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INFRARED STUDY OF HELIX STRANDEDNESS IN THE POLY A-POLY U[1] SYSTEM

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The reaction of poly A with poly U to form two- or three-stranded helices has been studied by a variety of methods (1-7). The most commonly used method of determining the stoichiometry of interaction or strandedness of the product has been the method of continuous variations or ultraviolet mixing curve (3,4). The question of strandedness in this system, however, still appears to be the subject of substantial disagreement (8). We report here the extension of earlier infrared studies to the determination of the kinds and amounts of the different polymer species and complexes from quantitative infrared spectra in D_2O solution.

The spectra were measured with a Beckman IR-7 spectrophotometer using matched CaF_2 cells of 55μ path length, as described in previous articles (9-11). The polynucleotides were obtained from Miles Laboratories and dialysed successively against versene, dilute NaCl, and triple distilled water as described previously (11), and then lyophilized from water and from D_2O before preparing stock solutions in D_2O for infrared study. To determine whether equilibrium had been

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[1] Abbreviations: Poly U, polyuridylic acid; poly A, polyadenylic acid; poly (A+U) or (A+U) or AU, two-stranded helix; poly (A+2U), or (A+2U), or AU_2, three-stranded helix.

reached, some of the solutions at 50% U and 66.7% U were allowed to stand for several months in 0.14 N Na^+, 0.02 N Mg^{++}, and their spectra were found to be the same as those observed soon after mixing. For analysis a number of the spectra were digitized and normalized to an extinction coefficient basis with a Honeywell-800 computer (Fig. 1 and 2), using the expression $\epsilon = A/C \times l \times E$, where A = absorbance, C = concentration, l = path length (determined from interference fringes), and E = linear expansion factor used on absorbance scale. It was then convenient to synthesize summation spectra or to analyse an experimental spectrum to obtain a least squares best fit in terms of component catalog spectra. We are indebted to Gordon Adams for writing the necessary computer programs and aiding in the numerical analyses. A more detailed account of the application of digital analysis to these and other spectra will be published later.

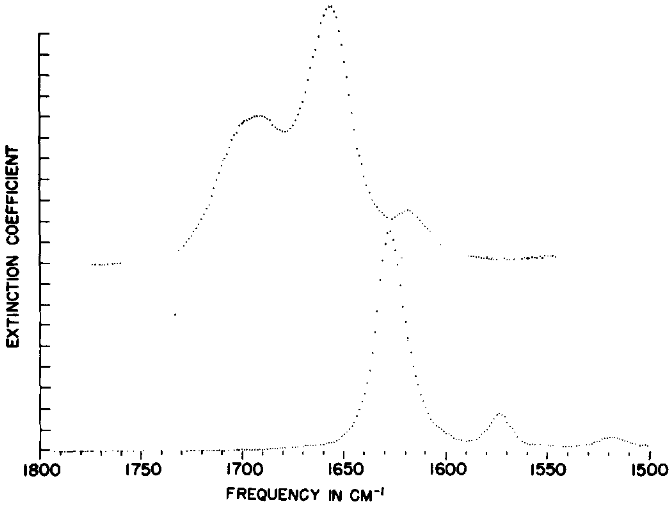

Figure 1. Spectra of poly U, upper, and poly A, lower, in 0.14 N Na^+ in D_2O, pD 7.3 ± 0.2. Digitized at intervals of 1.25 cm^{-1} and normalized by computer. Vertical index marks are 100 units apart.

A major advantage of the infrared method for the present study is that the vibrational spectra of the two-stranded and the three-stranded helices are qualitatively and quantitatively quite different (Fig. 2; Table I). In particular we call attention to the following characteristic differences: Poly (A+U) has uridine bands at 1691 cm^{-1} and 1672 cm^{-1}. The adenosine band is at 1631 cm^{-1}. Poly (A+2U), on the other hand, has uridine bands at 1696 cm^{-1}, ~ 1677 cm^{-1}, and a very strong band at 1657 cm^{-1}. The intensity of the adenosine band at ~ 1630 cm^{-1} has decreased almost to zero.

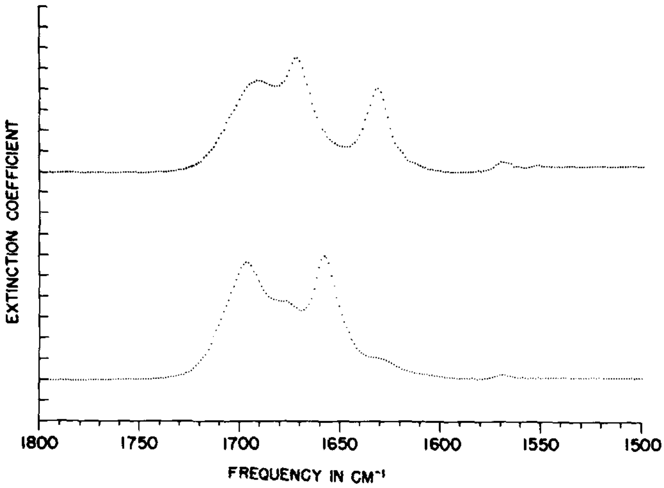

Figure 2. Spectra of poly (A+U), upper, and poly (A+2U), lower, under conditions of Fig. 1. The concentrations used in normalizing the spectra were based on total polymer phosphate.

The spectrum presented as that of poly (A+2U) is observed under conditions (e.g., 0.14 N Na^+, 0.02 N Mg^{++} at the 2:1 U:A ratio) which are generally agreed to produce a three-stranded helix.

Taking the correspondence of this spectrum to poly (A+2U) as our starting point we can then synthesize a spectrum corresponding to that which would exist at the 1:1 U/A ratio if we actually had (A+2U) + A (Fig. 3). We take the marked discrepancy of this spectrum from that presented here and previously (9) as that of (A+U) to be a clear demonstration that the structure is two-stranded and not three-stranded at the 1:1 ratio, under the conditions set forth below.

TABLE I

CATALOG INFRARED SPECTRA OF POLYNUCLEOTIDES*

	νmax	εmax	νmax	εmax	νmax	εmax	νmax	εmax
Poly A							1628	1057
Poly U	1692	710	1657	1230				
Poly (A+U)	1691	436	1672	544			1631	398
Poly (A+2U)	1696	567	1677	378	1657	599		

* Solutions in D_2O, 0.14N Na^+, ~ 0.04N polymer phosphate, pD 7.3 ± 0.2, temp. ~ 30° C. Concentrations based on total polymer phosphate in solution. Units of νmax, cm^{-1}. Units of εmax, $moles^{-1}$ liter cm^{-1}.

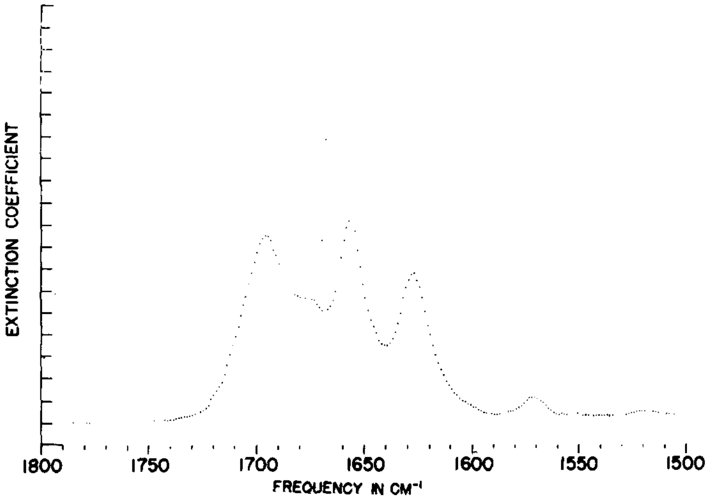

Figure 3. Synthetic spectrum formed by adding catalog spectrum of poly (A+2U) to that of poly A. Ordinate scale as in Figs. 1 and 2.

Fig. 4 shows the spectra of mixtures with varying ratios of poly A to poly U (0.14 N Na^+ pD 7.3 ± 0.2; very similar spectra are obtained for most of the points at room temperature if the solution is also 0.02 N in Mg^{++}). The fact that no 1657 cm^{-1} band is observed at or

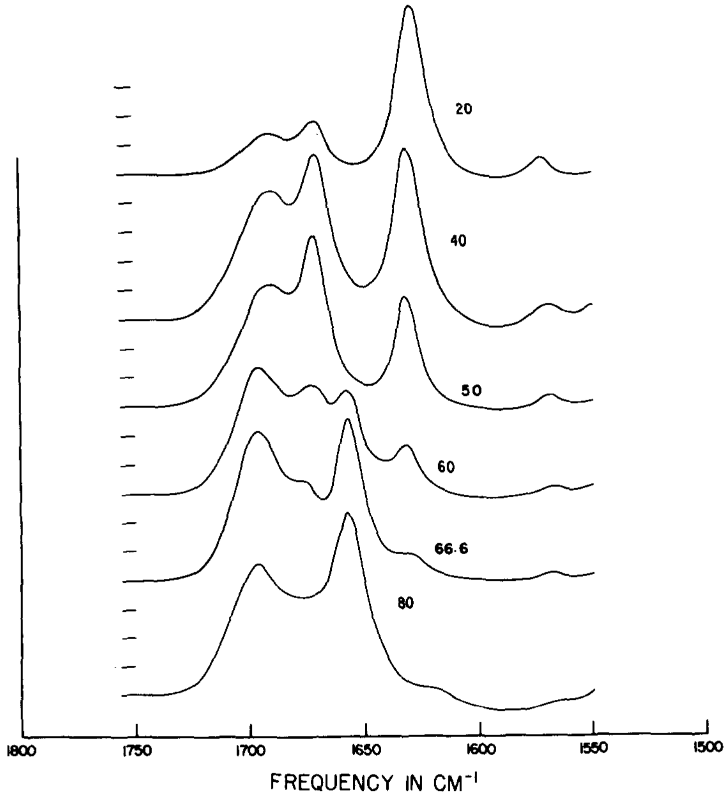

Figure 4. Infrared spectra of mixtures of poly A and poly U in D_2O, 0.14 N Na^+, pD 7.3 ± 0.2. The number above each spectrum indicates the mole % of poly U. The ordinate is absorbance, but since different scale expansions and polymer concentrations were used, the absolute intensities are not directly comparable among spectra in this figure. These spectra were normalized to a common extinction coefficient basis and analysed by computer to give results shown in Fig. 5 and Table II.

below 0.5 U/A indicates that there is neither free poly U nor poly (A+2U) in any of these solutions. Similarly, the high frequency peak is uniformly observed at 1691 cm^{-1} when U/A $\leq$ 0.5, but this peak shows the shift toward 1696 cm^{-1} at 0.6 U/A, which is complete at 0.67 U/A. The marked diminution in the intensity of the A peak at 1631 cm^{-1} is observed as soon as U/A ratio exceeds 0.5.

These qualitative observations may be more quantitatively assessed and summarized by normalizing the experimental curves shown in Fig. 4 to an extinction coefficient basis and analyzing each of them in terms of the standard components: A, U, (A+U), and (A+2U), Figs. 1 and 2. The results of these analyses are presented in Fig. 5 and Table 2. At U/A ratios between 0.5 and 0.67 all of the polymers are present as a mixture of the two helical forms.

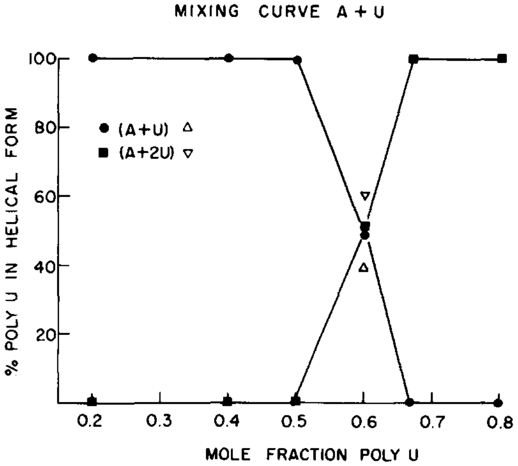

Figure 5. The points in the mixing curve were obtained by computer analysis of normalized curves from Fig. 4. The AU_2 point at 0.8 mole fraction U/A was calculated by subtracting the excess of U indicated for this ratio in Table II. The points indicated by triangles were taken from spectra 0.02 N in Mg^{++} as well as 0.14 N in Na^{+}. At the other ratios the presence of Mg^{++} appeared to make no difference in the spectra.

We have observed the same spectrum for the 1:1 mixture of A and U at 25-35° C under the following salt conditions: 0.04 N Na^{+}; 0.14 N Na^{+}; 0.44 N Na^{+}; 0.14 N Na^{+} + 0.02 N Mg^{++} (this was ~ 0.5 equivalents of Mg^{++} per phosphate). When the equivalents of Mg^{++} exceeded the equivalents of polymer phosphate present (0.14 N Na^{+} 0.04 N Mg^{++}, representing a normality ratio of ~ 1.5, the material was three-stranded at room temperature and had a spectrum identical with the synthetic spectrum shown in Fig. 3.

We consider our results to be incompatible with a recent conclusion (8), "..... that the conditions previously used to form

TABLE II

Analysis of Spectra of Varying U/A Ratio

Mole Fract. of U*	%A	%U	%AU	$\%AU_2$
.20	64.5	0	33.5	0
.40	17.8	0	82.2	0
.50	0	0	100	0
.60	0	0	49	51
.666	0	0	0	100
.80	0	38.4	0	61.6

* Solutions in D_2O, 0.14 N Na^+, ~ 0.04 N polymer phosphate, pD 7.3 ± 0.2, temp., ~ 30° C.

The values at .50 and .667 U/A ratios are by definition.

The values at .60 U/A are presumably slightly in error. A solution of this molar ratio and Na^+ concentration which was also 0.02 N in Mg^{++} gave a result of 39.5% AU and 60.5% AU_2.

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such a [two-stranded] complex (25° C, 0.1-0.2 M Na^+) lead to the three-stranded complex, poly (A+U+U) as the equilibrium form regardless of the relative composition of the interacting species in the mixture." We note that our polymer concentrations are much greater (~ 0.01 to 0.1 M in polymer phosphate) than those generally used in other methods, though recent ultraviolet experiments (12) are in agreement with the results presented here, suggesting that helix strandedness is essentially independent of polymer concentration over a wide range.

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