Fuwa, K., and Vallee, B. L. (1963), *Anal. Chem. 35*, 942.
Levinthal, C., Signer, E. R., and Fetheroff, K. (1962), *Proc. Natl. Acad. Sci. U. S. 48*, 1230.
Lindskog, S., and Nyman, P. O. (1964), *Biochim. Biophys. Acta 85*, 462.
Meyerhof, O., and Green, H. (1949), *J. Biol. Chem. 178*, 655.
Piras, R., and Vallee, B. L. (1967), *Biochemistry 6*, 348.
Plocke, D. J., Levinthal, C., and Vallee, B. L. (1962), *Biochemistry 1*, 373.
Plocke, D. J., and Vallee, B. L. (1962), *Biochemistry 1*, 1039.
Reynolds, J. A., and Schlesinger, M. J. (1969), *Biochemistry 8*, (in press).
Schwartz, J. H., and Lipmann, F. (1961), *Proc. Natl. Acad. Sci. U. S. 47*, 1996.
Sillen, L. G., and Martell, A. (1964), *in* Stability Constants of Metal Ion Complexes, Special Publication No. 17, London, The Chemical Society.
Simpson, R. T., and Vallee, B. L. (1968), *Federation Proc. 27*, 241.
Simpson, R. T., Vallee, B. L., and Tait, G. H. (1968), *Biochemistry 7*, 4336 (this issue; preceding paper).
Stein, S. S., and Koshland, D. E. (1952), *Arch. Biochem. Biophys. 39*, 229.
Theirs, R. E. (1957), *Methods Biochem. Anal. 5*, 273.
Vallee, B. L., and Wacker, W. E. C. (1968), *Proteins* (in press).
Vallee, B. L., and Williams, R. J. P. (1968a), *Proc. Natl. Acad. Sci. U. S. 59*, 498.
Vallee, B. L., and Williams, R. J. P. (1968b), *Chem. Britain 4*, 397.
Vallee, B. L., Williams, R. J. P., and Coleman, J. E. (1961), *Nature 190*, 633.

Conformational Studies on Polyriboadenylic Acid in Ethylene Glycol[*]

Sue Hanlon and E. O. Major

ABSTRACT: The conformation of polyriboadenylic acid in ethylene glycol in the presence and the absence of a 4- to 12-fold ratio of protons to adenine has been examined by spectral and optical rotatory techniques. When the glycol solvent is slightly basic, the stacking of the adenine residues in polyriboadenylic acid is completely abolished. In the presence of a small excess of hydrogen ions, however, a base-stacked helical conformation is preserved at glycol concentrations as high as 97–99.7%.

The optical properties as well as the thermal stability of this conformation in this nonaqueous solvent are very similar to those exhibited by the conformation of polyriboadenylic acid in acidified aqueous solvents at low ionic strength. The form present in the acid glycol solvent can be converted into the base-unstacked conformation by the addition of approximately 1 mole of hydroxide ion/mole residue of polymer. Despite the fact that glycol is normally a polynucleotide denaturing solvent, the conformation of polyriboadenylic acid present in the acidified glycol is similar to if not identical with the structures formed in acidified aqueous solvents under comparable conditions of ionic strength and acid concentration. The latter is presumably a subtle variant of the structure proposed by Rich *et al.* (Rich, A., Davies, D. R., Crick, F. H. C., and Watson, J. D. (1961), *J. Mol. Biol. 3*, 71). The stability of such a structure in a solvent which normally destroys base-stacked polynucleotide conformations can be ascribed to the effect of the lower dielectric constant of ethylene glycol on the strength of the internal ion pair which maintains this conformation.

A convenient index of the stability of an organized polynucleotide conformation is the standard free-energy change, $\Delta G°$, for the helix–coil transition

$$\begin{bmatrix}\text{organized,}\\ \text{base stacked,}\\ \text{helical form}\end{bmatrix} \rightleftharpoons [\text{random coil}] \qquad (1)$$

As information about polynucleotide conformations in both solution and the solid state has accumulated, it has become apparent that a unique conformation reflects a balance of many types of interactions of the constituent parts of the polymer both with each other and with the solvent. Thus, $\Delta G°$ for reaction 1 represents the difference in contribution of these constituent interactions between the helix and the coil form.

There are three general categories of self-interactions which make major contributions to the free-energy difference between the helix and the coil forms of poly-

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nucleotides *in vacuo* (DeVoe and Tinoco, 1962a). These are: (1) the base-stacking interactions, *i.e.*, the tendency of purines and pyrimidines to associate plane to plane, (2) hydrogen-bonding interactions involving the functional groups and nitrogen atoms of the bases as well as the 2′-OH and the phosphate oxygen of the polynucleotide backbone, and (3) electrostatic interactions between charged groups of the polynucleotide. The contribution of these interactions to the free-energy change, ΔG°, in reaction 1 can be formally represented as a simple sum

$$\Delta G^\circ = \Delta G_S{}^\circ + \Delta G_H{}^\circ + \Delta G_E{}^\circ \qquad (2)$$

where the subscripts, S, H, and E, refer to the base stacking, the hydrogen bonding, and the electrostatic contributions, respectively. The effect of solvent on conformation can be ascribed to perturbations of these self-interactions. Conformational changes in a given polynucleotide can be effected by an environmental reduction or change in sign of any one or all of the three terms on the right hand side of eq 2.

There is, however, another consequence of eq 2. Under conditions where the several categories of interactions of a polynucleotide are affected in a compensatory manner such that the magnitude of the total, ΔG°, remains constant, the conformation of the polynucleotide should remain unperturbed by a change in environmental conditions.

The acid form of poly rA is a case in which the variations in the interaction free energies might be compensatory. In neutral and alkaline aqueous solution, the polymer possesses a partially stacked, single-stranded helical structure (Holcomb and Tinoco, 1965; Witz and Luzzati, 1965; Leng and Felsenfeld, 1966). As the pH is lowered, with the consequent protonation of the adenine residues, a double-stranded base-stacked conformation forms (Fresco and Doty, 1957; Fresco, 1959; Fresco and Klemperer, 1959; Holcomb and Tinoco, 1965). It has been assumed that this conformation is similar to if not identical (Fresco, 1959) with that found by Rich and coworkers (1961) in fibers of poly rA pulled from acid solution. This structure (on the average) is a double-stranded helix with parallel chains which is maintained by base-stacking, interchain hydrogen bonds, and an internal salt linkage between the positively charged adenine residues in one strand and the negatively charged phosphate groups in the adjacent strand. When this particular conformation is surrounded by an organic solvent whose polarizability is greater than that of water, one would predict that the $\Delta G_S{}^\circ$ term would be considerably reduced, perhaps to zero or even a negative value (Hanlon, 1966). If at all significant, the $\Delta G_H{}^\circ$ should become slightly more positive since most organic solvents are poorer competitors, compared with water, for the functional groups of the bases. The $\Delta G_E{}^\circ$ term in this particular case actually consists of at least two parts, one accounting for the electrostatic repulsion of the negatively charged phosphates in the adjacent strands of the helical conformation (Schildkraut and Lifson, 1965) and the other reflecting the attractive interaction of the internal ion pair. In view of the ionic strength effects on the stability of the acid conformation of poly rA (Steiner and Beers, 1961; Holcomb and Timasheff, 1968), the latter attractive term obviously predominates. Thus, if the effective dielectric constant of this organic solvent in the neighborhood of the polymer is lower than that of water, the $\Delta G_E{}^\circ$ term should become correspondingly more positive for reaction 1 as written. The total ΔG° for reaction 1 might consequently remain unchanged in this situation. Thus, the acidified form of poly rA, in contrast to other polynucleotides, should exhibit the same conformation in such an organic solvent as it shows in an aqueous one.

In order to test this prediction, we undertook an examination of the optical properties of poly rA in organic solvents whose dielectric constants were lower than that of water. The solvent which was found to be most suitable for these experiments was ethylene glycol. It is transparent in the ultraviolet region and it solvates both the zwitterion and the basic form of the polymer. In addition, there was already good evidence in the literature that poly rA, in the acid form, could maintain its base-stacked conformation at intermediate concentrations (50%) of ethylene glycol (Rahn *et al.*, 1966).

Materials and Methods

All chemicals employed were reagent grade. Reagent grade ethylene glycol (Fisher) was further purified by distillation at reduced pressure, and the last half of the distillate was used in the preparation of the glycol solutions. In order to obtain reproducible results in both the glycol solutions as well as those at low ionic strength in H_2O, it was necessary to employ doubly distilled water in the preparation of all aqueous solutions as well as in the cleaning of all glassware. Chromatographically pure 5′-AMP from Sigma was used without further purification. Concentrations of stock solutions of AMP were estimated from the absorbance at the maximum of aqueous dilutions in phosphate buffer at pH 7. The extinction coefficient at this position was taken from the data provided in a Pabst publication (Pabst Laboratory Circular, OR-7, 1955).

Two lots (15634 and 11740) of high molecular weight poly rA (potassium salt) were purchased from Miles Chemical Co. The $s^0_{50\%}$ of these samples at pH 7 were 8.6 and 6.5 S, respectively. Using the data of Fresco and Doty (1957), the molecular weights corresponding to these sedimentation coefficients were estimated as 500,000 and 300,000, respectively.

Stock solutions of the polymer were usually prepared at a weight concentration of 1 mg/ml in either 0.10 M NaCl or 0.10 M $NaClO_4$. In some instances they were used without further purification. In other cases, these stocks were dialyzed against successive changes of 0.01 M EDTA (sodium salt) at pH 7.5, 1 M NaCl, and 0.10 M NaCl. Essentially similar results were obtained with these more elaborately purified samples as long as the pH of the resulting stock solution was readjusted to pH 7 after dialysis. Residue concentrations of these stock solutions were estimated from the absorbance at

TABLE I: Spectral Characteristics of Poly rA in Various Solvents.

Solvent	λ_{max} (mμ)	$E_{(\lambda_{max})}$ $\times 10^{-3}$ $\pm$ 0.15 (l./(mole cm))	% H $\pm$ 1%
0.10 M NaCl–0.05 M NaH_2PO_4–Na_2HPO_4, pH 7 in H_2O	257	10.1[a]	29
0.10 M NaCl–0.10 M CH_3COOH–CH_3-COONa, pH 4.6 in H_2O	252	8.57	38
24 × 10^{-5} M HCl in H_2O,[b] pH 3.7	252	8.89	36
97% $(CH_2OH)_2$–24 × 10^{-5} M NaOH[b]	261	14.8	3.2
97% $(CH_2OH)_2$–24 × 10^{-5} M HCl[b]	254	9.19	31
97% $(CH_2OH)_2$–75 × 10^{-5} M HCl[b]	253	8.77	33
97% $(CH_2OH)_2$–75 × 10^{-5} M $HClO_4$[c]	253	8.91	35
99.7% $(CH_2OH)_2$–75 × 10^{-5} M $HClO_4$[d]	253	8.75	36

[a] Value taken from the data of Holcomb and Tinoco (1965). [b] Solutions also contained 240 × 10^{-5} M NaCl. [c] Solutions also contained 240 × 10^{-5} M $NaClO_4$. [d] Solution also contained 24 × 10^{-5} M $NaClO_4$.

the maximum of a dilution in 0.10 M NaCl, 0.05 M NaH_2PO_4, and Na_2HPO_4 (pH 7) at 25°. An extinction coefficient of 10.1 × 10^3 l./(mole cm), taken from the data of Holcomb and Tinoco (1965) at 22°, was employed in these calculations. All poly rA solutions were prepared by diluting aliquots of the aqueous stock solution with the appropriate solvent containing acid or base. For most of the studies in ethylene glycol, the dilution factor was $^1/_{41}$ which resulted in an ethylene glycol concentration of 97.5% v/v. In a few cases, the dilution factor was $^1/_{401}$, resulting in a higher glycol concentration of 99.7%. Concentrations of poly rA in these solutions were 6 × 10^{-5} or 6 × 10^{-6} M, respectively. In order to keep the H_2O concentration as low as possible in the glycol solutions, the solvents were generally acidified by the addition of 1–3 μl of 1 N HCl or 1 N $HClO_4$. This usually introduced an uncertainty in the final acid concentration of ±4 × 10^{-5} M.

Spectra were obtained in a Cary spectrophotometer, Model 14CMR, equipped with thermostatted cell adaptors and a 0–1- and 0–0.1-optical density slide wire. The temperature of the cell contents was taken as the temperature of the adaptors which was measured with a thermistor and bridge assembly manufactured by the Yellow Springs Instrument Co. Light paths of 1.00 cm were normally employed. Difference spectra were obtained in the conventional fashion with a matched set of four cylindrical cells of 1-cm path length. Extinction coefficients, E, are reported in terms of 1/(mole cm) at 25°. Spectra obtained at temperatures other than 25° are so indicated and were corrected for the volume change in the solvent if this change was significant. The hypochromism, % H, of the polymer transitions between 230 and 300 mμ in a given solvent was calculated from the spectrum of poly rA and that of the monomer, 5′-AMP, under the same conditions. For these calculations, we employed a modified form of the equation given by DeVoe and Tinoco (1962b). Our spectra are recorded in terms of wavelengths, λ, rather than frequencies or wave numbers. Hence, we employed the expression

$$\% H = 100\left[1 - \frac{\Delta\lambda \sum_{230}^{300} \frac{E_P(\lambda)}{\lambda^2}}{\Delta\lambda \sum_{230}^{300} \frac{E_M(\lambda)}{\lambda^2}}\right]$$

where $E_P(\lambda)$ and $E_M(\lambda)$ are the extinction coefficients at λ of the polymer residue and the isolated monomer, respectively. The wavelength intervals, $\Delta\lambda$, were 2.50 mμ.

Optical rotatory dispersions were measured at 22° with either a Jasco spectropolarimeter, Model ORD/UV-5, or a Cary 60 spectropolarimeter. The Lorentz correction was not applied to the molar rotations, which are reported in (degrees deciliters per decimeter mole).

Sedimentation velocity experiments were performed with a Spinco Model E analytical ultracentrifuge. Absorption optics were employed in following the polynucleotide boundaries.

Results and Discussion

Optical Properties. The spectral properties of poly rA were first examined in aqueous solvents in order to assure ourselves that these particular samples exhibited the optical characteristics reported by other (Brahms and Mommaerts, 1964; Holcomb and Tinoco, 1965; Van Holde *et al.*, 1965; Leng and Felsenfeld, 1966). The solvents selected for these studies were the buffered media of moderate ionic strength, 0.10 M NaCl–0.05 M NaH_2PO_4–Na_2HPO_4 buffer (pH 7.0) and 0.10 M NaCl–0.10 M CH_3COONa–CH_3COOH (pH 4.6). Since the polymer is not completely protonated in the latter solvent at pH 4.6 (Steiner and Beers, 1961; Holcomb and Timasheff, 1968), aqueous solutions at lower pH values were also examined. To prevent extensive aggregation and precipitation, we employed unbuffered aqueous media of low ionic strength containing 25–73 × 10^{-5} M HCl, 240 × 10^{-5} M NaCl, or similar molarities of $HClO_4$ and $NaClO_4$. These solutions were usually allowed to stand for 1–3 hr before being examined optically. Within the time period in which the spectra and optical rotatory dispersion were obtained, the absorbance and optical rotation were constant. The absorbance, however, usually decreased by about 2% over a 24–48-hr

TABLE II: Optical Rotatory Dispersion Characteristics of Poly rA in Various Acid Solvents.

	Optical Rotatory Dispersion			
	Peak		Trough	
Solvent	λ (mμ)	[φ] × 10^{-4} ((deg dl)/(dm mole))	λ (mμ)	[φ] × 10^{-4} ((deg dl)/(dm mole))
0.10 M NaCl–0.10 M CH_3COOH–CH_3COONa, pH 4.6 in H_2O	281	+5.0	250	−10.6
24 × 10^{-5} M HCl–240 × 10^{-5} M NaCl, in H_2O	280	+5.2	250	−9.4
97% $(CH_2OH)_2$–73 × 10^{-5} M HCl–240 × 10^{-5} M NaCl	279	+4.7	249	−8.4

period. The latter was probably caused by a slow aggregation process.

The spectral characteristics of poly rA in some of these solvents are reported in Table I. The optical rotatory dispersion characteristics of the polymer in acid solvents are reported in Table II. As these data show, the optical properties of the polymer in the buffered solutions at moderate ionic strength compare reasonably well with those found by others in similar solvents. In the unbuffered solution at low ionic strength, the extinction coefficient at the maximum was higher and the over-all hypochromism was lower, compared with moderate ionic strength solutions at acid pH. Correspondingly, the residue rotation at the trough in the optical rotatory dispersion, was also slightly reduced.

When the aqueous solvents were replaced by mixtures of ethylene glycol and water containing a small excess of NaOH (24 × 10^{-5} M) to neutralize carbon dioxide or other acid contaminants, the extinction coefficients of the poly rA spectra increased as the volume per cent ethylene glycol increased. In contrast to this situation, however, similar experiments conducted in the presence of a small excess of HCl (24–73 × 10^{-5} M) revealed that relatively little change occurred in the absorption properties of the polymer as the solvent was changed from an aqueous acid solvent to one containing 97–99% ethylene glycol. These points are demonstrated in Figure 1 which represents the behavior of the extinction coefficients of poly rA at a fixed wavelength (260 mμ) in the mixed ethylene glycol–H_2O solutions as a function of solvent composition.

In Figure 2 the spectra of the polymer and 5′-AMP in the 97% ethylene glycol solvent containing NaOH are shown together with the spectrum in the aqueous solvent at pH 7. As can be seen, ethylene glycol in the ab-

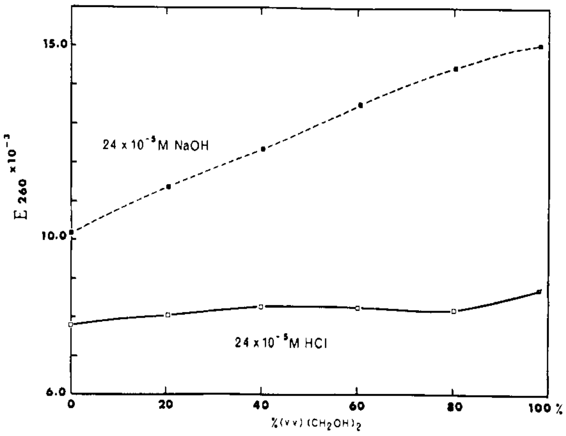

FIGURE 1: Effects of ethylene glycol on the extinction coefficients at 260 mμ of poly rA spectra. Data were taken from spectra of poly rA at a concentration of 5.64 × 10^{-5} M in mixed aqueous glycol solvents containing 240 × 10^{-5} M NaCl and either 24 × 10^{-5} M NaOH (■) or 24 × 10^{-5} M HCl (□).

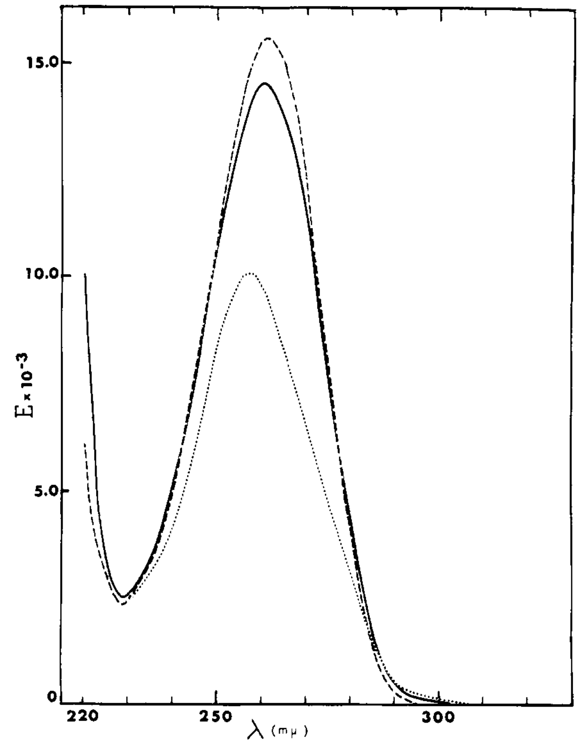

FIGURE 2: Spectra of poly rA and 5′-AMP in the absence of added acid. (···) Poly rA in 0.10 M NaCl–0.05 M NaH_2PO_4 Na_2HPO_4 (pH 7) in H_2O; (——) poly rA in 24 × 10^{-5} M NaOH–240 × 10^{-5} M NaCl, in 97% $(CH_2OH)_2$; (– – –) 5′-AMP in 24 × 10^{-5} M NaOH 240 × 10^{-5} M NaCl in 97% $(CH_2OH)_2$.

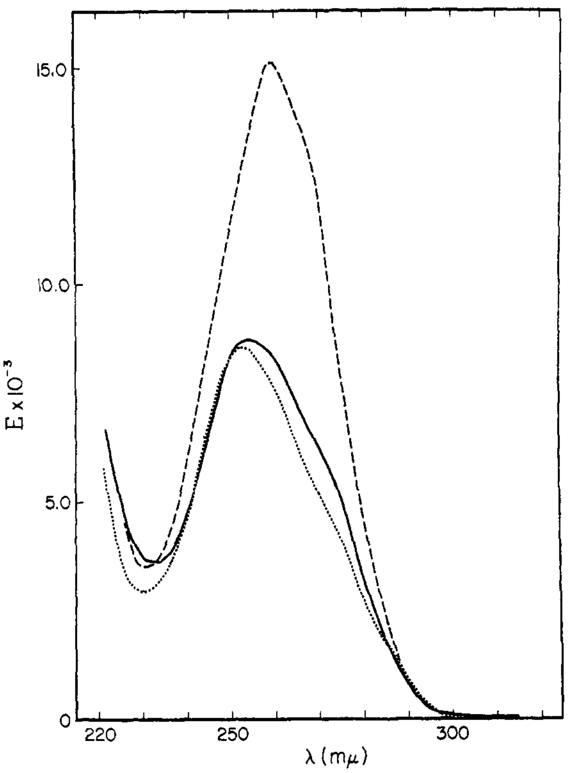

FIGURE 3: Spectra of poly rA and 5′-AMP in the presence of excess acid. (····) Poly rA in 0.10 M NaCl–0.10 M CH_3-$COOH$–CH_3COONa, at pH 4.6 in H_2O; (——) poly rA in 73 × 10^{-5} M HCl–240 × 10^{-5} M NaCl in 97% $(CH_2OH)_2$; (– – –) 5′-AMP in 0.003 M HCl–0.0024 M NaCl in 97% $(CH_2OH)_2$.

sence of any acidity, effectively abolishes the base-stacking interaction. Its interaction with the polymer leads to a conformation whose spectral properties are almost identical with that of the monomer, 5′-AMP, in the same solvent. The hypochroism of the polymer in this solvent, reported in Table I, is only 3.2 ± 1% and the position and extinction coefficient at the maximum are very close to 5′-AMP whose λ_{max} falls at 262 mμ and whose E_{max} is 15.5 × 10^3 l./(mole cm) in the same solvent.

In the presence of an 11-fold excess of hydrogen ions (73 × 10^{-5} M), however, the spectral properties in ethylene glycol did not differ markedly from those in the aqueous acid solutions and were clearly different from the monomer in the same solvent. This is demonstrated by the spectra shown in Figure 3 as well as the general optical characteristics of poly rA in various ethylene glycol–acid solvents reported in Tables I and II.

As was the case for the low pH–low ionic strength aqueous solutions, small decreases in the absorbance of the polymer in these acidified ethylene glycol solutions (*ca.* 2%) were usually observed after 24 hr. This was also probably due to a slow aggregation. Even the freshly prepared solutions (approximately 3-hr old) seemed to be extensively aggregated as the major share of the polymer sedimented at 10,000 rpm. There was no visible turbidity or other macroscopic evidence of aggregation in the glycol solutions, however, and the absorbance remained constant over the course of an experiment.

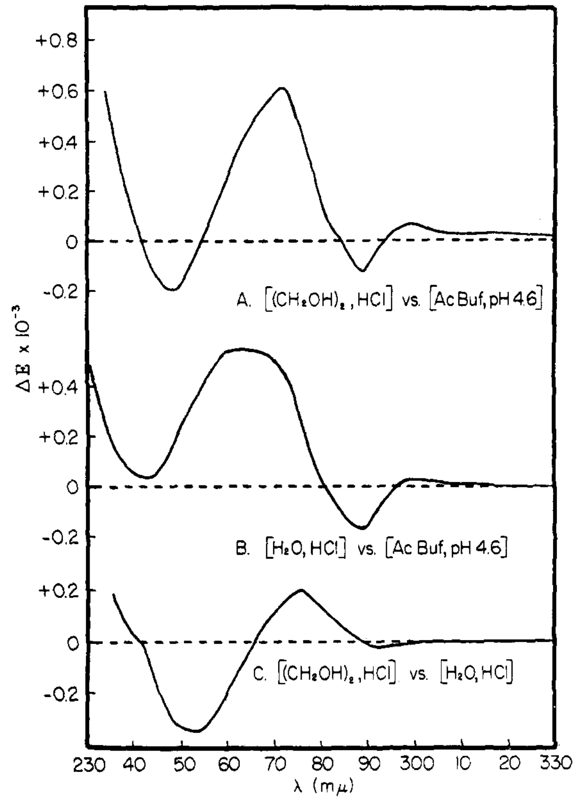

FIGURE 4: Difference spectra of poly rA in acid solvents. Spectral experiments were conducted with 5.43 × 10^{-5} M poly rA in the solvents cited. (A) 73 × 10^{-5} M HCl–240 × 10^{-5} M NaCl in 97% $(CH_2OH)_2$ in sample chamber against 0.10 M NaCl–0.10 M CH_3COOH–CH_3COONa (pH 4.6) in H_2O in reference chamber. (B) 60 × 10^{-5} M HCl–240 × 10^{-5} M NaCl in H_2O (pH 3.27) in sample chamber against 0.10 M NaCl–0.10 M CH_3COOH–CH_3COONa (pH 4.6) in H_2O in reference chamber. (C) 73 × 10^{-5} M HCl–240 × 10^{-5} M NaCl in 97% $(CH_2OH)_2$ in sample chamber against 60 × 10^{-5} M HCl–240 × 10^{-5} M NaCl in H_2O (pH 3.27) in reference chamber.

Difference spectra, shown in Figure 4, proved to be useful in selecting the appropriate aqueous solvent for comparison with the ethylene glycol solvent. They could also be used to detect small but significant changes in the spectra themselves. For instance, a difference spectrum obtained by running a solution of poly rA in 73 × 10^{-5} M HCl in 97% $(CH_2OH)_2$ against the polymer in 0.10 M NaCl–0.10 M CH_3COOH–CH_3COONa (pH 4.6) in H_2O is shown in the upper part (A) of Figure 4. This spectrum exhibits positive values of ΔE in the 260-mμ region, as well as a negative value at 288 mμ proportionately greater in view of the smaller value of E at this wavelength. A difference spectrum (Figure 4B) of similar shape although somewhat different characteristics was also observed when a solution of poly rA at low ionic strength in the unbuffered aqueous solvent, *ca.* 60 × 10^{-5} M HCl–240 × 10^{-5} M NaCl (pH 3.27), was run against the solution of poly rA in 0.10 M NaCl–0.10 M CH_3COOH–CH_3COONa (pH 4.6).

When the poly rA in the acidified ethylene glycol solvent was run against poly rA in the acidified aqueous

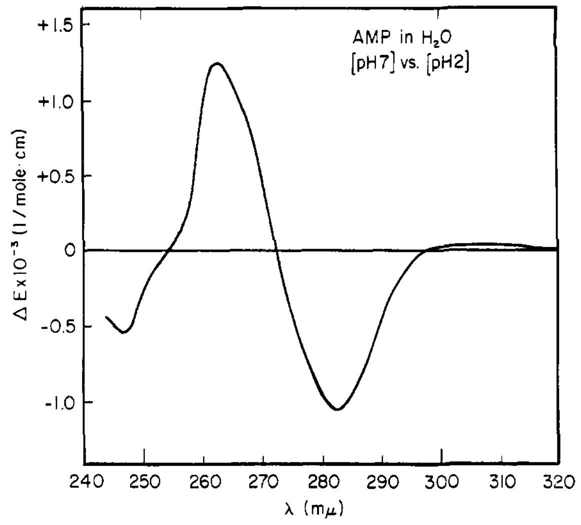

FIGURE 5: Difference spectrum of 5′-AMP in aqueous solvents. The spectrum above is observed when AMP in 0.01 M NaH_2PO_4–Na_2HPO_4 (pH 7) in the sample chamber is read against the same concentration in 0.01 M HCl–0.001 M NaCl (pH 2) in the reference chamber.

solvent at low ionic strength, the difference spectrum shown in Figure 4C was observed. The spectrum shows little change in the 290-mμ region indicating that the long-wavelength band has been equally affected in both solvents. In general there appears to have been a red shift of the entire absorption curve of the polymer in ethylene glycol compared with water. This is reasonable in view of the greater polarizability of the latter solvent as well as its lesser capacity to hydrogen bond.

The general shapes of the difference spectra seen in Figure 4A,B resemble, to a certain extent, a difference spectrum of AMP or adenosine in the unprotonated and the protonated forms. Such a spectrum for 5′-AMP of the *lesser* protonated *vs.* the *more* protonated is shown in Figure 5. The ΔE values in this figure are much larger than those observed in the difference spectra of poly rA. If the extent of protonation in the [$(CH_2OH)_2$, HCl] and in the [H_2O, HCl] solutions of poly rA was less than that in the reference, [acetate buffer, pH 4.6], solution, then the negative absorption at 290 mμ shown in Figure 4A,B might be merely a reflection of the inequity in extent of protonation. However, this cannot be true for the following reasons. (1) Titration studies of other workers (Steiner and Beers, 1961; Holcomb and Timasheff, 1968) have demonstrated that the polymer is *more* protonated in the [H_2O, HCl] solvent than in the [acetate buffer, pH 4.6] solvent. (In fact, at pH 3.27 and low ionic strength, all of the adenine residues in poly rA are protonated.) If extent of protonation were a significant factor, one should, if anything, observe a positive ΔE at 290 mμ rather than the negative one actually observed. (2) The negative absorption in the 290-mμ region in Figure 4A,B is relatively insensitive to acid concentration below the titration end point in the [CH_2OH, HCl] and the [H_2O, HCl] solvents.

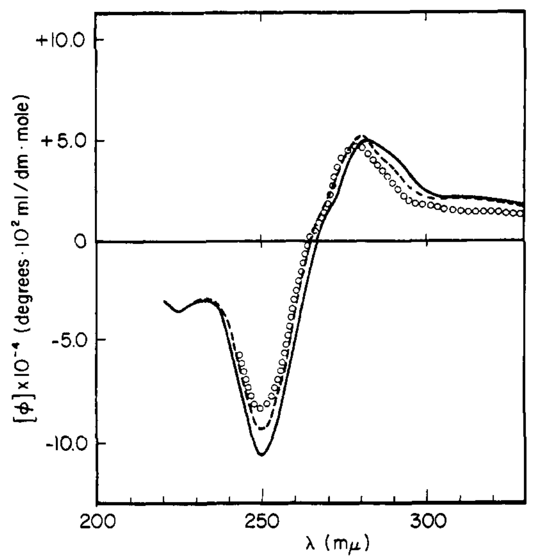

FIGURE 6: Optical rotatory dispersion of poly rA in acid solvents: ——, 0.10 M NaCl–0.10 M CH_3COOH–CH_3COONa, pH 4.6 in H_2O; – – –, 24×10^{-5} M HCl–240×10^{-5} M NaCl, pH 3.8 in H_2O; ○ ○ ○, 73×10^{-5} M HCl–240×10^{-5} M NaCl in 97% $(CH_2OH)_2$.

The optical rotatory dispersions of the polymer in the two aqueous acid solvents and in the ethylene glycol–acid solvent are shown in Figure 6. The effects seen in the absorption spectra in the 290-mμ region are also observed in these curves. In Figure 6, there appears to be the beginning of a Cotton effect at around 290 mμ for the polymer in 0.10 M NaCl–0.10 M CH_3COOH–CH_3COONa (pH 4.6). These effects in that wavelength region seem to be reduced in the case of the polymer in the low ionic strength aqueous solvent (24×10^{-5} M HCl–240×10^{-5} M NaCl, pH 3.8) and in the acid-glycol solvent (73×10^{-5} M HCl–240×10^{-5} M NaCl in 97% $(CH_2OH)_2$). The shapes of the patterns for the latter two solutions are quite similar, although the residue rotations, [φ], for the polymer in ethylene glycol–HCl are lower than those exhibited by the aqueous solution.[1]

In the presence of 24×10^{-5} M NaOH, the values of $[\varphi]_\lambda$ of the polymer in 97% $(CH_2OH)_2$ were indistinguishable from that of the monomer, 5′-AMP.

Titration Studies. The conformation of poly rA in the ethylene glycol–HCl solvent can be converted into the form whose spectral properties are similar to that of the monomer, 5′-AMP, by neutralizing the acid with NaOH. The spectral changes accompanying this addition of base are presented in Figure 7.

A more precise titration experiment was conducted with a solution of poly rA in excess $HClO_4$ in 97% ethylene glycol. The poly rA solution was first prepared at a concentration of 6×10^{-5} M in 73×10^{-5} M $HClO_4$–240×10^{-5} M $NaClO_4$ in 97% $(CH_2OH)_2$. The excess acidity was partially neutralized by the addition of a small volume of 1 M NaOH. The titration was then continued by the addition of 0.030 N NaOH to a 3.00-ml aliquot of the solution containing 18.1×10^{-5} mmole of adenine. The results of a typical experiment are pre-

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[1] On the scale employed in this figure, the rotation of the monomer, AMP, in any of the solvents cited is negligible.

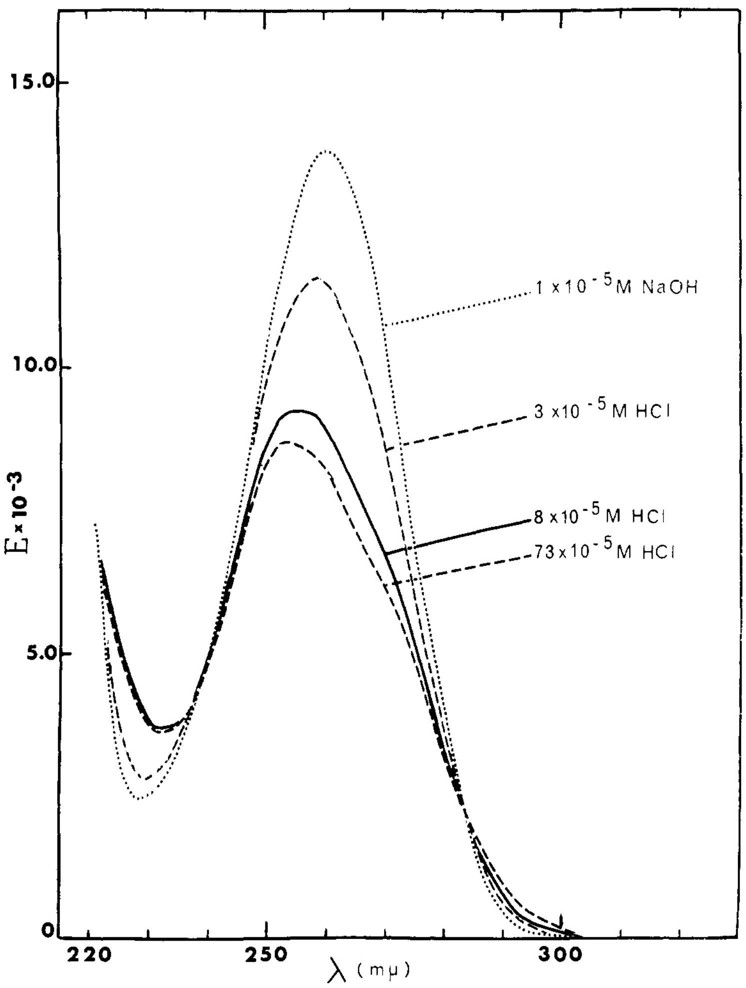

FIGURE 7: Spectral changes in acid solutions of poly rA in 97% $(CH_2OH)_2$ upon addition of NaOH: ---, 73 × 10^{-5} M HCl; ——, 8 × 10^{-5} M HCl; – – –, 3 × 10^{-5} M HCl; and · · ·, 1 × 10^{-5} M NaOH. The NaCl concentration varied from 240 × 10^{-5} M at the highest acid concentration to 313 × 10^{-5} M in 1 × 10^{-5} M NaOH.

sented in Figure 8 as a plot of the absorbance at 260 mμ as a function of the volume of 0.030 N NaOH added. The optical transformation observed corresponds to the spectral changes seen in the preceding figure (Figure 7) as the HCl concentration changed from 8 × 10^{-5} to 0 M. The end points of this transition are assumed to fall at the position of the crosses. The difference between these two points corresponds to 16.2 × 10^{-5} mmole of OH^-, or, effectively, 0.9 mmole of hydroxide ion per adenine residue. The average for several such titrations in $HClO_4$ as well as HCl was 0.95 ± 0.05. Similar results were also obtained in the low ionic strength aqueous solvent. The titrations in the ethylene glycol as well as in the aqueous solutions under comparable ionic strength conditions are only partially reversible within a 24-hr period[2] and, hence, attempts to extract additional information from this type of experiment were abandoned.

The disappearance of the optical rotatory dispersion properties shown in Table II also parallels the addition of base to the acid form in ethylene glycol.

Melting curves for poly rA in the several solvents employed in these studies are shown in Figure 9 as plots of the extinction coefficients at 260 mμ as a function of temperature. The absence of a large change in the extinction coefficient in ethylene glycol–NaOH supports the contention that there are no short-range stacking interactions between the adenine residues when this solvent is acid free.

If solutions of poly rA in ethylene glycol at acid concentrations of 73–24 × 10^{-5} M HCl are heated, precipitation occurs. In order to avoid this, it was necessary to titrate a small fraction of the residues in order to produce a slightly negatively charged species. Hence, the data so obtained represent the behavior of the form at the toe of the spectral transition shown in Figure 8. Despite this, however, there is no evidence of melting of such a poly rA structure in the organic solvent below 80°. (Above 80°, slow spectral changes appeared with time, but these were probably due to thermal degradation.) The aqueous solution of poly rA, prepared in 24 × 10^{-5} M HCl–240 × 10^{-5} M NaCl, was also ex-

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[2] Reacidification of these titrated solutions will result in a spectrum which shows marked hypochromism but does not go through the isosbestic points exhibited in Figure 7.

ceptionally stable, and did not exhibit a melting point below 85°. We thus feel that the absence of thermal changes below 80° justifies the assumption that the polymer conformation exhibited at low ionic strengths in H_2O and in ethylene glycol is the most thermodynamically stable form in those media.

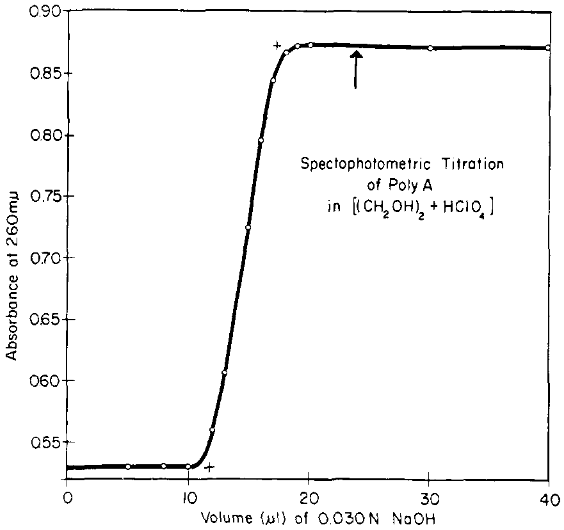

FIGURE 8: Spectrophotometric titration of poly rA in $(CH_2OH)_2$. Absorbance at 260 mμ of 3.00 ml of 6.03×10^{-5} M poly rA in 97% $(CH_2OH)_2$ in excess $HClO_4$ is plotted on the ordinate against the volume (in microliters) of 0.030 N NaOH added to this solution. The beginning and end of the transition are indicated by the crosses. Complete neutralization of the added acid should have occurred at the arrow at 24 μl.

Conclusion

The various optical properties of poly rA in the acidified ethylene glycol solutions clearly demonstrate that a base-stacked helical conformation is present in these solvents. The similarity of these properties as well as the stability of this conformation (as reflected in its absence of a T_m below 80°) when compared with those of the polymer in the aqueous solution at low ionic strength support the conclusion that the two conformations are very similar if not identical. In view of the red shift in the spectral properties exhibited in the ethylene glycol solutions, it can also be concluded that the optical properties of the polynucleotide bases are affected by the solvent environment even though they are embedded in the core of the polynucleotide helix.

The conformation found in the low ionic strength aqueous solutions and in the acidified glycol solution is somewhat different, however, from that formed at moderate ionic strength in water at pH 4.6. We cannot, at the present time, offer an unequivocal explanation for these differences. The difference spectra taken alone could be interpreted in terms of either a decrease in the oscillator strength or a hypsochromic shift of a band in the 290-mμ region in the case of the conformation present in acidified glycol and in acidified aqueous solutions at low ionic strength. The optical rotatory dispersion properties, however, show no signs of a blue shift in the rotational properties in this wavelength range and hence we have concluded that the change in the optical properties of the polymer in these two solvents compared with similar properties in aqueous acid solutions at moderate ionic strength is due to the reduction of both the oscillator and the rotational strength of a transition around 290 mμ. The latter may be an n → π* transition as it falls in a wavelength region where one would expect to find such a transition in purines and also exhibits hyperchromicity, when present, in the helical conformation (Rich and Kasha, 1960). If so, it should be very sensitive to minor alterations in the position of the adenine residues relative to the helix axis. On the basis of this line of reasoning, we tentatively offer the following explanation for these results. In the model of the acid form of poly rA proposed by Rich *et al.* (1961), the adenine bases are arranged as a propeller-like dimer about the helix axis. The slight tilt facilitates van der Waals contacts between the planes of the bases. This arrangement would tend to enhance the interaction properties of an n → π* transition, leading to hyperchromicity in the absorption spectrum and an increased rotational strength in the optical rotatory dispersion properties in the spectral region of the transition. Presumably this is the form present in the aqueous solutions containing 0.10 M NaCl–0.10 M acetate buffer (pH 4.6). The reduction of these effects in the low ionic strength aqueous acid and in the glycol–acid solutions leads to the conclusion that in the conformation present in these solutions, the bases have rotated so as to become more coplanar and more perpendicular to the axis of the helix. The coplanarity, however, had led to a slight increase in the center to center distances between the bases. Since the n → π* band is more sensitive to contact distance (Schellman and Oriel, 1962), this transition is more affected, on a proportional basis, than are the π → π* bands. The increase in center to center distance between the bases, however, also accounts for the increased hyperchromicity and lower rotational strength of these latter transitions in the 260-mμ region.

The inability of the two strands to come together once separated in ethylene glycol or in the low ionic strength aqueous solvents is likely to be due to a high activation energy resulting from the approach of two or more negatively charged phosphate ions as well as the large viscous drag of the highly charged coils. Since both these effects are particularly pronounced in ethylene glycol, we may well ask how the base-stacked double-stranded conformation is ever formed in this solvent. We feel that the crucial point is the method of preparation of the solution. Upon diluting an aliquot of the aqueous stock solution of poly rA in 0.10 M NaCl with ethylene glycol containing acid, the more rapidly diffusible protons from the glycol immediately protonate the adenine residues in the water-rich drop. The base-stacked helical conformation of poly rA thus quickly forms at a moderate ionic strength as the aqueous drop mixes with the glycol solvent.

The spectrum, in the absence of acid, shows that ethylene glycol abolishes the base-stacking interaction between the adenine residues and hence $\Delta G_S{}^\circ$ is approxi-

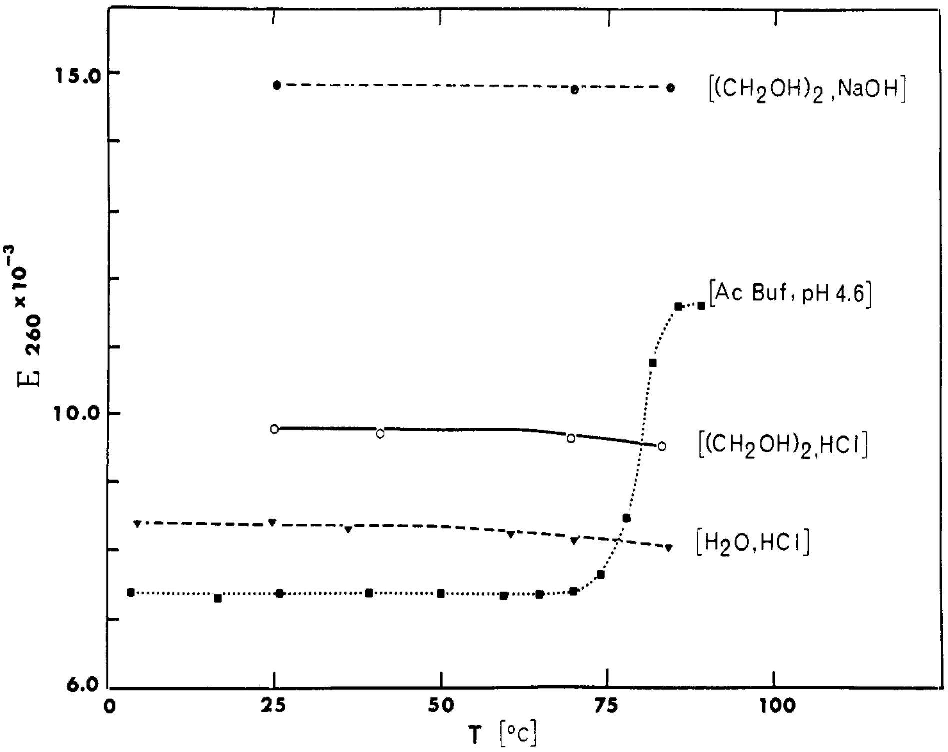

FIGURE 9: Temperature dependence of the spectral properties of poly rA in a variety of solvents. Extinction coefficients at 260 mμ are plotted as a function of temperature for poly rA at a concentration of 6×10^{-5} M in the following solvents: ●, 16×10^{-5} M NaOH–264×10^{-5} M NaCl in 97% $(CH_2OH)_2$; ○, *ca.* 6×10^{-5} M HCl–288×10^{-5} M NaCl in 97% $(CH_2OH)_2$; ▼, 24×10^{-5} M HCl–240×10^{-5} M NaCl, pH 3.8 in H_2O; ■, 0.10 M NaCl–0.10 M CH_3COOH–CH_3COONa, pH 4.6 in H_2O.

mately 0 in this system. The energy of stabilization in the acidified glycol solvent must derive principally from the enhanced strength of the internal ion pair interaction. We thus feel that we have supported our initial predictions that under such conditions, where compensatory effects are possible, the grosser features, at least, of an organized polynucleotide conformation will be preserved. This approach may prove useful in providing model systems for the study of various optical and thermodynamic properties of base-stacked helical polynucleotide conformations in solvent environments markedly different from water.

Acknowledgment

We thank Dr. Stelios Aktipis for the use of his Jasco ORD/UV-5 spectropolarimeter.

References

Brahms, J., and Mommaerts, W. F. H. M. (1964), *J. Mol. Biol. 10*, 73.

DeVoe, H., and Tinoco, Jr., I. (1962a), *J. Mol. Biol. 4*, 500.

DeVoe, H., and Tinoco, Jr., I. (1962b), *J. Mol. Biol. 4*, 518.

Fresco, J. R. (1959), *J. Mol. Biol. 1*, 106.

Fresco, J. R., and Doty, P. (1957), *J. Am. Chem. Soc. 79*, 3928.

Fresco, J. R., and Klemperer, E. (1959), *Ann. N. Y. Acad. Sci. 81*, 730.

Hanlon, S. (1966), *Biochem. Biophys. Res. Commun. 23*, 861.

Holcomb, D. N., and Timasheff, S. N. (1968), *Biopolymers 6*, 513.

Holcomb, D. N., and Tinoco, Jr., I. (1965), *Biopolymers 3*, 121.

Leng, M., and Felsenfeld, G. (1966), *J. Mol. Biol. 15*, 455.

Pabst Laboratory Circular OR-7 (1955), Pabst Brewing Co., Milwaukee, Wis.

Rahn, R. O., Yamane, T., Einsinger, J., Longworth, J. W., and Shulman, R. G. (1966), *J. Chem. Phys. 45*, 2947.

Rich, A., Davies, D. R., Crick, F. H. C., and Watson, J. D. (1961), *J. Mol. Biol. 3*, 71.

Rich, A., and Kasha, M. (1960), *J. Am. Chem. Soc. 82*, 6197.

Schellman, J. A., and Oriel, P. (1962), *J. Chem. Phys. 37*, 2114.

Schildkraut, C., and Lifson, S. (1965), *Biopolymers 3*, 195.

Steiner, R. F., and Beers, Jr., R. F. (1961), Polynucleotides, New York, N. Y., Elsevier.

Van Holde, K. E., Brahms, J., and Michelson, A. M. (1965), *J. Mol. Biol. 12*, 726.

Witz, J., and Luzzati, V. (1965), *J. Mol. Biol. 11*, 620.