Interaction of Poly(A) and Poly(I). A Reinvestigation[†]

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ABSTRACT: The stoichiometry of the poly(A), poly(I) system has been investigated by computer-based analytical methods which systematically examine all wavelengths of the ultraviolet spectra and of the derivative spectra, $d\epsilon/d\lambda$ vs. λ. Intersection angles (θ_1, θ_2) of mixing curves are defined for different polymer ratios and calculated as continuous functions of wavelength. Maxima and minima of the dispersion curves, θ vs. λ, indicate wavelengths of optimum sensitivity for demonstrating stoichiometry of those complexes which are formed. When for a given combining ratio a complex is not formed, the angle θ will be 180° over the entire spectral range. We have now extended this method to derivative spectra, $d\epsilon/d\lambda$ vs. λ, of mixtures of poly(A) and poly(I). We find that excellent derivative mixing curves can be obtained, often at wavelengths at which stoichiometry cannot be demonstrated by using un-

differentiated ultraviolet spectra. Application of these methods to the poly(A), poly(I) system in 0.1 M Na⁺ has established that a 1:1 complex is not formed at equilibrium and confirmed formation of the triple helix. Time dependence of absorbance–composition curves was observed in 0.05 M Na⁺ at 23 °C. These curves do not show transient formation of a 1:1 complex, in contrast to previous reports, and, in general, such curves cannot provide reliable evidence of stoichiometry at times significantly removed from equilibrium. A low salt condition (0.01 M Na⁺, 23 °C) previously reported to lead only to a 1:1 complex was found not to permit any interaction at all. The indicated temperature is approximately 6 °C above T_m of the 1:2 complex. We conclude that a 1:2 complex is the only interaction product formed by poly(A) and poly(I).

Interaction of poly(A) and poly(I) is interesting and unusual in that both components of the helix are purines and in the fact that the interglycosidic distance of the first and second strands is about 2 Å longer than the usual distance of about 10.8 Å found in Watson–Crick helices. In a separate study (Howard et al., 1977), we have investigated the interaction of poly(I) with chemically modified poly(A) molecules, but found, in interpreting our results, that much of the evidence in the literature on the poly(I), poly(A) system itself was contradictory and inconclusive. We have therefore reinvestigated this parent system in order to remove the uncertainty that persists about the poly(A), poly(I) interaction and in order to have a reliable baseline for interpreting other systems.

In the original descriptions of the poly(A), poly(I) interaction Rich (1958, 1959) reported that both 1:1 and 1:2 complexes are formed. The former complex was reported to be formed in 0.05 M Na⁺ (23 °C) as a transient intermediate at 30 min, in the first paper, and at 20 s in the second paper. The triple helix has been confirmed by other investigators (e.g., Sigler et al., 1962), but, since the original papers, there has been no experimental support for a 1:1 complex. The existence of an A·I double helix has nevertheless often been accepted in discussions of A, I pairing interactions (see, for example, Sakore and Sobell (1969); Crick (1968); Orgel (1968)).

Rich also reported x-ray diffraction of fibers obtained from a 1:1 mixture of poly(A) and poly(I) and interpreted the pattern as that of a double helix. A subsequent study (Arnott and Bond, 1973) reported a sharper diffraction pattern from a 1:2 A, I mixture, which evidently contains the features of the original pattern, but concluded that it resulted from a triple rather than a double helix.

We report here an examination of the stoichiometry of the A, I system using new methods of spectroscopic analysis, and observing the time course of the reaction at intermediate and longer times.

Experimental Section

The method used for the mixing curves has been described previously (Howard et al., 1971, 1976). A Cary Model 118 spectrophotometer was used to measure spectra as a function of time until absorbance became constant. Data were collected and recorded with a Honeywell Model DDP-516 computer simultaneously with measurement of the spectra (Shapiro and Shultz, 1971). Use of the computer to calculate wavelength dispersions of angles of intersection of mixing curves has been described (Howard et al., 1976). Calculation of derivative spectra by computer was also described in the same paper. Oscilloscope displays of data were used for comparing observed spectra with summations of other spectra. Most of the programs used for data reduction were written by Mrs. Marie Chang.

Poly(I) was purchased from Miles Chemical Co. (lot no. 33411) and poly(A) from P-L Biochemicals (lot no. 179-14, reported to have a sedimentation coefficient 7.7 S). Polymers were purified and dialyzed as described previously (Howard et al., 1971).

Results and Discussion

Poly(A), Poly(I). Stoichiometry. We have recently described a new method of analyzing ultraviolet spectra to establish the combining ratio of interacting polynucleotides (Howard et al., 1976). Briefly, the angles of intersection of the mixing curves at relevant mole fractions of one component (usually 0.5 and 0.67) are defined as dependent variables, θ_1 and θ_2, which are continuous functions of the wavelength. Spectroscopic data are collected at 0.2-nm intervals by an on-line computer, which then calculates the wavelength dispersion of θ_1 and θ_2. Maxima and minima in these dispersion curves correspond to optimum wavelengths for plotting the mixing curves, the sensitivity increasing with $|180° - \theta|$. When $\theta_i = 180°$ over the entire spectrum, there is no break in the mixing curve at any wavelength, and we conclude that a complex corresponding to mole fraction X_i is not formed. We see in Figure 1 that θ_2 has a minimum at 250 nm and maxima
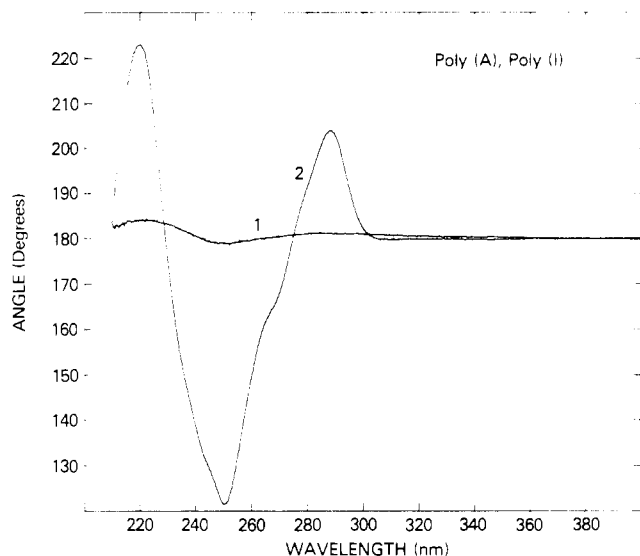
FIGURE 1: Wavelength dependence of the angles of intersection of the mixing curves of poly(A), poly(I) (8×10^{-5} M total polymer P; Na^+, 0.1 M; sodium cacodylate, 0.002 M; pH 7.0; 25 °C). Curve 1 gives the angle of intersection, θ_1, at 50 mol % poly(I), and curve 2 gives the angle, θ_2, at 67% poly(I). The fact that $\theta_1 \simeq 180°$ over the spectral range indicates that there is no wavelength capable of demonstrating a 1:1 complex. Curve 2 shows that 220, 250, and 288 nm are favorable wavelengths for demonstrating the 1:2 complex. 250 nm being the most sensitive.

FIGURE 2: Ultraviolet mixing curves show formation of 1:2 complex. poly(A)·2poly(I).

at 220 and 288 nm. All of these wavelengths are therefore favorable for demonstrating or detecting the 1:2 complex, but 250 nm, having the largest value of $|180° - \theta_2|$, is the most sensitive. Since $\theta_2 = 180°$ at 229, 275, and 302 nm, the complex cannot be detected at or near these wavelengths.

Mixing curves plotted at 220, 250, and 288 nm (Figure 2) clearly show formation of a 1:2 complex. θ_1, however, is equal to 180° within experimental error over the entire spectral range, indicating that a 1:1 complex is not formed.

The full ultraviolet spectra of mixtures of different compositions were drawn on the same scale by a Cal Comp plotter and examined for isosbestic points (Figure 3, Supplementary Material). When spectra of two absorbing species intersect, then the spectra of all mixtures of these species must also pass through this point of intersection, provided the total concentration is constant (see, for example, Jaffé and Orchin, 1962; Miles, 1971). The existence of an isosbestic point is therefore an indication that two, and no more than two, species are present over the range of composition for which the spectra pass through this point. In Figure 3 (Supplementary Material) two pairs of such points were observed. All spectra of mol % poly(I) in the range 0–66.7% pass through a point at 242 nm (ϵ 6000) and another at 282 nm (ϵ 2500). The two species present in this composition range are poly(A) and 1:2 complex. All spectra in the range 66.7–100% poly(I) pass through points at 227 nm (ϵ 4100) and at 259 nm (ϵ 6000). Here the two species are 1:2 complex and poly(I). Had a 1:1 complex been formed, spectra of mole fraction poly(I) between 0.5 and 0.67 would not have passed through the isosbestic points observed between 0 and 67% poly(I).

While it is often relatively straightforward to show that a given complex is formed, it is more difficult to establish that a complex having a plausible combining ratio is not formed. Particularly when major chemical conclusions depend directly upon such a negative result it is desirable to have confirmatory evidence. Since the principal conclusion of our study of the poly(2NH$_2$A), poly(I) system (Howard et al., 1977) does depend directly upon such a negative result, we have developed
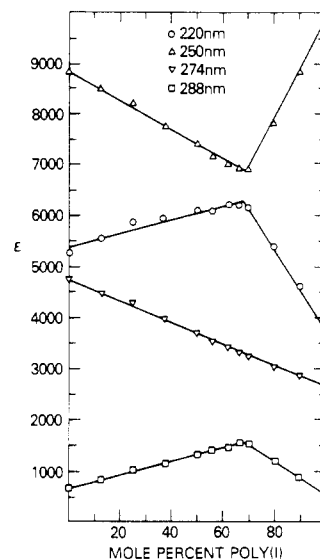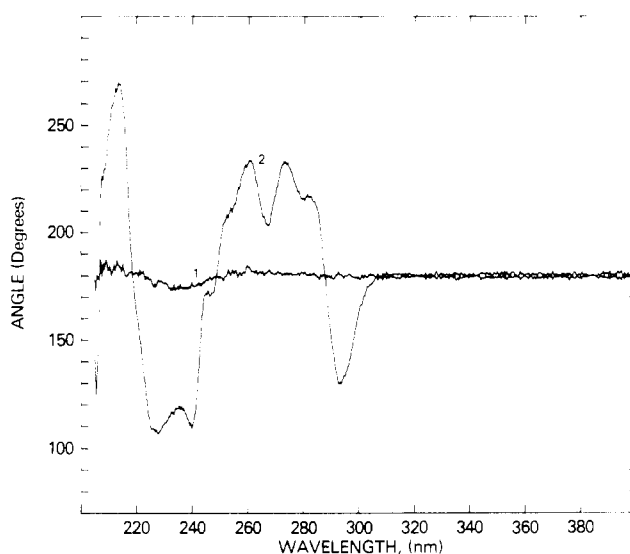

FIGURE 4: Wavelength dependence of angle of intersection of the derivative mixing curves (i.e., curves based upon the derivative spectra, $d\epsilon/d\lambda$ vs. λ, of the ultraviolet spectra) of the system poly(A), poly(I). Curve 2 refers to θ_2, defined as the angle of intersection at 67% poly(I). Maxima and minima indicate the most favorable wavelengths for detection of the 1:2 complex. Curve 1: $\theta_1 \simeq 180°$ over the spectral range, indicating a 1:1 complex is not formed.

another analytical method to provide confirmatory evidence and describe here its application to the poly(A), poly(I) system. In this procedure, the derivative spectra, $d\epsilon/d\lambda$ vs. λ, are calculated by computer at 0.2-nm intervals (cf. Howard et al., 1976) for all the spectra used in the mixing curves shown in Figure 2. These derivative spectra when plotted in overlay format exhibit isosbestic points at 223, 260, and 287 nm over the range 0–66.7% poly(I). In the range 66.7–100% poly(I) isosbestic points occur at 217, 240, and 265 nm. No other isosbestic points occur in any other concentration range (e.g., 0–50 or 50–100% poly(I)).

The angles θ_1 and θ_2 are defined for the intersections of limbs of mixing curves of the derivative spectra at 50 and 66.7% poly(I), respectively, as in the analogous treatment of the absorption spectra described above. The wavelength dispersion of these angles is calculated by computer. Over the range 220–300 nm, θ_1 (detection of 1:1 complex) = 180° within ex-
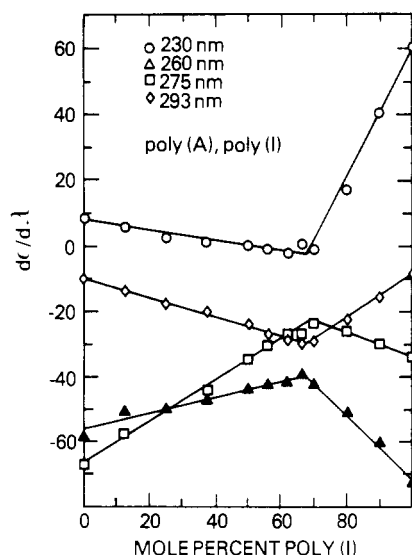
FIGURE 5: Derivative mixing curves for poly(A), poly(I) demonstrate formation of 1:2 complex.

perimental error, again indicating that a 1:1 complex is not formed (Figure 4). θ_2 has minima at 230 and 293 nm, maxima in the range 260–280 nm, and a sharp maximum at 210 nm. (The fine structure (i.e., maxima, minima, shoulders) in the dispersion curve appears to be real and reproducible. The corresponding curve for the closely similar poly(2MeA), poly(I) system (Howard et al., 1977) shows all of the same features but has very small wavelength shifts.) Mixing curves plotted at these wavelengths (Figure 5) show straight lines, with negligible scatter of data, intersecting sharply at 67% poly(I). It is interesting and potentially valuable that wavelengths near minima of the UV spectra and near the high-wavelength limit of absorption give excellent mixing curves, whereas mixing curves from the ultraviolet absorption spectra themselves are unable to show interaction at these wavelengths. Taken together these analytical methods extract all of the information the ultraviolet spectra contain on stoichiometry. The methods place stringent demands upon the precision and consistency of the spectral data and can assure, as less systematic methods do not, that inability to detect a particular complex is not merely due to failure to discover an appropriate wavelength. These analyses show clearly that at equilibrium a 1:2 complex is formed between poly(A) and poly(I) and that a 1:1 complex is not formed at any ratio of polymers in 0.1 M Na^+.

Kinetic Profile Mixing Experiments. Interaction in 0.05 M Na^+. The experiments reported to show the formation of a 1:1 A·I complex were carried out in 0.05 M Na^+ at 23 °C (Rich, 1958, 1959). The first of these papers showed a sharp minimum in a mixing curve (254 nm) at a mole fraction of poly(I) = 0.67 after 5 h, at which time the reaction was reported to be at equilibrium. A 1:1 minimum was reported at 30 min, and the conclusion that a two-stranded complex was formed was based upon this observation. The second paper (Rich, 1959) also presented a mixing experiment under the same conditions, but a minimum was observed, $X_I = 0.5$ at 20 s, rather than at 30 min. After 40 s, a second minimum appeared at $X_I = 0.67$. Equilibrium, with formation of a 1:2 complex, was reported to be reached in 30 min rather than 5 h, as in the first paper. The series of absorbance-composition curves was termed a kinetic profile and was said to show the consecutive formation of an initial 1:1 complex followed by a 2:1 complex.

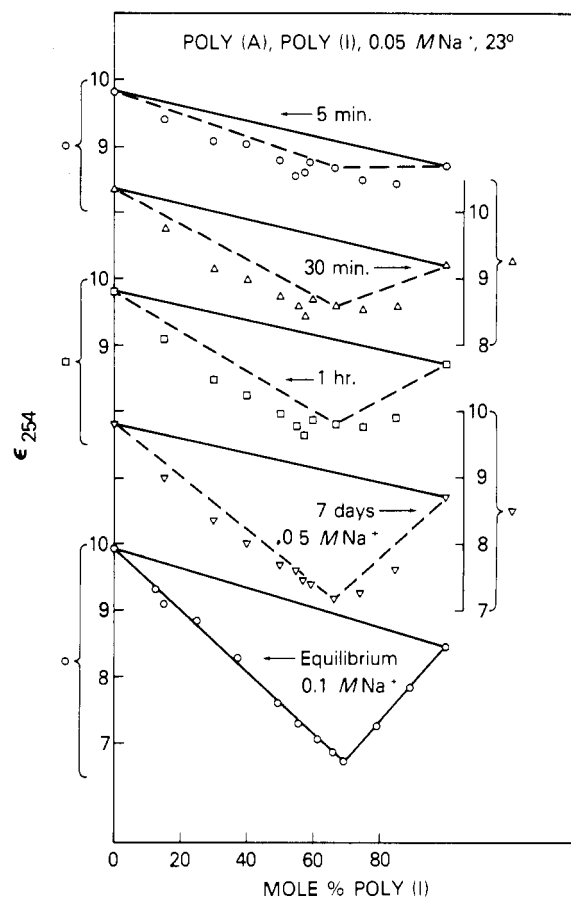Before proceeding to our mixing experiments in 0.05 M Na,

FIGURE 6: Absorbance-composition curves at varying time intervals after mixing poly(A) and poly(I) in 0.05 M Na^+ at 23 °C. At early times the data show only that interaction is occurring and not what the combining ratio is. Even after 7 days the reaction has not reached equilibrium. Complete interaction was observed only in 0.1 M Na^+ (bottom curve).

we wish to emphasize that neither these nor other mixing curves can provide reliable evidence of stoichiometry except at equilibrium. In a kinetic profile mixing experiment, solutions of different compositions approach equilibrium at different rates because of dependence of rate on concentration of reactants. At equal times after mixing we do not have equal extents of reaction at different compositions but a distribution of values. This distribution will change with time in a complex way and will invalidate a simple analysis appropriate only for the condition of equilibrium. The above comments apply when only a single complex is formed. When two or more complexes are being formed concurrently, the complexity is, of course, much greater. A mixing curve is thus not a valid method for demonstrating stoichiometry either of transient or of stable species at times significantly removed from equilibrium.

In some cases in which the stoichiometry has been previously established it is possible to follow the change in composition with time of a mixture containing several components, provided spectroscopic methods are available which are selective for each of the components in the presence of all others (cf. Blake et al., 1968; Howard et al., 1971). Such cases must be distinguished, however, from attempts to use kinetic profiles as mixing curves in order to determine stoichiometry in systems where the combining ratio is not already known. It is not possible to establish specificity of a spectroscopic method for a complex whose existence cannot itself be demonstrated.

We have observed the change of absorbance with time of poly(A), poly(I) mixtures of varying compositions (Figure 6). No attempt was made to determine initial rates, since we were

principally concerned with the interval between ~5 min and equilibrium and with changes in shape of the absorbance-composition curves with time. Though the experimental conditions are the same (Na^+, 0.05 M; 23 °C), our results are quite different from either set of data reported previously (Rich, 1958, 1959). Our data do not fall on straight lines at early and intermediate times, but instead fall below lines drawn from $X_1 = 0.67$ to $X_1 = 1.0$ and from $X_1 = 0.67$ to $X_1 = 0$. We observe that the approach to equilibrium is slowest at the stoichiometric ratio $X_1 = 0.67$, where neither reactant is present in excess. In these experiments, $t_{1/2}$ is longest at $X_1 = 0.67$ (0.6 h) and has increasingly shorter values as one departs from this stoichiometric ratio in either direction. Equilibrium was not reached in 5 h or in 30 min as reported previously (Rich, 1958, 1959, respectively). Even at 7 days the data indicate extents of reaction which are less than equilibrium values by ~15%. These experiments indicate that there is no convincing minimum at either a 1:1 or a 1:2 A, I ratio at times well removed from equilibrium (e.g. 5 min to several hours). Even if the results had shown apparent minima at these ratios, however, it would be invalid to draw stoichiometric conclusions from them for the reasons outlined above.

Since even lower salt (0.01 M Na^+) was reported to lead exclusively to a 1:1 complex in 18 h (Rich, 1958), we also examined the interaction of poly(A) and poly(I) in this solvent. The ultraviolet spectrum of a 1:1 poly(A), poly(I) mixture was displayed on an oscilloscope and superimposed on a computer summation of the two components (cf. Methods). The experimental and calculated curves were identical (as was the curve measured at 3 min), indicating that no interaction at all occurs under these conditions. Observations were continued at intervals, and at 8 days there was still no interaction. Extrapolation of the salt-dependence curve (Howard et al., 1977) to 0.01 M Na^+ gives a T_m of 17 °C, showing that the reason for lack of interaction under this condition is that the system is above T_m at ambient temperature. We therefore conclude that there is no valid evidence supporting the existence of a 1:1 poly(A), poly(I) complex under any condition.

Supplementary Material Available

Figure 3 (1 page). Ordering information is given on any current masthead page.

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