_{38}N_2O_6S$: C, 64.96; H, 6.91; S, 5.79. Found: C, 65.01; H, 6.85; S, 6.02.

**Interrelation of Spegazzinidine and Pyrifolidine.**—A solution of 143 mg. of pyrifolidine (antipode of V)[3c] in 10 cc. of ether was added dropwise to 0.5 g. of lithium aluminum hydride suspended in 20 cc. of the same solvent. After heating under reflux for 8 hr., excess reagent was decomposed by the dropwise addition of an aqueous saturated sodium sulfate solution and the product extracted with ether. The substance (antipode of IX) could not be crystallized and it was distilled at 115–120° (7 × $10^{-6}$ mm.), The infrared spectrum showed complete absence of the amide carbonyl band and thin-layer chromatography (1:1 acetone–ethyl acetate on silica gel using ceric sulfate reagent for spotting) revealed the presence of a single spot ($R_f$ 0.72); $\lambda_{max}^{EtOH}$ 218, 263 and 305 m$\mu$; log $\epsilon$ 4.36, 3.80 and 3.48; $[\alpha]^{22}D$ +19.8°.

*Anal.* Calcd. for $C_{23}H_{34}N_2O_2$: C, 74.55; H, 9.25; mol. wt., 370. Found: C, 74.23; H, 9.16; mol. wt., 370 (mass spec.).

When the reduction of 172 mg. of the tosylate VIII was performed in the same manner, there was obtained 86 mg. of crude product, which exhibited two spots in a thin-layer chromatogram ($R_f$ 0.72 and 0.51). The mixture was chromatographed on 10 g. of neutral alumina (activity IV) and the first fraction (32 mg.) eluted with benzene was distilled at 100–110° (3 × $10^{-6}$ mm.). The distillate represented 16-methoxy-N-deacetyl-N-ethylaspidospermine (IX) and proved to be identical in all respects (thin-layer chromatographic mobility, infrared and mass spectra) except for sign of rotation ($[\alpha]D$ −20.6°) with the above antipode derived from (+)-pyrifolidine.

*Anal.* Found: C, 74.51; H, 9.30; mol. wt., 370 (mass spec.).

**2-Deuteriostrychanone (Xb).**—Strychanone Xa (20 mg.) was heated under reflux in a nitrogen atmosphere with the same deuterium exchange mixture (NaOD–$D_2O$) described above. The procedure was repeated three times and the usual work-up afforded 15 mg. of crystalline residue, which was separated into two portions. One portion was used directly for mass spectrometry, while the other was warmed at 45° for 2 hr. with 10 cc. of 95% aqueous methanol, then taken to dryness and submitted for mass spectrometry. Both samples gave identical mass spectra with a molecular ion peak at *m/e* 283, indicating the incorporation of one deuterium atom.

**Acknowledgment.**—We are indebted to the National Institutes of Health of the U. S. Public Health Service for financial assistance (grants No. 2G-682 and A-4257) and to Prof. José F. Molfino for help with the botanical collection.

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

# Studies on Synthetic Polypeptide Antigens. VI. The Synthesis and Physical Chemical Properties of a New Group of Linear-chain Antigens[1,2]

BY EDWARD FRIEDMAN, THOMAS J. GILL III[3] AND PAUL DOTY

RECEIVED MARCH 19, 1962

A group of synthetic polypeptides of immunological interest containing different combinations and proportions of L-glutamic acid, L-lysine, L-tyrosine, L-phenylalanine and L-alanine has been synthesized and the physical chemical properties determined. The polymers were in the molecular weight range of 30,000 to 550,000 and exhibited helical contents varying from 0–40% helix as determined by optical rotatory dispersion; all of the polymers were studied under physiological conditions of *p*H and salt. The intrinsic viscosities and sedimentation coefficients of the polymers showed a good correlation with the degrees of polymerization. Alanine residues were found to increase the amount and stability of the $\alpha$-helical conformation in aqueous solution. One copolymer of L-glutamic acid, L-lysine and L-tyrosine in a mole ratio of 51:33:16 was found to associate in solution as a result of conversion to the $\beta$-form in a manner analogous to protein denaturation.

## Introduction

Several of the preceding papers in this series[4–6] have described the immunological and physical chemical properties of a group of linear synthetic polypeptides. The present paper will describe the synthesis and physical chemical properties of a new group of polypeptide antigens of immunological interest.[7] Three of the new polypeptides contained alanine so that the effect of a residue with an aliphatic side chain on the physical chemical and antigenic properties could be studied. Copolymers composed mainly of L-lysine or L-glutamic acid and small amounts of either L-alanine or L-tyrosine were studied to determine whether or not small amounts of alanine or tyrosine could convert the non-antigenic poly-L-lysine and poly-L-glutamic acid into antigens.[7] The properties of additional copolymers of L-glutamic acid, L-lysine and either L-tyrosine or L-phenylalanine were also studied.[6]

The large variation in composition of these polymers also afforded the opportunity to investigate the effects of different residues on the helical stability of the polypeptides.

## Experimental

**Polymer Preparation.**—The method of polymerization has been previously described in detail.[6] The polymers were prepared by the reaction of the appropriate mixtures of the

(1) Work supported by a grant from the National Science Foundation (G-7487).

(2) Preceding paper in this series; W. F. Anderson and T. J. Gill III, *Biochim. et Biophys. Acta*, **58**, 558 (1962).

(3) Junior Fellow of the Society of Fellows, Harvard University.

(4) T. J. Gill III and P. Doty, *J. Mol. Biol.*, **2**, 65 (1960).

(5) T. J. Gill III and P. Doty, *J. Biol. Chem.*, **236**, 2677 (1961).

(6) E. Friedman, T. J. Gill III, and P. Doty, *J. Am. Chem. Soc.*, **83**, 4050 (1961).

(7) The immunological properties will be described in an article by T. J. Gill III and P. Doty (in preparation).

TABLE I

COMPOSITION OF THE POLYMERS

| No. | Polymer symbol | Starting mole per cent. Glu | Lys | Phe | Tyr | Ala | Final mole per cent. Glu | Lys | Phe | Tyr | Ala | Final weight per cent. Glu | Lys | Phe | Tyr | Ala |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9A | $G_{56}L_{43}T_1$-2 | 58 | 41 | .. | 1 | .. | 56.0 | 42.6 | .. | 1.4 | .. | 53.9 | 44.7 | .. | 1.4 | .. |
| 11 | $G_{59}L_{40}P_1$ | 58 | 41 | 1 | .. | .. | 58.6 | 40.3 | 1.1 | .. | .. | 56.6 | 42.4 | 1.0 | .. | .. |
| 13 | $G_{51}L_{33}T_{16}$ | 52 | 32 | .. | 16 | .. | 51.3 | 32.5 | .. | 16.2 | .. | 49.2 | 34.0 | .. | 16.8 | .. |
| 18 | $G_{96}T_4$ | 96 | .. | .. | 4 | .. | 96 | .. | .. | 4 | .. | 95.7 | .. | .. | 4.3 | .. |
| 19 | $L_{70}A_{30}$ | .. | 70 | .. | .. | 30 | .. | 70 | .. | .. | 30 | .. | 84.4 | .. | .. | 15.6 |
| 20 | $L_{96}T_4$ | .. | 96 | .. | 4 | .. | .. | 96 | .. | 4 | .. | .. | 96.0 | .. | 4.0 | .. |
| 21 | $G_{70}A_{30}$ | 70 | .. | .. | .. | 30 | 70 | .. | .. | .. | 30 | 83.2 | .. | .. | .. | 16.8 |
| 22 | $G_{42}L_{28}A_{30}$ | 42 | 28 | .. | .. | 30 | 42 | 28 | .. | .. | 30 | 48.5 | 35.2 | .. | .. | 16.3 |

TABLE II

PROPERTIES OF THE POLYMERS

| No. | Polymer symbol | % N | $\bar{v}$ | R.W.[a] | $[\eta]$ | $S^0_{20,w}$ | Mol. wt. | D.P.[b] | $\frac{S^0_{20,w}}{1-\bar{v}\rho}$ | $b_0$ | $[\alpha]_D$ | $a_0$ | % helix[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9A | $G_{56}L_{43}T_1$-2 | 12.56 | 0.67 | 156.9 | 0.434 | 2.72 | 59,000 | 376 | 8.20 | −111 | −70 | −571 | 20 |
| 11 | $G_{59}L_{40}P_1$ | 12.40 | .66 | 156.4 | .682 | 3.09 | 87,000 | 556 | 9.20 | −170 | −80 | −656 | 25 |
| 13 | $G_{51}L_{33}T_{16}$ | 11.68 | .67 | 157.3 | .312 | 1.97 | 31,000 | 197 | 5.92 | −32 | −68 | −559 | 5 |
| 18 | $G_{96}T_4$ | 9.24 | .57 | 151.5 | 1.827 | 3.38 | 114,000 | 752 | 7.92 | +63 | −97 | −805 | 0 |
| 19 | $L_{70}A_{30}$ | 17.83 | .78 | 136.5 | 2.765 | 4.37 | 564,000 | 4130 | 20.0 | −58 | −108 | −776 | 10 |
| 20 | $L_{96}T_4$ | 16.68 | .79 | 164.4 | 2.228 | 4.14 | 487,000 | 2960 | 19.5 | +9 | −93 | −820 | 0 |
| 21 | $G_{70}A_{30}$ | 12.41 | .60 | 127.0 | 1.078 | 2.51 | 61,000 | 480 | 6.20 | −9 | −129 | −890 | 0 |
| 22 | $G_{42}L_{28}A_{30}$ | 14.58 | .67 | 130.8 | 0.692 | 2.69 | 74,000 | 566 | 8.23 | −265 | −72 | −471 | 40 |

[a] R.W. is the residue weight. [b] D.P. is the degree of polymerization. [c] Calculated from the $b_0$-values.

N-carboxy anhydrides of the α-L-amino acids at a total concentration of 1 g./100 cc. in benzene and an anhydride:initiator mole ratio of 400. The polymers to be described will be designated by formulas in which G refers to glutamic acid, L, to lysine, T, to tyrosine, P, to phenylalanine and A, to alanine. The subscripts refer to the mole percentage of each amino acid residue. A non-subscript number indicates a subsequent preparation having the same composition as a previous sample.

The polymerizations of $G_{56}L_{43}T_1$-2 and $G_{59}L_{40}P_1$ were stopped after 36 minutes when they were 90% complete. The polymerizations of $G_{96}T_4$, $L_{70}A_{30}$, $L_{96}T_4$, $G_{70}A_{30}$ and $G_{42}L_{28}A_{30}$ (Pilot Chemical Co., Watertown, Mass.) were allowed to proceed to completion. $G_{51}L_{33}T_{16}$ was prepared with an anhydride:initiator ratio of 75 and the reaction was stopped after 13 minutes, when kinetic studies showed that the reaction was 90% complete.

The polypeptides, which were all soluble in water, were dialyzed against 5 changes of 0.05 *M* NaCl at *p*H 7.6 and then against 4 changes of distilled water at *p*H 7.6. The polymer solutions were then filtered through a medium porosity sintered glass filter, lyophilized, and stored at room temperature until further use.

**Check for Removal of Protecting Groups.**—The protecting groups of L-glutamic acid, L-lysine and L-tyrosine were removed by bubbling hydrogen bromide through the reaction mixture.[6] The infrared spectrum of each polymer dissolved in $D_2O$ at *p*D 7–8 was taken using a Perkin–Elmer model 21 double beam spectrophotometer and the removal of protecting groups was established by the absence of an ester band at 1700–1730 cm.$^{-1}$. The absence of protecting groups in $L_{70}A_{30}$, $G_{70}A_{30}$ and $G_{42}L_{28}A_{30}$ was also established by the absence of aromatic absorption in the ultraviolet spectrum (240–300 mμ) of 0.25 g./100 cc. solutions taken using a Beckman DK2 recording spectrophotometer.

**Amino Acid Analysis.**—The compositions of $G_{56}L_{43}T_1$-2, $G_{59}L_{40}P_1$ and $G_{51}L_{33}T_{16}$ were determined by analysis with the Spinco amino acid analyzer according to the procedure of Spackman, Stein and Moore.[8] The other polymers were not analyzed since they were polymerized to completion. A comparison between the compositions of the reaction mixtures and the polymers is shown in Table I.

**Molecular Weight Determinations.**—All of the polymers were dissolved in 0.15 *M* saline–phosphate buffer at *p*H 7.6, except for $G_{51}L_{33}T_{16}$ which was dissolved in 0.15 *M* carbonate at *p*H 10.0, because of its tendency to precipitate at lower *p*H's. The sedimentation velocity of each polymer was determined at 25° in the Spinco model E Ultracentrifuge using schlieren optics and its intrinsic viscosity was determined at 23.4° using an Ubbelhode viscometer. The partial specific volumes of the polymers, including the appropriate counterions, were calculated in the manner previously described.[6] Using these data, the molecular weights were calculated from the Scheraga–Mandelkern equation using a β-value of $2.5 \times 10^6$, which is the value for a random coil.[9] The existence of some helical structure does not affect the β-value since the short helical regions would not significantly alter the spacial distribution of monomeric units. The physical chemical data are summarized in Table II.

**Anomalous Behavior of $G_{51}L_{33}T_{16}$.**—The polypeptide richest in tyrosine, $G_{51}L_{33}T_{16}$, was soluble at *p*H 7.6 to about the same extent as the other polypeptides, but its properties were strongly time dependent at this *p*H. For example, the viscosity of a 1.2 g./100 cc. solution increased over a 7-day period as shown in Fig. 1; the solvent flow time under the same conditions was 113.5 seconds. Violent agitation would cause the flow time to diminish sharply.

After 2–3 weeks the solution of $G_{51}L_{33}T_{16}$ formed a thick gel. The rate of gelation under several different conditions was studied. Aliquots of a 1 g./100 cc. solution were placed at 3° and 40° respectively, and the rate of gelation was found to be much faster at 40° than at 3°. The flow time of a 1 g./100 cc. solution in 8 *M* urea decreased slowly over a period of 4 days and then leveled off. The effects of salt and high temperature were studied by preparing two 1.2 g./100 cc. solutions of the polymer at *p*H 7–8, one in water and one in 2 *M* NaCl. Both solutions were heated to boiling for 45 minutes and then slowly cooled to room temperature. The salt solution had formed a thick gel after standing for 16 hours and the water solution had become more viscous but had not formed a gel. The infrared spectrum in $D_2O$ at *p*D 7.35 showed an increase in absorption at 1610 cm.$^{-1}$ over a period of 18 days, indicating the formation of β-structure during this period.[10–13] A typical set of spectra is shown in Fig. 2.

(8) D. H. Spackman, W. A. Stein and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(9) H. A. Scheraga and L. Mandelkern, *J. Am. Chem. Soc.*, **75**, 179 (1953).

(10) H. Lenormant, A. Baudras and E. R. Blout, *ibid.*, **80**, 6191 (1958).

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

(12) E. R. Blout and M. Idelson, *J. Am. Chem. Soc.*, **80**, 4909 (1958).

(13) J. Applequist and P. Doty. "Polyamino Acids, Polypeptides and Proteins," ed. by M. A. Stahmann, Univ. of Wisc. Press, Madison, 1962, p. 161.

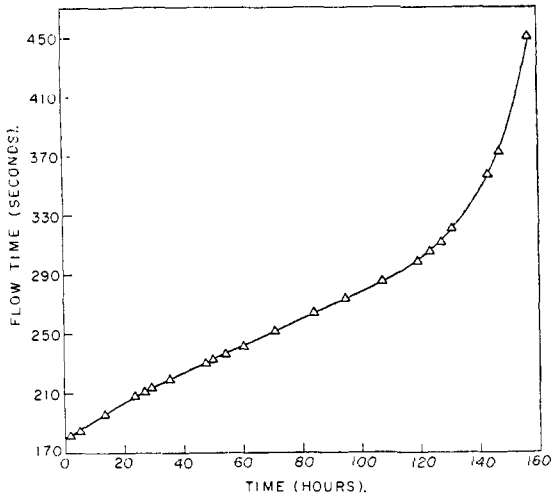


Fig. 1.—Flow time of a 1.2 g./100 cc. solution of $G_{51}L_{33}T_{16}$ at *p*H 7.6 in 0.15 *M* saline-phosphate buffer showing its increase as a function of time.

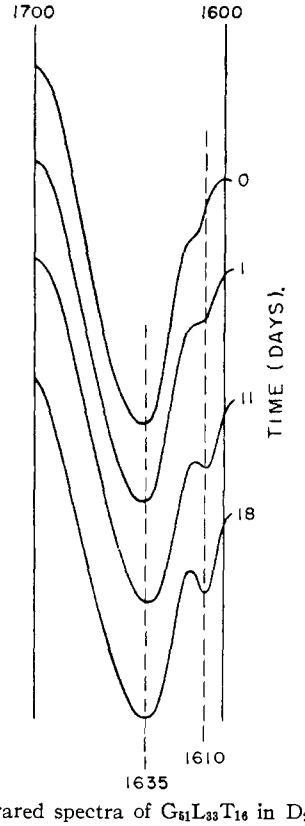


Fig. 2.—Infrared spectra of $G_{51}L_{33}T_{16}$ in $D_2O$ at *p*D 7.35 taken immediately after solution and 1, 11 and 18 days later. The increase in amplitude of the absorption band at 1610 cm.$^{-1}$ with time indicates the gradual formation of $\beta$-structure.

To determine the solubility of the polymer as a function of *p*H, another solution of $G_{51}L_{33}T_{16}$ was titrated with 0.2 *M* HCl. The solution became cloudy when the *p*H was lowered below 6.0 and precipitation occurred in increasing degree as the *p*H was lowered to 4.7. Below this value the solution partially cleared.

**Optical Rotatory Dispersion.**—The dispersion measurements were made on the Rudolph model 80S spectropolarimeter using a Sylvania "concentrated arc" zirconium lamp over the wave length range 320–620 m$\mu$. The polymers were

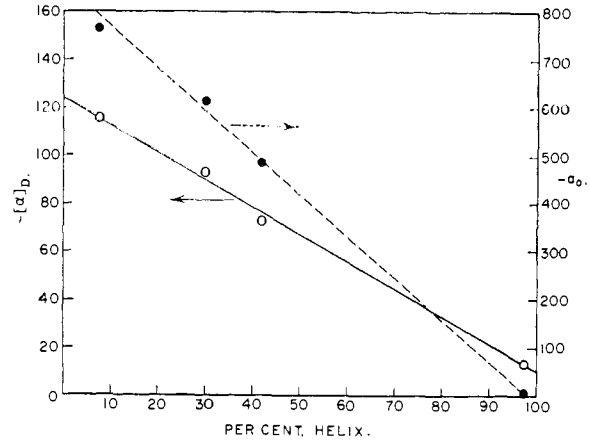


*Fig. 3.—Variation of* $-[\alpha]_D$ *and* $-a_0$ *with helical content* for the copolymer $G_{42}L_{28}A_{30}$. The open circles represent data for $-[\alpha]_D$ and the solid circles for $-a_0$. Both of the parameters decrease as the helical content increases.

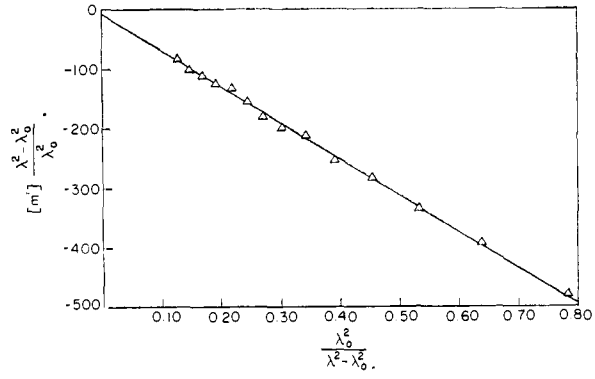

Fig. 4.—Optical rotatory dispersion of $G_{42}L_{28}A_{30}$ at *p*H 1.4 plotted according to Moffitt's equation.[14]

dissolved in the same solvents used for the molecular weight determinations to give 0.20–0.25 g./100 cc. solutions. The constants $a_0$ and $b_0$ from the Moffitt equation[14] were calculated from the data, assuming the refractive indices of the solution to be independent of wave length over the range studied, and are listed in Table II together with specific rotation $[\alpha]_D$.

Since the specific rotations and the $a_0$-values are dependent on the effects of solvation, segmental interactions and side chain chromophores, the values of $b_0$ were considered to be a better index of $\alpha$-helical structure[15] and the helical contents of the polymers so calculated are listed in Table II.

An extended study of the dispersion of $G_{42}L_{28}A_{30}$ was undertaken to determine the effect of alanine on the stability of the helical conformation. The polymer was soluble over the entire *p*H range studied except between *p*H 4.5–4.9. Dispersion measurements were made at *p*H 1.4 and 11.7 and in 8 *M* urea using a General Electric AH 6 high pressure mercury lamp. The values of $a_0$, $b_0$, $[\alpha]_D$ and the percentage helix calculated from the $b_0$-values are listed in Table III; the $[\alpha]_D$ at *p*H 3.75 is also listed. Figure 3 shows the plots of $a_0$ and $[\alpha]_D$ against helical content and Fig. 4 shows the Moffitt plot of $G_{42}L_{28}A_{30}$ at *p*H 1.4.[14]

## Discussion

**Correlation of $S^\circ_{20,w}$ and $[\eta]$ with Degree of Polymerization.**—When a polypeptide is composed primarily of L-glutamic acid and L-lysine at approximately a 3:2 mole ratio, the mean conformation

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

(15) P. Doty, "Fourth International Congress of Biochemistry," VIII, 1958, Vienna, Pergamon Press, New York, N. Y., 1959, p. 8.

TABLE III
OPTICAL ROTATORY DISPERSION DATA OF $G_{42}L_{28}A_{30}$ IN DIFFERENT SOLUTIONS

| Solution | $a_0$ | $b_0$ | $[\alpha]_D$ | % helix[a] |
|---|---|---|---|---|
| *p*H 1.4 | −7 | −609 | −14 | 95 |
| *p*H 3.75 | | | −13 | |
| *p*H 7.6 | −471 | −265 | −72 | 40 |
| *p*H 11.65 | −619 | −185 | −93 | 30 |
| 8 *M* urea | −767 | −47 | −116 | 5 |

[a] Calculated from $b_0$ measurements.

and degree of expansion of the polymer coil should not be greatly influenced by modest amounts of either a third component or helical sections. Consequently, $G_{56}L_{43}T_1$-2, $G_{59}L_{40}P_1$ and $G_{42}L_{28}A_{30}$ and five copolymers previously studied[6] should show a common dependence of the sedimentation constant and intrinsic viscosity on chain length. To check on this, the data for the three relevant polymers from this study and the five from the previous study are plotted in Fig. 5. The de-

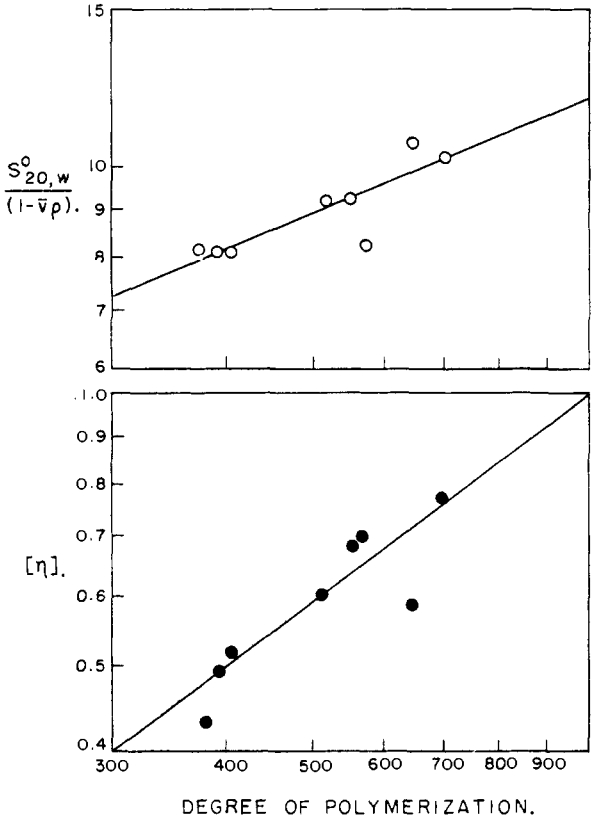


Fig. 5.—$S^\circ_{20,w/(1-\bar{v}\rho)}$ and $[\eta]$ as a function of the degree of polymerization for a series of copolymers containing L-glutamic acid and L-lysine in approximately a 3:2 molar ratio at *p*H 7.6 in 0.15 *M* saline–phosphate buffer. Open circles represent the sedimentation data and the solid circles the viscosity data.

pendence is seen to be linear and the least squares lines correspond to the relations

$$\frac{S^\circ_{20,w}}{(1-\bar{v}\rho)} = 0.77\ \text{D.P.}^{0.39} \quad (1)$$

$$[\eta] = 0.0061\ \text{D.P.}^{0.73} \quad (2)$$

Thus, our expectation is borne out and the resulting empirical relations should be useful in estimating the degrees of polymerization of copolymers of this general nature when studied in the solvent employed here.

**Observations on the Relationship of Composition to Conformation in Aqueous Solution.**—The optical rotatory dispersion results for the four copolymers that contained only two components did not indicate anything unusual. The origin of the small positive value of $b_0$ for $G_{96}T_4$ and the small negative value for $L_{70}A_{30}$ are compatable with zero helical contents, although there is 10% helix calculated from the usual scale in the latter polymer.[15] Urnes and Doty[16] have pointed out that if $\lambda_c$ is different from $\lambda_0$ for a given polypeptide, the value of $b_0$ consistent with no helical structure would be different from zero. The values of $\lambda_c$ for poly-L-lysine and poly-L-glutamic acid previously studied[6] were 218 and 205 m$\mu$, respectively, and hence it is not surprising that $L_{70}A_{30}$ and $G_{96}T_4$ have $b_0$-values different from zero.

The copolymer $G_{56}L_{43}T_1$-2 has essentially the same physical chemical properties, except for molecular weight, as the previous preparation $G_{56}L_{43}T_1$-1.[6]

The alanine-containing copolymer $G_{42}L_{28}A_{30}$ has nearly the highest alanine content consistent with water and saline solubility and represents an interesting protein model since it contains a proportion of hydrophobic groups comparable to many proteins. The most evident effect of the 30% alanine is to increase considerably the helical content of the polypeptide. In 8 *M* urea, the value of $\lambda_c$ was found to be 218 m$\mu$, thereby making the observed value of $b_0(-47)$ compatible with no helical structure. Thus, this polypeptide is seen to denature completely in urea.

The behavior of $G_{42}L_{28}A_{30}$ at the extremes of *p*H was particularly interesting. On the acid side of the isoelectric point, where there is a net positive charge on 28% of the residues, the helical conformation increased to include essentially the whole molecule. On the alkaline side, where there is a net negative charge of 42%, the helical content diminished to 30%. These results are in contrast to those obtained by Doty, Imahori and Klemperer[11] and by Blout and Idelson.[12] Doty, Imahori and Klemperer observed that a 50:50 copolymer of L-glutamic acid and L-lysine was 50% helix in acid solution, 15% helix at neutrality and 0% helix at *p*H 12. Subsequently, Blout and Idelson observed that another 50:50 copolymer of L-glutamic acid and L-lysine was 50% helix at *p*H 3 and 30% helix at *p*H 8. The latter investigators also reported that a 60:40 copolymer of L-glutamic acid and L-lysine was 70% helix at *p*H 3 and 25% helix at *p*H 8. All of these studies indicate that glutamic acid residues form a more stable helix than lysine residues and the studies with $G_{42}L_{28}A_{30}$ reported here show that the alanine residues further stabilize the $\alpha$-helix in aqueous solution over the entire *p*H range. As a result, one has available in this polypeptide both a higher helical content in neutral saline–phosphate solution than in a copolymer of glutamic acid and lysine with the same net negative charge and a high

(16) P. Urnes and P. Doty, *Adv. Protein Chem.*, **16**, 401 (1961).

mole fraction of residues with uncharged side-chains. It should therefore offer a more faithful model of protein behavior than previous polypeptides.

The tyrosine-containing copolymer $G_{51}L_{33}T_{16}$ offers some interesting contrasts to the alanine-containing copolymer $G_{42}L_{28}A_{30}$. Although the mole fraction of hydrophobic groups is less in $G_{51}L_{33}T_{16}$, the weight fraction of hydrophobic groups is higher and it is this hydrophobic burden that is probably decisive in favoring the $\beta$-conformation which gradually develops in these solutions. The rate of formation of the $\beta$-structure was found to increase at elevated temperatures and at high salt concentrations. This increased rate of formation of the $\beta$-structure with temperature, its culmination in gel formation, and its resolution by 8 $M$ urea are all reminiscent of easily denatured proteins.

While the investigation of the properties of $G_{42}L_{28}A_{30}$ and $G_{51}L_{33}T_{16}$ is still in its early stages, it is clear that between them they exhibit in aqueous solution the whole range of characteristic protein properties deriving from interconversions among disordered coils, $\alpha$-helical structures and $\beta$-conformations. Thus, these polypeptides represent a continual evolution toward the reproduction of the full range of the conformational properties of proteins in synthetic polymers that had its start with the display of the more limited features in poly-L-glutamic acid[17] and copoly-L-glutamic acid–L-lysine.[11]

**Acknowledgment.**—The authors would like to thank Mr. Peter Urnes for many helpful discussions concerning the results of the optical rotatory dispersion measurements.

(17) P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Polymer Sci.*, **23**, 851 (1957).

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[CONTRIBUTION FROM THE ARGONNE NATIONAL LABORATORY, ARGONNE, ILLINOIS, AND THE GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM 54, MASSACHUSETTS]

# Use of Fully Deuteriated Algae Extracts for the Isolation of Nucleic Acids[1,2]

BY HENRY L. CRESPI, JULIUS MARMUR AND JOSEPH J. KATZ

RECEIVED MARCH 15, 1962

The composition of aqueous extracts of fully deuteriated algae is discussed and the utility of such extracts for the growth of fully deuteriated fastidious microörganisms is described. Fully deuteriated organisms grown in this way provide an excellent source of heavy DNA.

## Introduction

Because essentially all of the hydrogen has been replaced by deuterium and because of a rich content of growth factors, extracts made from algae grown in $D_2O$ have been found ideally suited for the growth of fully deuteriated, nutritionally-demanding microörganisms. These microörganisms serve as a very convenient source of isotopically altered compounds. In particular, a basal medium containing inorganic salts, and which is fortified by the addition of fully deuteriated carbohydrates and algae extract (Table I) has been successfully used to grow microörganisms (as well as their bacteriophage lysates) for the isolation of deoxyribonucleic acid (DNA). The medium will be referred to as DAEG. Fastidious organisms such as *Hemophilus influenzae* can be grown if the medium is supplemented with several vitamins as well as with diphosphopyridine nucleotide and hemin. Bacteriophage T7 will infect and lyse *Escherichia coli* grown in the medium described; moreover, it is possible to prepare bacteriophage lysates much more readily in DAEG than in the mineral salts–$N^{15}H_4Cl$–$D_2O$ medium described previously.[3] Another advantage of the much more nutritionally-adequate DAEG medium in growing *E. coli* and other microörganisms is that the lag period and generation time in high concentrations of $D_2O$ are greatly reduced.

(1) One of us (J. M.) was aided by a grant from the National Institutes of Health (RG-7985) and the National Science Foundation (G-13990).

(2) Based in part on work performed under the auspices of the U. S. Atomic Energy Commission.

(3) J. Marmur and C. L. Schildkraut, *Nature*, **189**, 636 (1961).

TABLE I

COMPOSITION OF MEDIUM FOR THE GROWTH OF ISOTOPICALLY SUBSTITUTED MICROÖRGANISMS

| | |
|---|---|
| $K_2HPO_4$[a] | 1.0 g. |
| $KH_2PO_4$[a] | 0.25 g. |
| KCl | 1.5 g. |
| NaCl | 5.0 g. |
| $Na_2SO_4$ | 0.05 g. |
| *Scenedesmus* extract[b] | 1.5 g. |
| Deuteriated sugars[c] | 0.25 g. |
| $D_2O$ (99+ per cent.) | 1000 cc. |

[a] The phosphate salts were dried down several times in $D_2O$ before addition to the medium. The pD was adjusted before and during growth to approximately 7.5 with DCl or NaOD. [b] In the growth of bacteria for the isolation of DNA substituted with both D and $N^{15}$, *Chlorella* extract at 4.0 g./l. has been used. [c] Pure deuterio-glucose was used in the *Hemophilus* cultures.

**Preparation and Characterization of Algae Extracts Preparation.**—The algae *Scenedesmus obliquus* and *Chlorella vulgaris* are grown in 99.6% $D_2O$ in mass culture,[4] harvested by centrifugation, washed once with $D_2O$ and stored at −20°. Periodically, 600 g. (wet weight) portions are thawed, thinned with $D_2O$ and autoclaved at 15 pounds pressure ($D_2O$) for 15 minutes. Upon cooling, the gelatinous mass is centrifuged. The cell residue is washed once with $D_2O$, centrifuged and the two supernatant solutions pooled. After reducing the volume of the extract from *S. obliquus* to about 200 ml. by lyophilization, the whole turbid extract is clarified by centrifuging out at 18,000 g. for 20 minutes. The clear supernatant is then lyophilized to dryness. The extract from *C. vulgaris* is first lyophilized to complete dryness. The residue is taken up in $D_2O$, and the insoluble, gelatinous material that now appears is centrifuged out at 18,000 g. for 20 minutes. The residue is washed once, centrifuged and the pooled supernatant

(4) H. F. DaBoll, H. L. Crespi and J. J. Katz, in press.