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POLYNUCLEOTIDES. I. MOLECULAR PROPERTIES AND CONFIGURATIONS OF POLYRIBOADENYLIC ACID IN SOLUTION[1]

Sir:

We wish to report the discovery of two configurations of polyriboadenylic acid (poly-A): one a flexible, randomly coiled, molecularly dispersed form and the other a rigid, presumably helical form composed of variable numbers of poly-A molecules.

Using polynucleotide phosphorylase from *Azotobacter vinelandii*,[2] samples of poly-A were prepared at various enzyme–substrate ratios and collected at different extents of reaction in order to provide products covering a wide molecular weight range. These samples in solution above *p*H 6.5 in water or above *p*H 5.7 in 0.15 *M* salt, exhibited a characteristic absorption spectrum having a maximum at 257 mμ ($E_{1\%} \cong 295$), were not birefringent, and displayed sedimentation constants, s^0, and intrinsic viscosities, $[\eta]$, that depended on molecular weight in the manner shown at the left side of Fig. 1. The molecular weights were calculated from the Flory–Mandelkern equation[3] using 2.3×10^6 for $\Phi^{1/3}/P$ and 0.55 for partial specific volume. The slopes, 0.45 and 0.65, respectively, are typical of randomly coiled chains: we conclude that this is the configuration under these conditions.

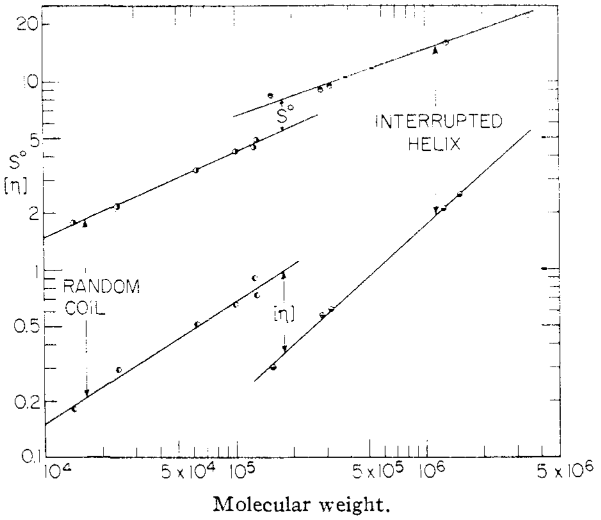

Fig. 1.—Molecular weight dependence of sedimentation constant, S^0_{20} in svedbergs and intrinsic viscosity $[\eta]_{25}$, in dl./g., of the two configurations of poly-A. For the curves on the left, the solvent was 0.15 *M* NaCl, 0.015 *M* citrate, *p*H 7.1; for those on the right it was 0.15 *M* acetate, *p*H 4.9. The open circle (O) represents measurements at *p*H 3.7 with no added salt present.

However, below *p*H 5 in 0.15 *M* salt and below *p*H 6.5 in water the poly-A samples displayed a different spectrum having a maximum at 252 mμ ($E_{1\%} \cong 255$) and had values of s^0 and $[\eta]$ that depended on the concentration and the temperature at which the transition had been brought about by lowering the *p*H.

By varying the concentration of poly-A (0.2 to 1.5 g./100 cc. for the sample having a molecular weight of 126,000) prior to lowering the *p*H to 4.9 (acetate buffer of ionic strength 0.15) the solutions were found to exhibit systematic variations in s^0 and $[\eta]$ that were clearly indicative of the formation of stable aggregates. The weights of these aggregates increased with the concentration of poly-A at which the transition was induced. Calculating the molecular weights (or particle weights) as before, it was found that the s^0 and $[\eta]$ varied with weight as shown in the right side of Fig. 2. The linear relations that result and the values of the slopes, 0.36 and 0.92, respectively, indicate that the species are homologous in structure and that the differences in weight are the result of different extents of linear growth. Unlike the randomly coiled configuration, this form does not show a marked increase in specific viscosity upon removal of salt. This and the behavior in flow birefringence lead to the conclusion that the structure is relatively rigid and rod-like, but not quite to the extent found for deoxyribonucleic acid (DNA). The stability of this structure appears to result from the lowering of the electrostatic energy brought about by titrating about half of the adenine groups. The failure to find spectra intermediate in character between the two described further suggests that the transition is abrupt and complete and that practically all the chromophoric groups (adenine) undergo the same change in environment. Moreover, at *p*H 4.9 in 0.15 *M* acetate, the transition to the randomly coiled form occurs in a very narrow temperature range near 75°, only a few degrees below the transition temperature observed for DNA. This coöperative behavior indicates a highly ordered arrangement of secondary bonds for the acid-stable form.

These observations are compatible with a structure in which poly-A molecules are associated through hydrogen-bonded base-pairing in a double stranded helix,[4] each strand of which has gaps where one poly-A molecule ends and another begins. This would resemble the interrupted helical model once proposed for DNA,[5] but the gaps in our case may be larger. The increased rotational freedom at the gaps would account for the smaller space filling properties exhibited by this configuration

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[1] This investigation was supported by a research grant (C-2170) from the National Cancer Institute, Public Health Service.

[2] M. Grunberg-Manago and S. Ochoa, THIS JOURNAL, **77**, 3165 (1955); M. Grunberg-Manago, P. J. Ortiz and S. Ochoa, *Biochim. Biophys. Acta*, **20**, 169 (1956). We are very grateful to Professor Ochoa for making available to us unpublished information on the purification of the enzyme.

[3] P. J. Flory and L. Mandelkern, *J. Chem. Phys.*, **20**, 212 (1952).

[4] A two-stranded structure of this type has been proposed by F. H. C. Crick, D. Davies, A. Rich and J. D. Watson and is discussed by J. D. Watson in "The Chemical Basis of Heredity," W. D. McElroy and B. Glass, editors, the Johns Hopkins Press, Baltimore, 1957 and by A. Rich in the *Proc. Acad. Sci., New York*, in press. It is not necessary that the detailed atomic arrangements of the helical configuration in solution be the same as that which may ultimately be established in the solid state.

[5] C. A. Dekker and H. Schachman, *Proc. Natl. Acad. Sci.*, **40**, 894 (1954).

relative to DNA. If this structural proposal is the correct one, it may be possible for ribonucleic acid, also, to assume a double-stranded configuration in solution through pairing of only the adenine nucleotides.[6]

[6] We are very much indebted to Mrs. Elizabeth Klemperer who was reponsible for many of the measurements in this investigation.

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INCORPORATION OF THE CARBON CHAIN OF METHIONINE INTO SPERMIDINE[1,2]

Sir:

The cumulative work of several laboratories has indicated the metabolic importance of the polyamines putrescine, spermidine and spermine in diverse biological systems. Tabor, *et al.*,[3] have shown that putrescine is the source of the four-carbon chain of spermidine and spermine. Recent results obtained by the author show that the side chain of methionine is an efficient precursor of the three carbon chain of spermidine in *Neurospora crassa*.

Neurospora crassa strains 74A-3b (wild type) and 38706 (blocked in the conversion of homocysteine to methionine) were grown at room temperature on Fries minimal medium supplemented with 50 μg./ml. of DL-methionine-$2C^{14}$. The spermidine was isolated by grinding the mold with sand and 5% TCA[4] and chromatography of the extract on 0.6 × 20 cm. columns of Dowex 50-X2 using an HCl gradient. The spermidine containing fractions were combined and dried; spermidine trihydrochloride was crystallized from methanolic hydrogen chloride and ethyl acetate. The specific activities of the added methionine and the isolated spermidine are compared in Table I. Amine concentrations were determined as described by Rosenthal and Tabor.[5] Radiopurity of the isolated spermidines was checked by paper ionophoresis in *p*H 8.2 borate buffer and by descending paper chromatography (3 parts 1-propanol:1 part 0.2*M* sodium acetate buffer). In all cases the radioactivity moved in one spot and was in the same position as added authentic spermidine trihydrochloride.

TABLE I

Strain	Specific Activities Methionine (initial) c.p.m./μmole	 Spermidine (isolated) c.p.m./μmole	Ratio Spermidine/ Methionine
74A-3b	6.36×10^4	3.38×10^4	0.53
38706	11.4×10^4	6.34×10^4	0.56

Spermidine was degraded by oxidation with alkaline permanganate. The small yield of succinate, from the four carbon chain, was isolated by chromatography on Dowex 1[6] and on paper using aqueous phenol.[7] The succinate isolated from the oxidation product of synthetic spermidine,[8] labeled in the four carbon chain, contained about 33% of the radioactivity put on the Dowex 1 column, while less than 0.5% of the radioactivity was found in the succinate from the oxidation products of isolated spermidine and synthetic spermidine[8] labeled in the three carbon chain. The low level of activity in the succinate shows that the isolated spermidine is not labeled in the four carbon chain and is consistent with labeling in the three carbon chain.

TABLE II

*p*H	Total radioactivity in spermidine fractions, c.p.m. + ATP	 − ATP
7.2	2.1×10^4	520
8.2	2.5×10^3	795

Incubation, four hours at 37°; incubation mixture, 100 μg. $2C^{14}$-DL-methionine (3.8×10^6 c.p.m.) per vessel, 0.01 *M* putrescine, 0.008 *M* glutathione, 0.003 *M* $MgCl_2$, 0.00001 *M* pyridoxal phosphate, 0.1 *M* buffer, 2.5 ml. 1:1 extract of 74A-3b, 0.02 *M* K_4ATP as indicated, total volume 5 ml.; buffers are *p*H 7.2, potassium phosphate + Tris hydrochloride (1:1), *p*H 8.2, Tris hydrochloride.

A possible mechanism for this reaction is the transfer of the methionine side chain from S-adenosyl methionine to putrescine in a manner similar to the transfer of the methyl group of this compound first observed by Cantoni.[9] The almost absolute ATP requirement for the incorporation of C^{14} methionine into spermidine by a cell free extract of 74A-3b as shown in Table II is consistent with this hypothesis. Further evidence for the presence of radioactivity in the spermidine from the *p*H 7.2 incubation mixture was obtained by paper ionophoresis in *p*H 8.2 borate buffer. Tabor, *et al.*,[10] have extended these studies in extracts of *E. coli* and have found further evidence that S-adenosylmethionine is an intermediate.

[1] A preliminary report of this work was presented before the American Society of Biological Chemists; *Federation Proc.*, **16**, 189 (1957).

[2] This work was supported in part by Research Grant C-3436(A) from the National Cancer Institute, National Institutes of Health, Bethesda 14, Md.

[3] H. Tabor, S. M. Rosenthal and C. W. Tabor, *Federation Proc.*, **15**, 367 (1956).

[4] Abbreviations used are: TCA, trichloroacetic acid; ATP, adenosine triphosphate; Tris, tris-hydroxymethylaminomethane.

[5] S. M. Rosenthal and C. W. Tabor, *J. Pharmacol. and Exptl. Ther.*, **116**, 131 (1956).

[6] J. K. Palmer, Bull. 589 Conn. Agric. Expt. Station (1955).

[7] H. K. Berry, H. E. Sutton, L. Cain and J. S. Berry, Biochemical Institute Studies IV, No. 5109, Univ. of Texas, Austin, Texas, 1951, p. 22.

[8] Kindly supplied by Dr. E. Jackson of the National Institute of Arthritis and Metabolic Diseases.

[9] G. L. Cantoni, "Phosphorus Metabolism," Vol. II, Johns Hopkins Press, Baltimore, Md., 1952, p. 129.

[10] H. Tabor, S. M. Rosenthal and C. W. Tabor, personal communication.

RADIOISOTOPE SERVICE, VETERANS ADMINISTRATION HOSPITAL, AND DEPARTMENT OF BIOCHEMISTRY, DUKE UNIVERSITY, SCHOOL OF MEDICINE, DURHAM, NORTH CAROLINA, AND NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MARYLAND
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THE HETEROGENEITY OF POLYSACCHARIDES AS REVEALED BY ELECTROPHORESIS ON GLASS-FIBER PAPER

Sir:

Glass-fiber paper[1] has been used in conjunction with a borate buffer *p*H 9–10 for the electrophoretic separation[2] of a wide variety of organic substances

[1] M. J. O'Leary, R. B. Hobbs, J. K. Missimer and J. J. Erving, *Tappi*, **37**, 446 (1954).

[2] D. R. Briggs, E. F. Garner, R. Montgomery and F. Smith, *Anal. Chem.*, **28**, 1333 (1956).