A STRAND DISPROPORTIONATION REACTION IN A HELICAL POLYNUCLEOTIDE SYSTEM

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Poly A[1/] and poly U react in aqueous salt solution to form two- or three-stranded helices (1-6). We have recently employed digital analysis of quantitative infrared spectra to determine the kinds and amounts of helical and random coil forms in poly A-poly U mixtures (7). In this report we employ quantitative melting curves measured in the infrared and analysis of digitized spectra to demonstrate a new disproportionation reaction, (1).

(1) 2 poly (A+U) ⟶ poly (A+2U) + poly A

This disproportionation occurs when poly (A+U) is heated under appropriate conditions (e.g., 0.14 N Na^+ + 0.02 N Mg^{++} or ~ 0.5 equivalents of Mg^{++} per phosphate), and was first detected by observing the infrared spectra of a 1:1 poly A-poly U mixture as a function of temperature (typical spectra shown in Fig. 1). There is a rapid change of spectrum at comparatively low temperature to a new spectrum which then does not change appreciably over a wide temperature range. A sensitive feature of this spectral change is the absorbance at 1657 cm^{-1}. When the absorbance at this frequency is plotted from spectra measured during the melting, a clear biphasic curve is observed (Fig. 2, light circles), suggesting the occurrence of two distinct processes during the heating. The identity of the plateau spectrum (e.g., the spectrum

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[1/] Abbreviations: Poly A, polyadenylic acid; poly U, polyuridylic acid; poly (A+U), two-stranded helix; poly (A+2U), three-stranded helix.

measured at 56.9°) with a synthetic curve calculated by summation of the spectra of poly (A+2U) and poly A (ref. 7, Fig. 3) demonstrates that the strand disproportionation reaction (1) had indeed occurred as the first step of the biphasic curve. The second step represents a simultaneous dissociation of the three-stranded helix (reaction (2)), as we shall show in a subsequent paper.

(2) poly (A+2U) $\longrightarrow$ poly A + 2 poly U

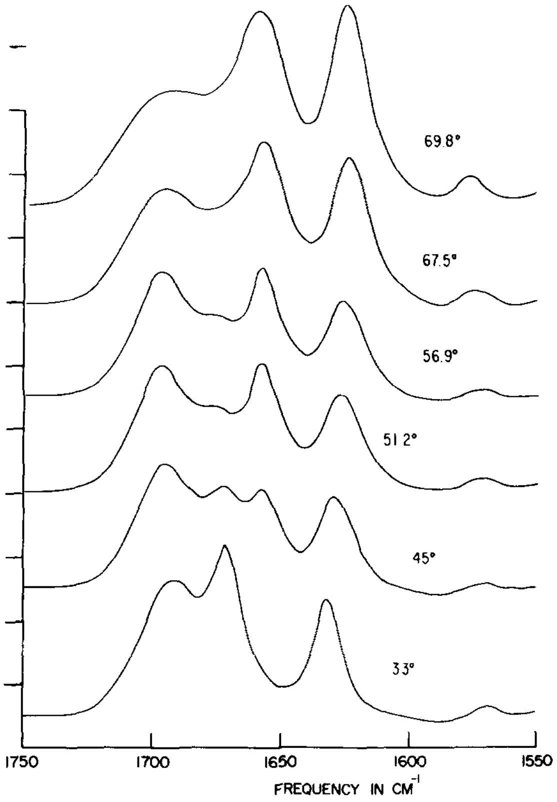

Fig. 1. Infrared spectra in D_2O solution of poly (A+U), 0.045 M in polymer phosphate; 0.14 N Na^+; 0.02 N Mg^{++}; 4.83 fold scale expansion; 55.6µ matched CaF_2 cells. Ordinate is absorbance, the index marks being 0.2 units apart as observed on the original spectra, uncorrected for scale expansion.

A further demonstration of the composition of the solution at ~ 57° was obtained from a computer analysis of the plateau spectrum to give a least squares best fit in terms of the standard catalog spectra,

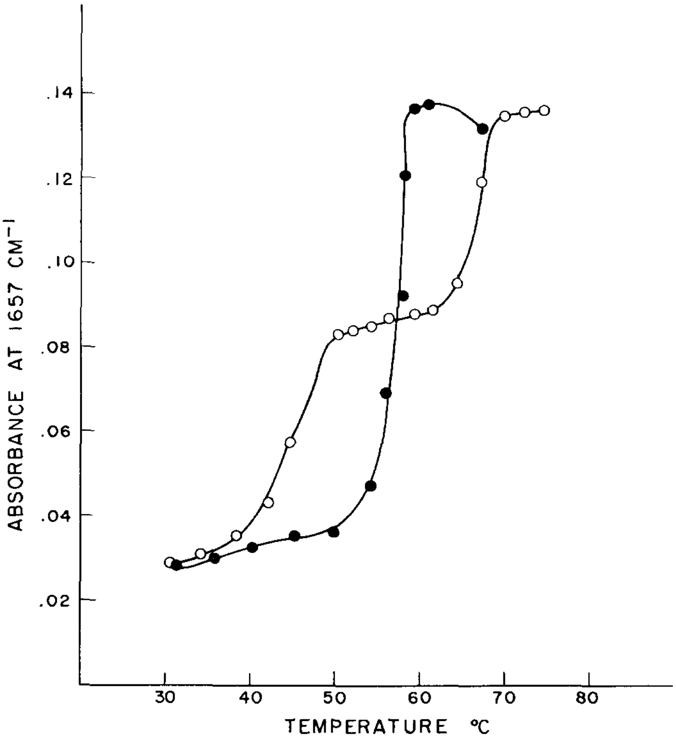

Fig. 2. Melting curves for poly (A+U) in presence Mg^{++} (conditions of Fig. 1; light circles) and in absence of Mg^{++} (conditions of Fig. 3; dark circles), In both cases the total polymer concentration (as phosphate) was 0.045 M, and the ordinate has been corrected for scale expansion to give true absorbance values.

poly A, poly U, poly (A+U), poly (A+2U). The result showed 23.8% poly A (in terms of polymer phosphate) 75.7% poly (A+2U) 0.5% poly (A+U) and 0% poly U. This method of analysis has been used in a previous report (7) and will be discussed at greater length in later papers.

We observe, by contrast with the foregoing results, that the spectrum of a 1:1 poly A-poly U mixture under somewhat different salt conditions (e.g., 0.14 N Na^+, no Mg^{++}) undergoes quite different changes with temperature (Fig. 3) (note, e.g., the much later appearance of a discrete 1657 cm^{-1} band and a continuous change of spectrum throughout heating rather than reaching a spectrum which remains fixed over a wide temperature range as in Fig. 1), and gives a monophasic

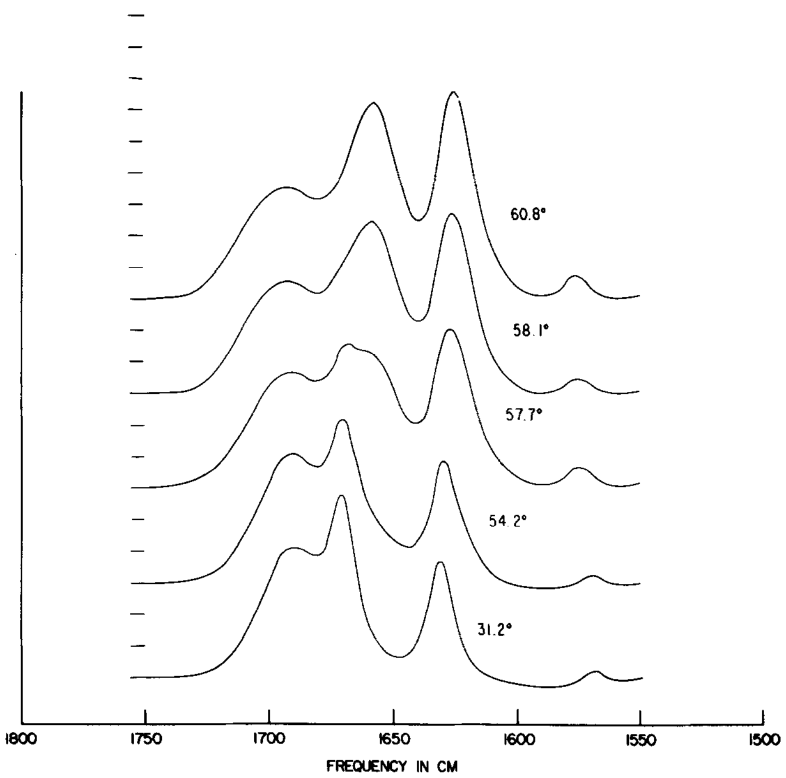

Fig. 3. Infrared spectra in D_2O solution of poly (A+U), 0.045 M; 0.14 N Na^+; no Mg^{++}; 4.50 fold scale expansion; 55.7μ path length. Ordinate is absorbance. The index marks being 0.1 units apart, uncorrected for scale expansion.

melting curve (Fig. 2, dark circles). A least squares analysis of several of the spectra in Fig. 3 permits us to determine directly the % poly (A+U) as a function of temperature (Fig. 4). The process observed in Fig. 2 (dark circles) is essentially the simple dissociation of the two-stranded helix:

(3) poly (A+U) $\longrightarrow$ poly A + poly U

We have briefly investigated the dependence of the occurrence of reaction (1) or (3) upon Na^+ and Mg^{++} concentration. On heating poly (A+U) at lower Na^+ concentrations the principal reaction is (3), but at higher Na^+ concentration (e.g., 0.44 N Na^+) the disproportionation reaction (1) occurs even in the absence of Mg^{++} (Fig. 5). A

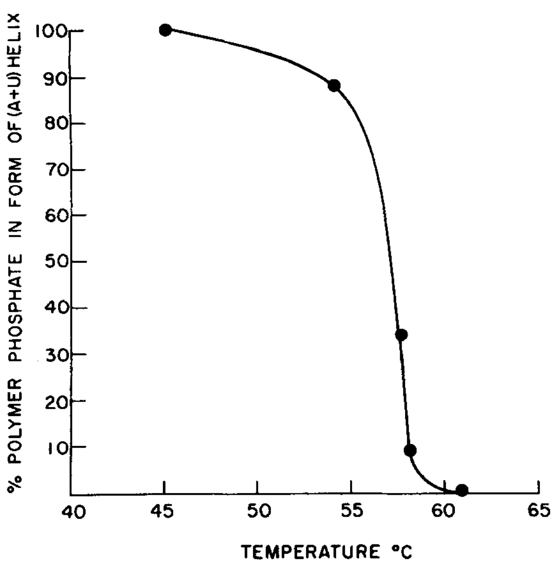

Fig. 4. The points on this curve were obtained from least squares analyses of some of the spectra in Fig. 3. The original spectra were digitized with a Gerber Digital XY Reader, scaled to an extinction coefficient basis, and analysed by computer in terms of standard catalog spectra, as indicated in the text and in ref. 7.

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few Tm values from monophasic melting curves of poly (A+U) (~ 0.05 N in polymer phosphate) in solutions of varying Na^+ concentration in the absence of Mg^{++} are as follows: 0.04 Na^+, 41°; 0.08 N Na^+, 52°; 0.14 N Na^+, 57°. When the equivalents of Mg^{++} exceed those of polymer phosphate (e.g., 0.06 N Mg^{++} or ~ 1.5 equivalents per phosphate; 0.14 N Na^+; ~ 0.04 N polymer phosphate), reaction (1) is complete at room temperature and the poly U exists entirely in three-stranded form. The solutions containing Na^+ as the only cation showed no hysteresis on cooling, but in the presence of Mg^{++} hysteresis was observed, particularly if the solution had been kept for some time at elevated temperature. Ultraviolet evidence for the occurrence of reaction (1) has also been obtained in a much lower concentration range (10^{-4} to 10^{-3} M in polymer phosphate) (8).

We wish to point out that by selecting infrared frequencies of well-resolved peaks definitely assignable to the separate polymers

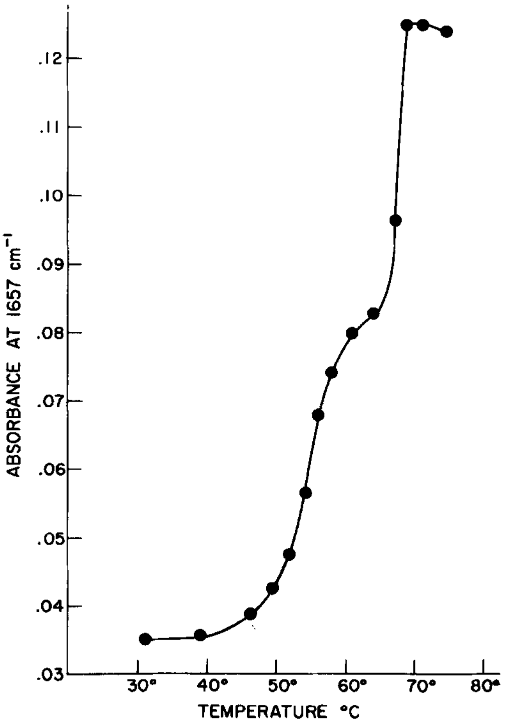

Fig. 5. Melting curve of poly (A+U), 0.035 M (in phosphate), in D_2O, 0.44 N Na^+; no Mg^{++}; path length, 55.6μ. The points were taken from spectra measured over the range 1550-1800 cm^{-1} and have been corrected for scale expansion to true absorbance.

(e.g., the 1657 cm^{-1} peak of uridine and the 1625 cm^{-1} peak of adenosine) we can demonstrate through the coincidence of the melting curves (Fig. 6) that the spectral changes specifically reflect an interaction between poly A and poly U and not, for example, independent structural changes in the separate polymers. There is no reason to doubt the nature of the interaction in this case, but we shall report the application of the method later in cases in which the specificity of the interaction is less obvious.

The concentrations of free polymers and helices are based on total polymer phosphate. The sodium ion concentrations represent the sum of added NaCl and the counter ion of the polymer, the latter contribution being important at the polymer concentrations employed (~ 0.02 - 0.06 M). The polymers were versene dialyzed, as described

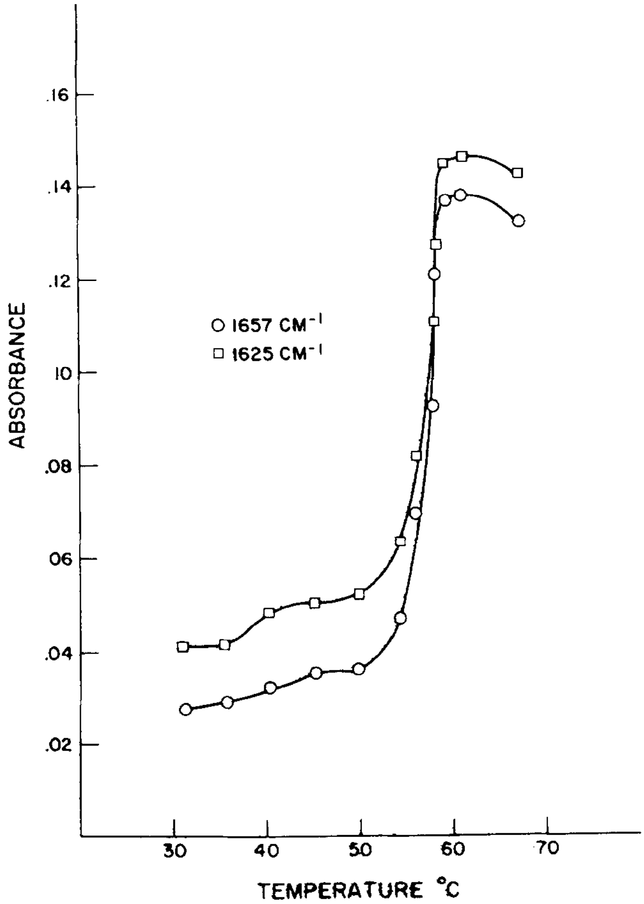

Fig. 6. Melting curve in D_2O of poly (A+U) under conditions of Fig. 2, dark circles. The 1657 cm^{-1} band is attributed to the uridine (primarily the C_4 carbonyl), and the 1625 cm^{-1} to the adenosine (ring vibration). The coincidence of the curves indicates that the interaction is specifically between the two different bases.

previously (7,9), and the solutions were buffered with 0.005 N sodium cacodylate, pH 7.3 ± 0.2.

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