thin mica windows. The optics of the camera was of the "infinite slit" type:[3] in interpreting the experimental results we performed the necessary corrections. Experimental and mathematical details will be given elsewhere.

Granted some assumptions (that are likely to hold true for serum albumin),[3] small-angle X-ray diffraction experiments can provide the values of the volume (V) and of the ratio external surface/volume (S/V), of one molecule, besides the length of the radius of gyration (R_0).[3] To eliminate interparticle scattering effects V and R_0 values have to be extrapolated to zero concentration; S/V is fairly independent of concentration.

The protein was dissolved in saline water (0.15 M NaCl). The pH was adjusted by adding some diluted HCl to the solution, and was checked before and after the X-ray diffraction experiment (the maximum shift was 0.15 pH unit). The experiments were carried out at pH values ranging from 5.6 to 3.5, where all the physico-chemical properties of the protein undergo reversible changes, and the molecular weight is constant.[1]

In a preliminary stage it was observed that the experimental parameters undergo continuous changes as a function of pH. A complete determination of R_0, V and S/V was then undertaken at pH 5.1 and 3.6. The experimental values of R_0, V and S/V, extrapolated to zero concentration, are reported in Table I. The major effect of decreasing pH from 5.1 to 3.6 seems to be to lower the volume by as much as 30%.

TABLE I

pH	R_0 (Å.)	V (Å.^{3})	$\frac{S}{V}$ (Å.$^{-1}$)
5.1	31.5	130,000	0.160
3.6	30.5	90,000	0.200

This result should be compared to previous work. In the same conditions the "hydrodynamic volume" of the molecule increases by lowering the pH: one of us[1] obtained V = 171,000 Å.^{3} at pH 5.3 and V = 262,000 Å.^{3} at pH 3.48, by viscosity, diffusion and sedimentation experiments.

These two sets of results are not necessarily incompatible, since X-ray and hydrodynamic techniques are not concerned exactly with the same "particle." The "X-ray particle" is the region of the solution where the electron density is higher than in the solvent; the "hydrodynamic particle" is likely to be bulkier since it includes the shell of solvent that the molecule drags along in its movement. So, if for instance some segments of the peptide chains unfolded at acidic pH, and took a random configuration (reversible denaturation), the "hydrodynamic volume" would become larger, while the "X-ray volume" would decrease, since the electron density of the solvent around one molecule would be hardly raised by the presence of a few random polypeptide chains.

This explanation is only tentative. We intend to compare X-ray and hydrodynamic results in more detail elsewhere.

(3) A. Guinier and G. Fournet, "Small-angle Scattering of X-Rays," John Wiley and Sons, New York, N. Y., 1955.

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THE FORMATION OF A HELICAL COMPLEX BETWEEN POLYINOSINIC ACID AND POLYCYTIDYLIC ACID

Sir:

The polyribonucleotides are a series of enzymatically produced polymers which have the same ribose-phosphate backbone as ribonucleic acid.[1] Previous work has shown that polyadenylic acid and polyuridylic acid can combine to form a two-stranded helical complex with a structure similar to that of deoxyribonucleic acid.[2] Evidence now has been obtained which demonstrates that polyinosinic acid (poly I) and polycytidylic acid (poly C) can also react together to form a helical complex.

The ultraviolet absorption spectra of poly I and poly C and a 1:1 mixture of the polymers (Fig. 1) clearly indicate that some interaction takes place. To determine the stoichiometry of the reaction, the optical density has been measured at 235 mμ for a series of mixtures containing varying proportions of poly I and poly C, while keeping the total nucleotide concentration constant. The resulting curve is composed of two straight lines which intersect sharply at a 1:1 mole ratio, thereby demonstrating the formation of a strong 1:1 complex. Ultracentrifuge studies at low concentrations using ultraviolet absorption measurements show that the sedimentation coefficient of the complex (S_{20} = 13.9) is considerably greater than that of the separate polymers (poly I, S_{20} = 7.4; poly C, S_{20} = 5.5). These results all apply to solutions containing 0.1 M NaCl and 0.01 M sodium cacodylate buffer at pH 6.7.

The reaction is controlled by the amount of electrolyte that is present. No reaction takes place at very low concentrations (*e.g.* $< 10^{-4}$ M NaCl, without buffer at pH 6.5). In the presence of 0.01 M sodium cacodylate at pH 6.7, the reaction proceeds very slowly, taking about two hours to reach completion, while in 0.1 M NaCl, equilibrium is reached within a few minutes. At high salt concentrations (*e.g.*, 1 M NaCl, 0.01 M sodium cacodylate at pH 6.7) no reaction takes place.

Fibers can be drawn from concentrated solutions of the 1:1 mixture and these yield a moderately well oriented X-ray diffraction pattern which is characteristic of a helix. This diffraction pattern is unlike that of the complex formed between polyadenylic acid and polyuridylic acid and in many respects resembles that of natural ribonucleic acid.[3] The layer line spacing is near 28 Å. There is a strong second layer line and two strong reflections on or near the meridion at 3.3 and 3.9 Å.

There are a number of possible two (or four) stranded configurations for this helical complex in

(1) M. Grunberg-Manago, P. J. Ortiz and S. Ochoa, *Science*, **122**, 907 (1955).

(2) A. Rich and D. R. Davies, THIS JOURNAL, **78**, 3548 (1956).

(3) A. Rich and J. D. Watson, *Proc. Nat. Acad. Sci.*, **40**, 759 (1954).

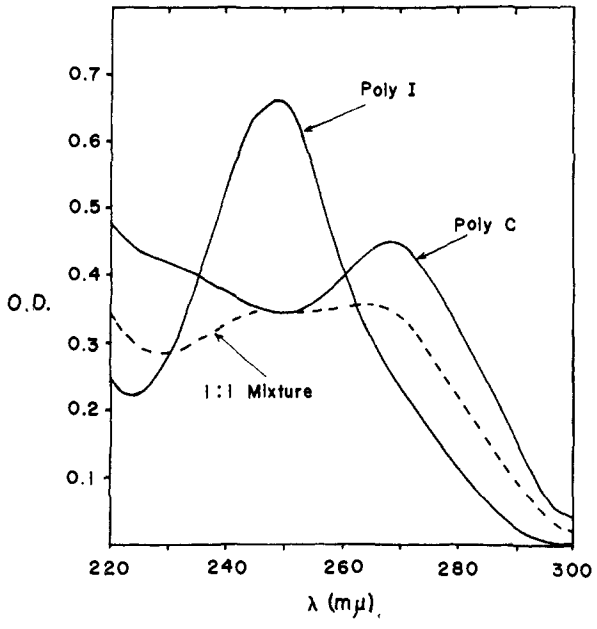

Fig. 1.—The ultraviolet spectra of equal concentrations polyinosinic acid, polycytidylic acid, and a 1:1 mixture of these solutions. All solutions are in 0.1 *M* NaCl, 0.01 *M* sodium cacodylate, *p*H 6.7, $T = 23°$.

which the bases are joined by systematic hydrogen bonding. Models of these helical configurations have been built and their Fourier transforms are being computed for comparison with the observed diffraction data.

We wish to thank Prof. S. Ochoa for the gift of some polynucleotide phosphorylase which was used to prepare the polymers. We are indebted to Mrs. Jean Johnson for technical assistance.

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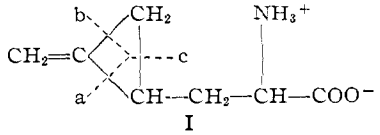
THE STRUCTURE AND BIOLOGICAL ACTIVITIES OF HYPOGLYCIN

Sir:

Hypoglycin A, a substance with hypoglycemic activity, has been isolated from the seeds of *Blighia sapida*.[1,2] It has been described as an amino acid with the empirical formula $C_7H_{11}NO_2$.[3] Work in these laboratories confirms these reported findings and supports structure I for hypoglycin.[4]



I

Hypoglycin is optically active, $[\alpha]^{25}D + 10.3$ (H_2O, *c*. 1.55). Calcd for $C_7H_{11}NO_2$: C, 59.62; H, 7.86: N, 9.94; mol. wt. 141. Found: C, 59.17; H, 7.81; N, 9.80; Van Slyke N, 11.87; C–CH_3, 0.4%; mol. wt., 139. Acetylation with acetic anhydride in acetic acid or ketene gave two isomeric N-acetyl derivatives: acetylhypoglycin, m.p. 92.5–95.5° (uncor.), $[\alpha]^{25}D + 28.5$ (*c* 1.44, acetone); Calcd. for $C_9H_{13}NO_3$: C, 59.00; H, 7.15; N, 7.65; 1 C–CH_3, 8.20; mol. wt., 183. Found: C, 58.95; H, 7.42; N, 7.93; neut. equiv., 186; Isoacetylhypoglycin, m.p. 120–121° (uncor.), $[\alpha]^{25}D - 0.08 \pm 0.06$ (*c* 5.3, acetone). Found: C, 58.93; H, 7.19; N, 7.53; C–CH_3, 6.59; mol. wt., 180; neut. equiv., 193.

Lack of any maxima in the ultraviolet indicated the absence of conjugated double bonds. The infrared spectra of hypoglycin, its salts and acetyl derivatives were consistent with an amino acid formulation and displayed bands at 11.25 and 5.70 μ (weak) indicative of a terminal methylene group. Confirmatory evidence for the latter was found in the formation of formaldehyde (10% yield) on ozonolysis of isoacetylhypoglycin and in the formation of a C-methyl on reduction of the double bond (*vide infra*).

Conversion of the acetylhypoglycin into isoacetylhypoglycin by treatment with ketene required a hydrogen atom on the α-carbon and established the epimeric relationship of the isomers. Confirmation of the α-amino acid formulation was obtained by the formation of thiohydantoin and phenylthiohydantoin derivatives.

The failure of D-amino acid oxidase to attack hypoglycin and the ready oxidation by L-amino acid oxidase further confirmed the presence of an α-hydrogen atom and demonstrated the L-configuration of hypoglycin.

The appearance in the nuclear magnetic resonance (n.m.r.) spectrum of potassium isoacetylhypoglycinate in D_2O of a low frequency triplet (1092 cycles) demonstrated the presence of the α-hydrogen of an α-acylamino acid with a β-carbon atom bearing two hydrogen atoms.[5] The formation of aspartic acid by neutral permanganate oxidation of isoacetylhypoglycin, followed by hydrolysis, was consistent with this formulation.

Evidence concerning the three as yet uncharacterized atoms was provided by hydrogenation. Catalytic hydrogenation of hypoglycin in methanol with platinum oxide resulted in the uptake of 1.2 moles of hydrogen per mole. The reduction product was found to be a mixture of three amino acids: A, B and C in the molar proportions of 1:3.5:10. A and B proved to be indistiguishable in the infrared and by paper chromatography from 2-aminoheptanoic acid and 2-amino-4-methylhexanoic acid,[6] respectively.

Compound C (calcd. for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78; 1 C–CH_3, 9.54. Found: C, 58.4; H, 9.16; N, 9.55; C–CH_3, 8.3) was shown to possess a ring by its resistance to further hydrogenation. It lacked the 11.25 μ band but showed a

(1) C. H. Hassall, K. Reyle and P. Feng, *Nature*, **173**, 356 (1954).

(2) C. H. Hassall and K. Reyle, *Biochem. J.*, **60**, 334 (1955).

(3) C. von Holt, W. Leppla, B. Kroner and L. von Holt, *Naturwissenschaften*, **43**, 279 (1956).

(4) As a simplification, we suggest that this new amino acid be referred to as hypoglycin.

(5) Additional peaks were also observed for the terminal methylene (1036 cycles) and the N-acetyl groupings (1177 cycles). The n.m.r. spectrum was taken by Dr. M. Saunders of Yale University on a Varian High Resolution Nuclear Magnetic Resonance Spectrometer at room temperature and 40 mc. The reference scale was based on the absorption of the aromatic and methyl hydrogens of toluene at 1000 and 1197 cycles, respectively.

(6) Both the synthetic and natural samples of the amino acid were probably mixtures of diastereoisomers.