a minimum heat of solution in this region of the solvent spectrum might have been expected if its solvation behavior were similar to that of tetramethylammonium chloride.[3]

It is tempting to theorize about the reason for the sudden solvation changes that occur in these solvent mixtures. However, further measurements that we are currently making on other solute–mixed solvent systems suggest that it will be prudent to defer speculation until more data are available.

Acknowledgments.—We are pleased to express our gratitude to Professors Raymond Craig, Henry Frank, Loren Heppler and Joseph Jordan for much helpful advice. We are grateful to Mr. Charles Douty for initial measurements on this system.

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POLYNUCLEOTIDES. V. HELIX-COIL TRANSITION OF POLYRIBOGUANYLIC ACID[1,2]

Sir:

We wish to present evidence that polyriboguanylic acid[3] (poly G), like other homopolynucleotides,[4] can exist in two distinct macromolecular conformations. One of these is apparently a single-stranded form, devoid of secondary structure, that occurs either in neutral solution of low ionic strength at temperatures close to 100° or in strongly alkaline solution. Under a wide range of solvent conditions removed from these extremes of temperature or pH, poly G exists as a multistranded helix. The two forms of poly G are interconvertible, and have been distinguished in very dilute polymer solution on the basis of several physical properties that are sensitive to secondary structure in polynucleotides.

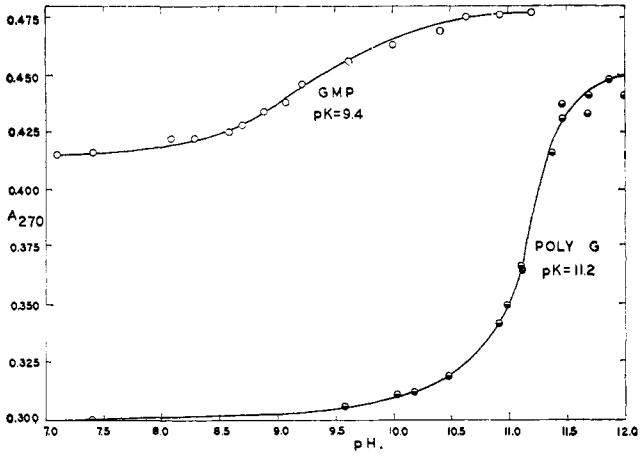

Fig. 1.—Spectrophotometric titration of poly G and its alkaline hydrolysis products (2′-GMP + 3′-GMP) at the same concentration, 4.3×10^{-5} *M*, in 0.2 *M* Na^+ at 25°.

Alkaline Transition.—On titration with alkali, the monomer, guanosine monophosphate (GMP) displays an isosbestic point at 260.5 mμ. The —HN—CO— group being titrated in this region has a p*K* of 9.4 (Fig. 1). By contrast, when poly G is so titrated in 0.2 *M* Na^+ at 25°, the p*K* is shifted to 11.2 (Fig. 1) and there is a significant hyperchromic change at 260.5 mμ (see Fig. 2). In addition, the complete titration of the polymer occurs within one pH unit. It can also be seen from Fig. 2 that poly G is markedly hypochromic, relative to GMP, at neutral pH, but only slightly so at high pH. Complete alkaline titration of poly G brings about other changes as well. There is a dramatic decrease in specific rotation (from 100° to −38° at 589 mμ) that does not occur with the monomer ($[\alpha]_D$ for 2′-GMP changes only from −26° to −32°), as well as a marked change in the rotatory dispersion. The sedimentation constant (in 0.2 *M* Na^+ to minimize charge repulsion effects) also changes, reversibly, by more than a factor of 2 (in one case from 3.6 $s_{w,20}$ to 1.6 $s_{w,20}$ and back to 3.4 $s_{w,20}$ on reneutralization).

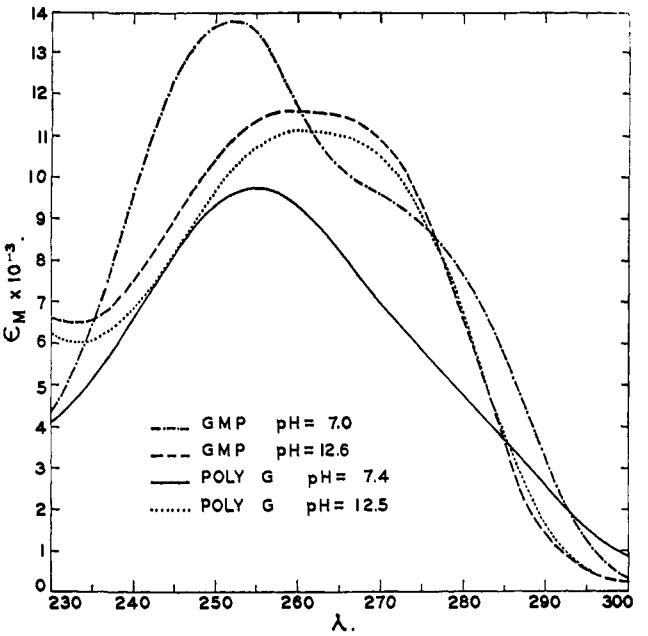

Fig. 2.—Near-ultraviolet absorption spectra of poly G and GMP in 0.6 *M* Na^+ at 25°.

These observations are most simply interpretated in terms of an alkali-induced, coöperative, helix-coil transition. We deduce that the helix is multistranded, has a right-handed sense of twist,[5] and contains stacked sets of hydrogen-bonded guanine residues.

Thermal Transition.—Denaturation of the helix can also be achieved thermally. At pH 7 the helix is exceptionally stable, and the transition, as indicated by the hyperchromic change, is not complete at 100°, even in a solvent of very low ionic strength (0.002 *M* Na^+). As the pH is raised, the transition occurs at lower temperatures. Increasing the ionic strength stabilizes the helical structure, and can counteract, in part, the effect of elevated pH. The addition of 1-butanol to the aqueous neutral solvent weakens the helix. The breadth of these helix-coil transitions is somewhat greater than usual for polynucleotide helices, and varies with the preparation of poly G. This is probably because of the very considerable molecular weight polydispersity among the single strands of poly G (observed in the ultracentrifuge) that interact to form helices and may also be due to imperfections in the helical structure. The hyperchromic change is es-

(1) The last paper in this series was by J. R. Fresco and D.-F. Su, *J. Biol. Chem.*, **237**, PC 3305 (1962).

(2) This investigation was supported by grants from the United States Public Health Service, the National Science Foundation, and the American Heart Association.

(3) Poly G, synthesized enzymatically,[4] was purified by phenol extraction and extensive dialysis to remove proteins, divalent cations and very short oligonucleotides.

(4) Polyadenylic acid: J. R. Fresco and P. Doty, *J. Am. Chem. Soc.*, **79**, 3928 (1957); polyuridylic acid: M. Lipsett, *Proc. Natl. Acad. Sci.*, **46**, 445 (1960); polycytidylic acid: J. R. Fresco, R. Brown and P. Doty, in preparation; polyinosinic acid: A. Rich, *Biochim. Biophys. Acta*, **29**, 502 (1958).

(5) This is indicated by the sign of the optical rotatory change; see J. R. Fresco, *Tetrahedron*, **13**, 185 (1961).

sentially reversible on cooling, but the rate of reformation of the complex diminishes markedly with increasing pH.

Although detailed data on the structural organization of the poly G complex are lacking, several observations suggest that it arises from interactions between *more than two* polynucleotide strands helically wound around the same axis: the exceptional thermal stability of the poly G helix; the very slow rate of reactivity with poly C to form poly (G + C) and poly (G + C + G)[6] at neutral pH; the difficulty in making perfect helices that melt sharply; and the tendency of poly G to aggregate and precipitate at neutral pH even at low polymer concentration. A three or four stranded model, not unlike those proposed for poly I,[7] but containing additional hydrogen bonds linking the 2-amino substituent of each base to the N-7 of the adjacent one at each base-plane level, would not be inconsistent with the properties of the poly G helix reported here.[8,9]

(6) These are helical complexes containing one strand of polycytidylic acid (poly C) and either one or two strands of poly G. J. R. Fresco, J. Massoulié and R. D. Blake, in preparation.

(7) A. Rich, *Biochim. Biophys. Acta*, **29**, 502 (1958).

(8) After completing this work, we learned that a 4-stranded model involving such a hydrogen bonding scheme has been proposed for helices formed in concentrated solutions of 5-GMP: M. Gellert, M. N. Lipsett and D. R. Davies, *Proc. Natl. Acad. Sci.*, **48**, 2013 (1962).

(9) We are indebted to Mr. Richard Blake for assistance in this investigation.

(10) Established Investigator of the American Heart Association.

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THE STRUCTURE OF COPPER TETRAPHENYLPORPHINE[1]

Sir:

We wish to substantiate the report of the nonplanarity of the porphyrin ring system in metalloporphyrin compounds.[2] The structure of copper tetraphenylporphine (Fig. 1) was determined by X-ray analysis.

Copper tetraphenylporphine crystals are tetragonal, space group $I\bar{4}2d$ (No. 122 International Tables), with the following cell edges: $a = 15.03 \pm 0.01$ Å.; $c = 13.99 \pm 0.01$ Å. There are four molecules of the porphyrin per unit cell. A set of 465 independent structure factors was determined using CuKα radiation; each independent structure factor is the average of two equivalent reflections.

The space group requires that the copper atom be at $x = 0, y = 0, z = 0$ on a fourfold inversion axis. The structure was solved by utilizing the copper atom to determine the phases of the structure factors. The structure was refined by least-squares analysis employing a weighting scheme of unit weights for all reflections and anisotropic temperature factors in the final refinement procedure. Hydrogen atoms were included in the refinement.

The final R-factor including all reflections was 6.6%, and the standard deviations of the atomic coördinates, not including the hydrogen atoms, ranged from 0.007 to 0.015 Å. Table I lists the bond distances of the molecule; Table II lists the fractional atomic coordinates. The seven hydrogen–carbon bond lengths averaged 1.1 ± 0.1 Å.

The geometry of the porphyrin molecule has the following interesting features. The phenyl group is both tilted down from the porphyrin ring and is twisted out of the "plane" of the porphyrin ring. The C_5–C_6 bond makes an angle of 13° with its projection on the 001 plane. The line through the atoms C_8 and C_{10} makes an angle of 72° with its projection on the 001 plane. Thus, the phenyl group is almost perpendicular to the porphyrin ring. The C_5–C_6 bond distance is 1.51 Å. This is strong evidence that the phenyl group is electronically isolated from the porphyrin ring.

(1) This research was supported by a Public Health Service Grant.

(2) E. B. Fleischer, *J. Am. Chem. Soc.*, **85**, 146 (1963).

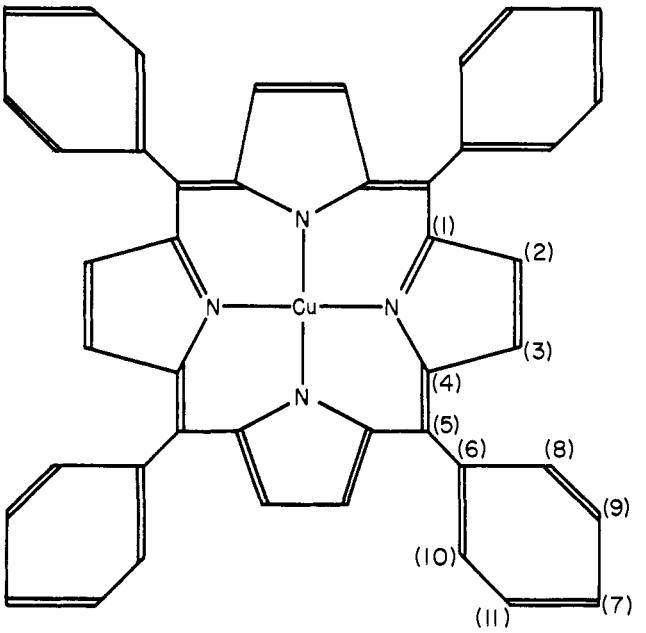

Fig. 1.—Copper tetraphenylporphine.

TABLE I

BOND LENGTHS IN COPPER TETRAPHENYLPORPHINE

Bond	Length in Å.[a]	Bond	Length in Å.[b]
Cu–N	1.98	C_5–C_8	1.38
N–C_1	1.38	C_8–C_9	1.40
N–C_4	1.39	C_9–C_7	1.39
C_1–C_2	1.44	C_7–C_{11}	1.36
C_2–C_3	1.35	C_{10}–C_{11}	1.42
C_3–C_4	1.45	C_{10}–C_6	1.40
C_4–C_5	1.36		
C_5–C_6	1.51		

[a] Standard deviation of bond length ≤0.013 Å. [b] Standard deviation of bond length ≤0.021 Å.

TABLE II

FRACTIONAL COÖRDINATES FOR COPPER TETRAPHENYLPORPHINE

Atom	x	y	z
Cu	0.000	0.000	0.000
N	.114	.066	− .003
C_1	.197	.032	.019
C_2	.261	.103	.017
C_3	.217	.178	− .007
C_4	.124	.157	− 017
C_5	.057	.217	− .030
C_6	.081	.311	− .055
C_7	.132	.484	− .102
C_8	.104	.332	− .147
C_9	.128	.419	− .172
C_{10}	.087	.376	.017
C_{11}	.111	.464	− .010

The porphyrin ring is non-planar. The extent of the non-planarity can be illustrated by the distance that atoms are from the plane parallel with the 001 plane and passing through the origin. These distances are given in Table III. It is evident from Table III that the molecule is highly distorted from a planar configuration.