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The Single-Stranded Polyadenylic Acid–Poly-L-lysine Complex. A Conformational Study and Characterization*

Betty Davidson and Gerald D. Fasman

ABSTRACT: The effect of poly-L-lysine on the conformation of single-stranded polyadenylic acid has been studied by optical rotatory dispersion and ultraviolet spectroscopy. The ultraviolet and optical rotatory dispersion spectra of polyadenylic acid are altered on addition of poly-L-lysine at pH 7.0. The ultraviolet maximum of 256 mμ for polyadenylic acid shifts to 262 mμ for the complex. The optical rotatory dispersion spectrum is also red shifted (4 mμ), and decreased in molar rotation ($[m]_{256}^{\text{poly A}} = -78{,}000$, $[m]_{260}^{\text{complex}} = -23{,}000$) and complexity as compared with polyadenylic acid. Complex formation occurs in two steps. The primary interaction involves single-chain association, which is followed by a secondary aggregative interaction. The primary association occurs with a 1:1 residue stoichiometry, a large association constant, relatively little insolubility, and an apparent insensitivity to both the degree of charge on the poly-L-lysine and to ionic strength (100–40% charge, H_2O to 0.2 M NaF). The secondary (aggregative) interaction, which occurs near residue equivalence, is marked by further red shifts of the optical rotatory spectra and increased insolubility. Aggregation occurs maximally when the poly-L-lysine is 50% helical. There is no complex formation in 0.2 M NaF when the poly-L-lysine has zero charge and is 100% helical.

It is concluded that poly-L-lysine forms a well-defined complex with polyadenylic acid, altering the conformation of the latter.

Interest in the mechanism of cellular differentiation has prompted extensive research, some of which considers the possible role of histones in gene expression. One proposal suggests that these basic proteins exert control by altering the physical character of that portion of the chromosome to which they are bound, thus affecting its properties as a template for RNA synthesis (Stedman and Stedman, 1950; Ts'o and Bonner, 1964).

Previous work has shown that nucleic acids and synthetic polynucleotides form complexes *in vitro* with a variety of cationic molecular species ranging in size from Mg^{2+} ion through diamines such as spermine and spermidine, through synthetic oligopeptides, and finally to the protamines, histones, and synthetic polypeptides (Spitnik *et al.*, 1955; Felsenfeld and Huang, 1959, 1960; Matsuo and Tsuboi, 1966; Tsuboi *et al.*, 1966; Leng and Felsenfeld, 1966; Latt and Sober, 1967; Olins *et al.*, 1967, 1968, and references therein; Gabbay, 1968). The complexing phenomenon shows some specificity as to the nature and size of the components of the system (Felsenfeld and Huang, 1959; Szer, 1966a,b; Latt and Sober, 1967; Olins *et al.*, 1968), and the complexes are composed of stoichiometric ratios of nucleotide residue to cationic ligand which are characteristic for the system (Sober *et al.*, 1966; Tsuboi, 1967). Such complexes show greater thermal stability than the free polynucleotide components (Szer, 1966a,b; Tsuboi, 1967; Olins *et al.*, 1967, 1968). Finally, the solubility of the complexes has been found to be highly dependent upon experimental conditions (Spitnik *et al.*, 1955; Leng and Felsenfeld, 1966; Olins *et al.*, 1967; Tsuboi, 1967).

The above characterizations have been obtained largely by

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measurements of turbidity and of relative hyperchromicity as a function of temperature (Felsenfeld and Sandeen, 1962; Mandel, 1962; Mahler and Mehrotra, 1963; Leng and Felsenfeld, 1966; Tsuboi, 1967; Olins *et al.*, 1967, 1968), although equilibrium dialysis has also been used (Akinrimisi *et al.*, 1965; Herschman *et al.*, 1967; Latt and Sober, 1967a,b).

The question of conformational changes attendant upon complex formation has been considered by Bradbury *et al.* (1962), Gratzer and McPhee (1966), McPhie and Gratzer (1966), Oriel (1966), Boublík *et al.* (1967), and Cohen and Kidson (1968). Several investigators conclude that binding to nucleic acid is required to stabilize the histone or ribonuclear protein component in a partial α-helical conformation. While the present study, which concerns itself with possible conformational changes of a single-strand model nucleic acid upon addition of a histone model, was being completed, Cohen and Kidson (1968) demonstrated the change of conformation of DNA upon complexing with poly-L-lysine.

In the present study, the conformational changes associated with binding of poly-L-lysine to single-stranded poly A have been studied by optical rotatory dispersion and ultraviolet spectrophotometry. Two well-characterized synthetic polymers, poly A and poly-L-lysine, have been used. Poly A was chosen because of its ability to undergo a transition from a double- to a single-stranded helical form over a pH range (5–6) where poly-L-lysine remains a cationic polymer. At higher pH values (8.5–11.2) complex formation between single-stranded poly A and poly-L-lysine can be studied as a function of the conformation of the polypeptide. A subsequent paper will describe the conformational changes associated with complex formation between the double-stranded form of poly A and poly-L-lysine.

As complex formation proceeds, the optical rotatory dispersion spectrum characteristic of single-stranded poly A (Holcomb and Tinoco, 1965; Sarkar and Yang, 1965) is replaced by a simpler optical rotatory dispersion spectrum, displaced to higher wavelengths and having a lower molar rotation than the original poly A. Ultraviolet studies also indicate the formation of a new species having a ultraviolet maximum displaced to higher wavelengths. This species (primary complex) displays a characteristic stoichiometry of 1:1 lysyl to adenyl residues. A subsequent aggregative step (secondary complex) then occurs, characterized by a further red shift in the optical rotatory dispersion and increasing insolubility.

Materials and Methods

Reagents

Solvents. Twice-distilled water was used for all work. The second distillation was performed in an all-glass apparatus.

Poly-L-lysine-HCl was synthesized as previously described (Fasman *et al.*, 1961). The material used for this study was the same sample (BD-1-10-28) as previously described (Davidson and Fasman, 1967). The molecular weight estimated by viscosity measurements was about 55,000.

Poly A was purchased from Miles Chemical Co. Almost all of the experiments reported herein were done with lot no. 112639, $s_0 = 9.75$, mol wt $\geq$ 100,000.

Poly-L-histidine. Sample GFG741 was prepared as previously described (Norland *et al.*, 1963); mol wt 30,000.

AMP-NH$_4$ was supplied by the Sigma Chemical Co.

L-Lysine-HCl and *tetraglycine* were purchased from the Nutritional Biochemical Corp. *Diglycyl methyl ester* was purchased from Mann Research Corp.

Methods

Concentration of Poly-L-lysine and Poly A. The concentrations of stock solutions of poly-L-lysine-HCl were determined either by a modified microbiuret procedure (Zamenhof and Chargaff, 1963) or by Nessler micro-Kjeldahl nitrogen analysis (Lang, 1958). Poly A concentrations were determined spectrophotometrically at pH ~7.2 (0.01 M sodium phosphate buffer) by relating the maximum absorbance (256 mμ) to the extinction coefficient (ϵ_{max} 10.1 $\times$ 10^3, Holcomb and Tinoco, 1965). Polymer concentrations are expressed as mole residues per liter.

pH Measurements. Routine measurements of pH were made with a Sargent Model DR pH meter equipped with a combination glass–AgCl microelectrode, 30070-10. Standard pH buffers at pH 4.0, 7.0, 9.6, and 10.6 from the Fisher Scientific and Beckman Instrument Co. were used.

Potentiometric Titrations in 0.2 M NaF. Titrations were performed using a Sargent 30070-10 combination glass–AgCl microelectrode attached to a Model EUW-20A Heathkit pH meter and recorder (full scale = 2 pH units = 10 in.). The pH scale was linear over 6 pH units. Temperature was maintained at 22° by use of a water-jacketed titration vessel connected to a Haake circulating water bath. Standard HCl (0.3 M, diluted from 1 M) was added from a Manostat ultramicro digital readout buret (capacity = 0.10 ml) to the vessel containing either 3.1 ml of 0.2 M NaF or 3.1 ml of 0.887 $\times$ 10^{-3} M poly-L-lysine in 0.2 M NaF, both at pH 11.3. Contamination by carbon dioxide was minimized by use of freshly distilled water and freshly prepared 10% NaOH solution for the initial pH adjustment and by performance of the titrations under a nitrogen atmosphere.

In Water. The procedures employed were those described for 0.2 M NaF, except that the titration vessel contained 5.2 ml of either water or 1.19 $\times$ 10^{-3} M poly-L-lysine in water (pH 11.2). A Model 25 SE Radiometer pH meter equipped with a scale-expanding attachment and a combination glass–calomel electrode (GK2021B) was used.

Optical Rotatory Dispersion. Measurements were made with a Cary Model 60 recording spectropolarimeter (slit width programmed to maintain a 15-Å half-band width). A fused-quartz optical cell of 1-cm path length was used. Measurements at temperatures other than 22° were made using a water-jacketed 1-cm cell connected to a circulating water bath, as previously described (Davidson and Fasman, 1967). Optical cells were purchased from the Optical Cell Co., Brentwood, Md. Data are reported as $[m]_\lambda$, molar rotation (deg cm^2/dmole) per adenyl residue.

Addition of Poly-L-lysine to Poly A. Poly-L-lysine (1.42 $\times$ 10^{-2} M, 0.233%) was added in 1-μl aliquots to a 1-cm path-length optical cell containing 3.0 ml of 5.1 $\times$ 10^{-5} M poly A in 0.01 M phosphate buffer, pH 7.23 (Figure 1). Additions were made with disposable micropipets supplied by Bonus Laboratories, Reading, Mass. The optical rotatory dispersion (300–200 mμ) was recorded after each addition of poly-L-lysine. The reference solution was the solvent, 0.01 M phosphate buffer (pH 7.2). The volume ranged from 3.000 to 3.013 ml. Dilutions in this and all other experiments were accounted for in calculations of concentration. The residue ratio, lysyl:adenyl, ranged from 0 to 1.2.

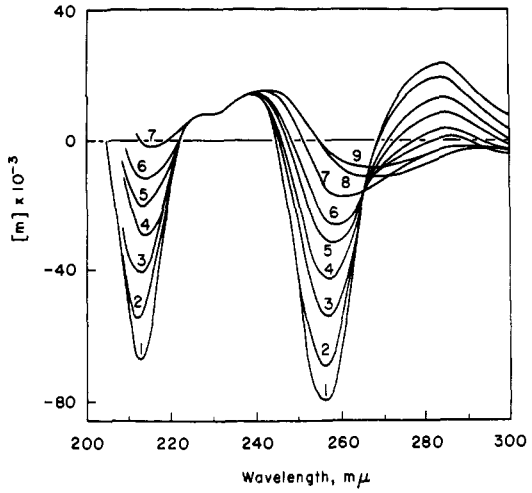

FIGURE 1: The effect of addition of poly-L-lysine on the optical rotatory dispersion of poly A. The experimental procedure is described in the Methods section. Solvent: 0.01 M phosphate buffer, pH 7.23. Spectrum 1, poly A alone (5.1×10^{-5} M); spectra 2 to 9, lysyl/adenyl ratios were 0.19, 0.46, 0.65, 0.83, 0.92, 1.0, 1.1, and 1.2, respectively.

Addition of Poly A to Poly-L-lysine. Poly A (4.05×10^{-4} M; 0.01 M phosphate, pH 7.23) was added in multiples of 0.05 ml to a 1-cm cell containing 3.0 ml of 6.1×10^{-4} M poly-L-lysine (0.01 M phosphate buffer, pH 7.2) (Figure 2). For each optical rotatory dispersion spectrum (300–240 mμ), the desired volume of poly A was added to a fresh 3.0-ml aliquot of poly-L-lysine. In this experiment the optical rotatory dispersion of poly-L-lysine (6.1×10^{-4} M) was used as the zero base line, since this concentration of poly-L-lysine has significant optical activity in the wavelength region of the experiment. The lysyl:adenyl residue ratio ranged from 41 to 10.

The Effect of pH on Complex Formation. H_2O. Poly-L-lysine was dissolved in water and this stock solution was diluted to a final concentration of 10^{-4} M after adjustment to the desired pH with standard 1 M NaOH. For each pH the optical rotatory dispersion of 2.7 ml of 10^{-4} M poly-L-lysine was measured. Poly A (0.3 ml in water, pH 9.3, final concentration 4.78×10^{-5} M) was then added and the optical rotatory dispersion measurement repeated. pH values were measured before addition of poly A and after completion of the poly A plus poly L-lysine optical rotatory dispersion spectra. The final residue ratio was 9×10^{-5} M lysyl/4.78×10^{-5} M adenyl = 1.9.

0.18 M NaF. The solvent was 0.18 M NaF containing 0.009 M each of phosphate and Tris buffers. The pH was adjusted by addition of standard 1 M NaOH or HCl. At each pH the optical rotatory dispersion of the solvent alone (3.0 ml) was recorded. Poly-L-lysine (0.01 ml) was then added to the cell (final concentration 10^{-4} M) and the optical rotatory dispersion spectrum repeated. Finally, stock poly A (0.3 ml) solution (final concentration 4.35×10^{-5} M) was introduced into the cell containing the poly-L-lysine and the optical rotatory dispersion was again recorded. The pH values were checked before the first and after the final optical rotatory dispersion spectra. The residue ratio was 9.1×10^{-5} M lysyl/4.35×10^{-5} M adenyl = 2.1. The presence of the single-stranded form of poly A was verified by optical rotatory dispersion at all reported values of pH and ionic strength.

Ultraviolet Absorption. Ultraviolet absorbance was measured with a Cary 14 spectrophotometer. The procedure, conditions, and reference solutions were the same as for the analogous optical rotatory dispersion experiments (Figure 3 is paired with Figure 1 and Figure 4 with Figure 2.) Absorption spectra were recorded from 370 to 230 mμ. These were corrected for light scattering as described below. The spectra for which scattering corrections were valid encompassed lysyl: adenyl ratios from 0.19 to 0.96 in Figure 3 (addition of poly-L-lysine to poly A) and residue ratios from 80 to 13 in Figure 4 (poly A added to poly-L-lysine).

Corrections for Light Scattering. Corrections for Rayleigh-type scattering were made for each spectrum by plotting the logarithm of the wavelength against log absorbance at that wavelength, from 370 to 300 mμ. The linear portion of the plot, usually 370–310 mμ, was extended to the wavelengths of sample absorbance. These extrapolated values were assumed to be the scattering contribution to the experimental spectra and were accordingly subtracted from them. In view of the reservations of Olins *et al.* (1967) about the validity of such corrections, the ultraviolet spectrum of a sample of poly A was measured in tandem with increasing amounts of a scattering material (glycogen) suspended in water. The combined optical path of poly A plus glycogen was 1 cm + 1 cm = 2 cm. The concentration of poly A and the range of scattering levels were the same as in the absorbance experiments of Figures 3 and 4. Apparent perturbation of the corrected absorption spectrum of poly A occurred as predicted by theory. As $A_{370\ m\mu}$ reached 0.11, the poly A spectrum appeared to undergo a 2-mμ red shift around a false "isosbestic point" at 256 mμ, the actual absorption maximum of poly A. In contrast, in the absorption experiment of Figure 3, the isosbestic point occurred at 262 mμ rather than 256 mμ; the red shift was more pronounced and the absorbance changes were from five to ten times greater in the experiment than in the appropriate scattering control. Finally, in Figure 4, no wavelength shift of the corrected absorption maximum is seen, although scattering levels are increasing. It is concluded that if $A_{370\ m\mu}$ remains below 0.1, perturbations of the type suggested by Olins *et al.* (1967) are small enough to allow scattering corrections in the region of an absorbance peak of $A_{\lambda_{max}}$ 0.5. In all the experiments reported here, $A_{370\ m\mu}$ did not exceed 0.08.

Calculation of the Optical Rotatory Dispersion Spectrum of the Primary Complex in the Presence of Excess Poly A. The following assumption was made. All added poly-L-lysine is converted into complex. (1) Concentration of complex = concentration of added poly-L-lysine. (2) Concentration of free poly A = initial poly A concentration − concentration of complex. (3) $\alpha_\lambda^{obsd} = \alpha_\lambda^{complex} + \alpha_\lambda^{free\ poly\ A}$. (4) $\alpha_\lambda^{free\ poly\ A}$ = concentration of free poly A $\times$ $[m]_\lambda^{poly\ A}$. (5) $\alpha_\lambda^{complex}$/concentration of complex = $[m]_\lambda^{complex}$. These computations of $[m]_\lambda^{complex}$ were done with the aid of an IBM 1620 computer for spectra 2–7 of Figure 1 to yield Figure 9.

Results

Optical Rotatory Dispersion Spectra. The optical rotatory dispersion spectrum of single-stranded poly A is altered upon the addition of small increments of poly-L-lysine. This is shown in Figure 1, a tracing of the optical rotatory dispersion spectra as recorded on the spectropolarimeter. As the ratio lysyl residues/adenyl residues approaches unity, the optical rotatory

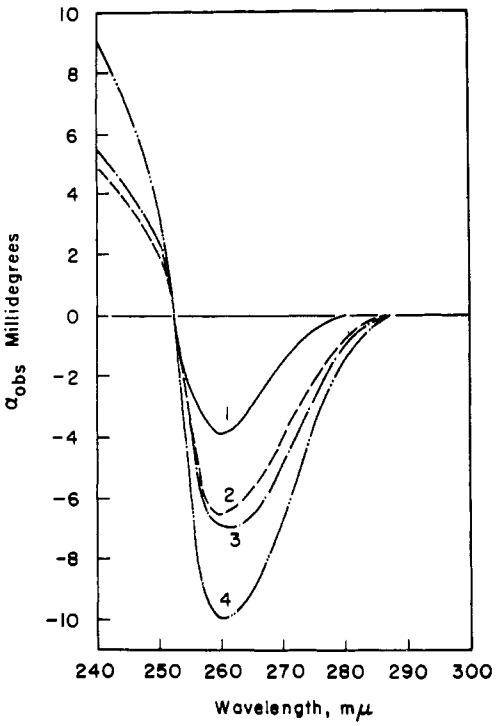

FIGURE 2: The optical rotatory dispersion of the complex formed upon addition of poly A to poly-L-lysine. The experimental procedure is described in the Methods section. Solvent: 0.01 M phosphate buffer, pH 7.35. Initial concentration of poly-L-lysine: 6.1×10^{-4} M. Lysyl/adenyl residue ratios in spectra 1-4 were, respectively, 41, 16.3, 13.7, and 10.2. The optical rotatory dispersion of the excess poly-L-lysine was subtracted from each spectrum.

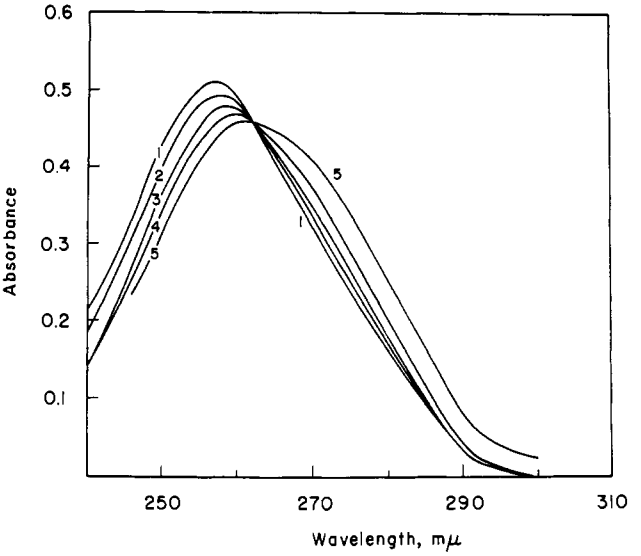

FIGURE 3: The effect of addition of poly-L-lysine on the ultraviolet spectrum of poly A. The experimental procedure is described in the Methods section. Solvent: 0.01 M phosphate buffer, pH 7.23. Spectrum 1, poly A alone, 5.08×10^{-5} M; spectra 2–5, lysyl/adenyl residue ratios were 0.19, 0.48, 0.67, and 0.96, respectively. The spectra have been corrected for scattering.

dispersion spectrum changes from one typical of single-stranded poly A ($[m]_{283\ m\mu} = +23{,}920$, $[m]_{256\ m\mu} = -78{,}500$, $[m]_{213\ m\mu} = -68{,}000$, and crossovers at 269 and 245 mμ (Holcomb and Tinoco, 1965; Sarkar and Yang, 1965)) to a simpler spectrum of lower molar rotation, centered at higher wavelength ($[m]_{260\ m\mu} = -18{,}000$; crossover at 249 mμ). The 213-mμ trough simultaneously decreases to near zero, with a possible red shift in the complex. Further, as the residue ratio approaches unity the cell contents become opalescent. Spectra 7, 8, and 9 (residue ratios 1.0, 1.1, and 1.2) were recorded on solutions which were noticeably opalescent. There is no further shift in wavelength of the Cotton effect after spectrum 8 (λ crossover = 255 mμ), but there is a decrease in rotation, probably due to precipitation of the aggregated complex. Spectra 1–7 have an "isorotatory" point at ~265.5 mμ. Spectra 8 and 9 do not share this point. The optical rotatory dispersion spectrum of the remaining dissolved material was not affected by the turbidity, in agreement with the findings of Gratzer and McPhie (1966). Under the conditions of this experiment, the maximal concentration of 6.1×10^{-5} M poly-L-lysine made no significant contribution ($\alpha^{obsd}_{260\ m\mu} < 1$ mdeg) to the optical rotation.

These experiments were conducted at neutral pH, using 0.01 M phosphate buffer. Under these conditions, poly A is in a single-stranded form with a portion of the nucleotide bases stacked in a helical array (Holcomb and Tinoco, 1965). The poly-L-lysine, which was added to the poly A, however, was not in the random conformation, as expected for this pH, but appeared to have an optical rotatory dispersion spectrum resembling a mixture of random, α-helical, and/or β conformation (Greenfield *et al.*, 1967). This unexpected behavior was observed only when 0.01 M phosphate buffer was the solvent. The optical rotatory dispersion of poly-L-lysine at neutral pH was that of a random coil in water, in 0.01 M Tris buffer, or in a mixture of 0.01 M Tris, 0.01 M phosphate, and 0.2 M NaF. This phenomenon probably reflects a specific binding of the phosphate ions to the positively charged ε-amino groups of the polypeptide. The resultant charge neutralization could then permit the poly-L-lysine to commence folding into a more compact structure. The optical rotatory dispersion spectrum of the [poly A–poly-L-lysine] complex was the same, regardless of inorganic ions present, indicating that initial poly-L-lysine conformation had no effect on complex formation.

Since the optical rotatory dispersion spectrum of the [poly A–poly-L-lysine] complex has a lower molar rotation than poly A, it is easily masked by an excess of uncomplexed polynucleotide. In order to avoid this difficulty while observing complex formation, increments of poly A were added to an excess of poly-L-lysine. The ratios of lysyl residues/adenyl residues ranged from 6.1×10^{-4} M/1.48×10^{-5} M = 41 to 5.3×10^{-4} M/5.3×10^{-5} M = 10 (Figure 2). The rotational contribution of the poly-L-lysine (α^{obsd} was −9 mdeg at 260 mμ) was subtracted from each of the optical rotatory dispersion spectra. Under the conditions of this experiment, all the added poly A would be expected to combine with the poly-L-lysine (present in excess), forming increasing amounts of a complex having the optical rotatory dispersion characteristics seen in Figure 1 (curve 7). These expectations were fulfilled. Figure 2 shows the formation of increasing amounts of a species characterized by a negative Cotton effect, $\lambda_{trough} = 260$ mμ, crossover at 252 mμ. It will be shown later that this 1:1 complex has the same optical rotatory dispersion spectrum ($[m]_{260\ m\mu} = -19{,}000$, crossover at 252 mμ) regardless of the order of addition of the components (Figure 9).

Ultraviolet Spectroscopy. The addition of poly-L-lysine to

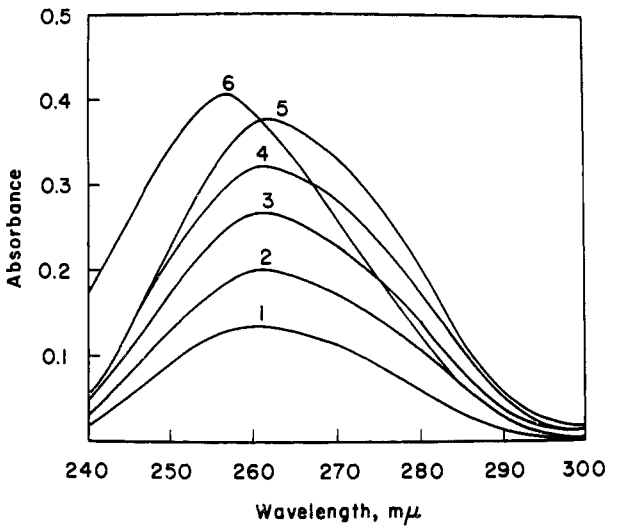

FIGURE 4: The ultraviolet spectrum of the complex formed upon the addition of poly A to poly-L-lysine. The conditions are described in the Methods section. Solvent: 0.01 M phosphate buffer, pH 7.35. Initial poly-L-lysine concentration, 6.1×10^{-4} M. Lysyl/adenyl ratios are 41, 27.4, 20.5, 16.3, and 13.7 (spectra 1–5). Spectrum 6 is poly A alone at the same adenyl residue concentration as in spectrum 5. The absorbance was corrected for scattering.

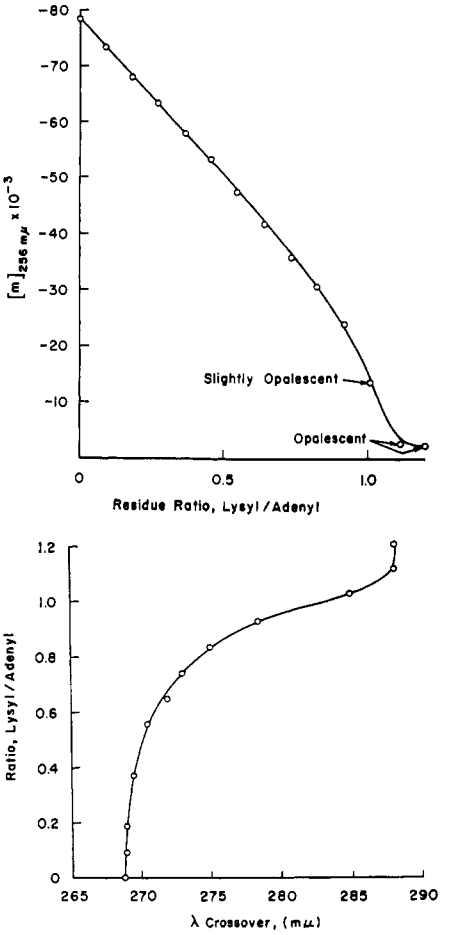

FIGURE 5: Complex formation studies. (a) The decrease in $[m]_{256}$ upon complex formation with increasing lysyl/adenyl residue ratios. The experimental conditions are those of Figure 1. (b) The red shift of λ crossover during complex formation with increasing lysyl/adenyl residue ratios. The experimental conditions are those of Figures 1 and 5a.

poly A at neutral pH was also followed by ultraviolet spectroscopy (Figure 3). The experimental conditions were analogous to those of the optical rotatory dispersion experiment of Figure 1.

Complex formation proceeds with a shift of the ultraviolet maximum of poly A from 257 to 262 mμ, and with an apparent 7% decrease in $\epsilon_{\lambda_{max}}$ (10.1×10^3 for poly A, 9.4×10^3 for the complex). There appears to be an isosbestic point at 262 mμ, indicating the presence of two species. These are believed to be free poly A and the [poly A–poly-L-lysine] complex.

If aliquots of poly A are added to an absorption cell containing excess poly-L-lysine (Figure 4), the only species observed is that having an absorption maximum at 262 mμ, and an absorbance which increases with additional poly A. Its calculated $\epsilon_{\lambda_{max}}$ is 9.4×10^3, as found in the experiment of Figure 3. The formation of this species, believed to be the [poly A–poly-L-lysine] complex, is shown in Figure 4. The spectrum of uncomplexed poly A is also shown in Figure 4. A comparison of this spectrum with that of the complex shows that the latter is hypochromic and red shifted. This experiment is analogous both in conditions and in results to the optical rotatory dispersion experiment of Figure 2.

The spectra of Figures 3 and 4 have been corrected for artifacts due to light scattering (see Materials and Methods section). In Figure 4 the peak absorbance of the complex followed Beer's law, showing that the small amount of insoluble material responsible for the scattering does not constitute a significant portion of the complex.

Stoichiometry of Complex Formation. The stoichiometry of this process may be estimated by following the change in the optical rotatory dispersion parameters, $[m]_{256\,m\mu}$ and crossover wavelength, as the polynucleotide and polypeptide components are mixed in varying proportions. Figure 5a shows the decrease in rotation at the ~256-mμ optical rotatory dispersion trough at increasing ratios of lysyl to adenyl residues (0–1.2). The decrease is linear and proceeds until approximately one lysyl residue has been added for every adenyl residue with $[m]_{256\,m\mu}$ changing from −78,000 to ≃−20,000. The next addition of poly-L-lysine results in a more precipitous decrease in rotation accompanied by progressive opalescence.

Figure 5b depicts the red shift of the 269-mμ crossover of the poly A optical rotatory dispersion spectrum upon addition of poly-L-lysine. It is seen that the greatest red shift occurs between residue ratios 0.7 and 1.1, where λ crossover shifts from 272 to 287 mμ. Very little change is noted at ratios below 0.7. Probably this parameter primarily reflects the secondary aggregative step.

Complex formation, then, is separable into two stages. The first appears to be a combination of single-stranded poly A with poly-L-lysine. This primary complex is characterized by solubility, as compared with the secondary complex, and an optical rotatory dispersion spectrum having a Cotton effect trough at 259–260 mμ. Its formation is accompanied by a linear decrease in rotation until a 1:1 residue stoichiometry is approached. The secondary stage occurs as residue equivalence

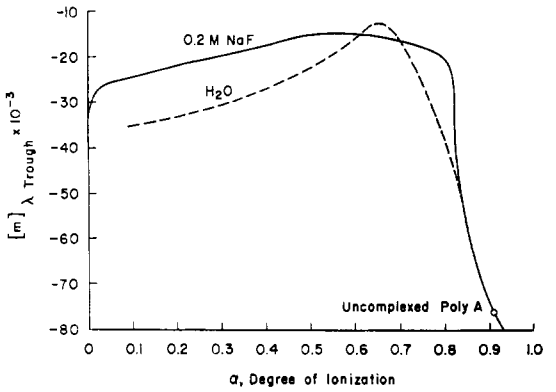

FIGURE 6: Complex formation of poly A as measured by $[m]_{\lambda_{trough}}$ as a function of α, the degree of ionization of poly-L-lysine, in H_2O and in 0.18 M NaF ($LysNH_3^+ \rightleftharpoons H^+ + LysNH_2$). Experimental conditions are described in the Methods section. The optical rotary dispersion was measured at several pH values. These were correlated with α by means of potentiometric titrations, as described in the Methods section. Residue ratios were 9×10^{-5} M lysyl/4.78×10^{-5} M adenyl = 1.9 for water and 9.1×10^{-5} M/4.35×10^{-5} M = 2.1 for 0.18 M NaF.

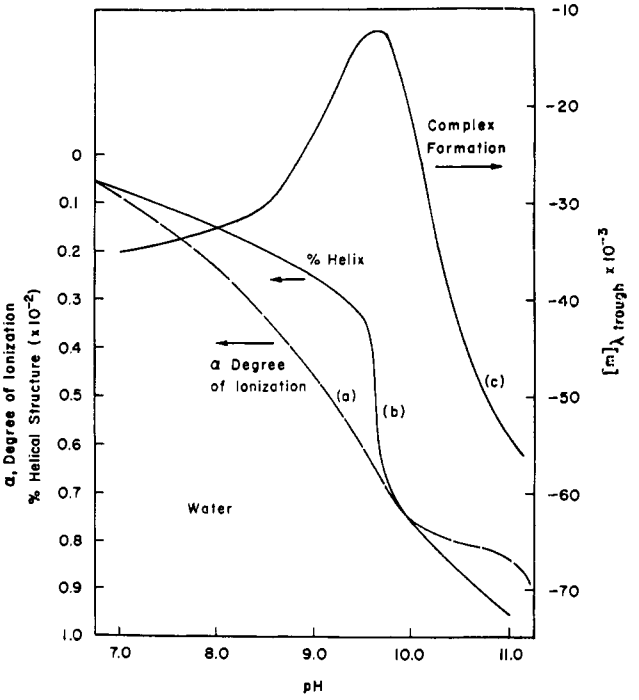

FIGURE 7: The pH dependence of poly-L-lysine degree of ionization, α-helical structure, and complex formation with poly A. Solvent: H_2O. The experimental details are described in the Methods section and in Figure 6. Curve a is the potentiometric titration (left ordinate); curve b represents per cent α-helical structure (left ordinate); and curve c (right ordinate) is $[m]_{\lambda_{trough}\ (\sim 260\ m\mu)}$, a parameter measuring complex formation.

is approached and exceeded. This is characterized by insolubility, a greater than linear rotational decrease, and a marked red shift of the primary complex optical rotatory dispersion spectrum. The new trough occurs at ~265–275 mμ. It will be shown later that the optical rotatory dispersion of the primary complex can be reversibly changed by temperature alterations, while that of the secondary complex is not recoverable on cooling. The properties of the secondary complex indicate that it is an intermolecular aggregate.

The Mechanism of Complex Formation. The following experiments were undertaken to determine whether combination of poly A and poly-L-lysine is simply a result of electrostatic attractions between phosphate and ε-amino groups or whether some other factors might also be important in complex formation.

Effect of pH, Ionic Strength, and Poly-L-lysine Conformation on Complex Formation. If charge attraction is the sole factor governing complex formation, then the phenomenon should decrease as a function of α, the degree of ionization of poly-L-lysine ($LysNH_3^+ \rightleftharpoons LysNH_2 + H^+$); moreover, charge shielding effects would be expected to decrease electrostatic interactions at increased ionic strength. Figure 6 shows that α can vary from 0 to about 0.8 without a concomitant decrease in complex formation ($[m]_{\lambda_{trough}}$ remains between −35,000 and −15,000, while uncomplexed poly A has $[m]_{\lambda_{trough}} \approx -78{,}000$). Over this range of poly-L-lysine ionization both primary and aggregative interactions occur. In water the primary association is complex ($[m]^{complex}_{\lambda_{trough}} = -35{,}000$) at $\alpha = 0.1$ (pH 7.2). In water the potentiometric titration curve of poly A is displaced to higher pH values, and 7.2 is the lowest pH at which the single-strand form of the polynucleotide is stable (Fresco and Klemperer, 1959; Holcomb and Tinoco, 1965; Holcomb and Timasheff, 1968). From $\alpha = 0.1$ to $\alpha = 0.3$ the primary form predominates ($[m]_{\lambda_{trough}}$ remains at around −30,000 with the optical rotatory dispersion minimum at around 260 mμ). From $\alpha = 0.3$ to $\alpha = 0.65$, however, there is an increase in aggregation as shown by a decrease in trough magnitude from $[m]_{\lambda_{trough}} =$ −30,000 to −15,000 and a red shift (λ_{trough} 260 mμ to 265 mμ). At $\alpha = >0.78$, λ_{trough} is again shifted to lower wavelength, indicating that the optimal ionization range for aggregation has been passed. The last experimental point, $\alpha = 0.85$, results in an ORD spectrum similar to poly A in position (λ_{trough} 256.5 mμ) but not in magnitude ($[m]_{\lambda_{trough}} = -56{,}000$).

In 0.18 M NaF the primary association is complete at $\alpha = 0$ (pH 6.1). In salt the single-strand form of poly A exists at this pH. Only at $\alpha = 0$ is the primary interaction uncomplicated by aggregation ($[m] = -32{,}000$, λ_{min} 260 mμ). At this ionic strength the ionization range for aggregation is broader than in water (at $\alpha = 0.05$, $[m]_{\lambda_{trough}}$ has become −25,000, λ_{trough} 262 mμ). At $\alpha \geq 0.8$ the range of maximal aggregation is passed and the optical rotatory dispersion spectrum at $\alpha = 0.82$ is again typical of the primary complex. At $\alpha = 1.0$ in 0.18 M NaF there is no complex formation and the optical rotatory dispersion spectrum is that of uncomplexed poly A. In the experiments using unbuffered water as solvent, no change of pH was observed upon complex formation indicating that the ionization properties of complexed and uncomplexed poly-L-lysine are not substantially different.

Complex formation ($[m]^{complex}_{\lambda_{trough}}$), degree of ionization, and per cent helical structure of poly-L-lysine are depicted as a function of pH in Figures 7 (H_2O) and 8 (0.18 M NaF). Complex formation does not seem related in any simple way to either the poly-L-lysine conformation or state of ionization. This derives partly from the complicated nature of the as-

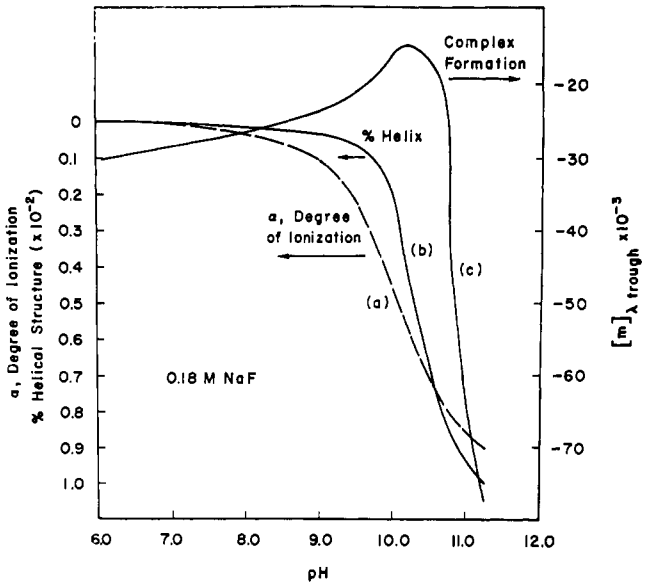

FIGURE 8: The pH dependence of poly-L-lysine degree of ionization, α-helical structure, and complex formation with poly A. Solvent: 0.18 M NaF. The experimental procedures are described in the Methods section and Figure 6. The curves are lettered and the ordinates arranged as in Figure 7.

sociation. The data of Figures 7 and 8 indicate that the stage two (aggregative) interaction occurs maximally when the poly-L-lysine is in a 50% random conformation ($[m]_{\lambda_{trough}}$ attains its least negative value). The primary interaction seems independent of the poly-L-lysine helical content up to about 80% helical structure, with $[m]^{complex}_{\lambda_{trough}} \simeq -30{,}000$. Changes in $[m]^{complex}_{\lambda_{trough}}$ between 0 and 80% helix represent aggregation. Completely helical poly-L-lysine does not form complexes with poly A in 0.18 M NaF; however, some residual association seems to occur in water, even when the polypeptide is maximally helical (Figure 7).

In contrast to the aggregative step, there is no substantive effect of ionic strength on primary complex formation. As seen in Figure 6, $[m]^{complex}_{\lambda_{trough}}$ values are similar in water at α = 0.1 and 0.18 M NaF at α = 0. Further evidence for the insensitivity of primary complex formation to ionic strength is seen in Table I, optical rotatory dispersion data obtained at neutral pH in aqueous solvents of varying ionic strength. In one set of experiments the lysyl/adenyl ratio was kept low (R = 0.38) in order to minimize aggregation. There was no difference in the optical rotatory dispersion spectra of the [poly A–poly-L-lysine] complex in water, 0.01 M phosphate buffer, 0.02 M NaF, or 0.2 M NaF. At a residue ratio near unity, where aggregation is more likely to occur, the optical rotatory dispersion of the complex remained essentially unchanged in water, 0.01 M phosphate buffer, 0.02 M NaF, and 0.1 M NaF. In 0.2 M NaF, however, the optical rotatory dispersion spectrum was typical of the aggregated form, both in magnitude and in spectral position.

The Stability of the [Poly A–Poly-L-lysine] Complex at Room Temperature. At room temperature the association constant for the formation of complex is apparently very large. It is possible to compute an optical rotatory dispersion spectrum for the primary complex, making the assumption that all of the poly-L-lysine added to an excess of poly A is converted to

TABLE I: The Effect of Ionic Strength on Complex Formation.

Lysyl/ Adenyl	Solvent	$\alpha_{obsd,p\text{-}t}$[b] (mdeg)	λ_{trough} (mμ)
0.38	0.01 M phosphate buffer	37	257
	H_2O[a]	36	257
	0.02 M NaF	36	257
	0.20 M NaF	33	257
0.98	0.01 M phosphate buffer	20.5	258
	H_2O[a]	21.5	258
	0.02 M NaF	18.5	258
	0.1 M NaF	18.5	260
	0.2 M NaF	11.6	265

[a] Poly-L-lysine (pH 7.5) added to poly A at pH ~8.6. [b] Amplitude of Cotton effect, 245-mμ peak to ~285-mμ trough. Poly A concentration was the same in all experiments (5.1×10^{-5} M).

complex: poly-L-lysine + poly A $\rightleftharpoons$ complex. At equilibrium, the concentration of poly-L-lysine $\approx$ 0 and K_{comb}[1] = [complex]/[poly-L-lysine][poly A] $\approx \infty$. This was done for the experiment of Figure 1, as described in the Methods section. Each ratio of lysyl to adenyl residues yielded an optical rotatory dispersion spectrum which could be treated as a linear combination of the optical rotatory dispersion of excess (uncomplexed) poly A and that of the [poly A–poly-L-lysine] complex. When the contribution of the free poly A was subtracted, the spectrum remaining was essentially proportional to the assumed concentration of the complex (concentration of poly-L-lysine added = concentration of complex). Figure 9 depicts the optical rotatory dispersion spectrum (solid curve) of the primary complex, composed of the averaged computed molar residue rotations at lysyl to adenyl ratios ranging from 0.09 to 1.0. Instances in which the calculated rotational contribution of the complex was less than 2 mdeg were omitted from the average. (These occurred mainly at wavelengths above 270 mμ, when lysyl to adenyl residue ratios were below 0.5.) The standard deviation from the mean is indicated by bars on the figure. Also included in Figure 9 are two sets of points, the first representing the optical rotatory dispersion of the 1:1 complex with no excess of either component (curve 7, Figure 1) and the second representing the 1:1 complex in the presence of a tenfold residue excess of poly-L-lysine (curve 4, Figure 2). Both sets of points are in excellent agreement, and they also correspond reasonably well to the averaged optical rotatory dispersion spectrum. The observed discrepancy probably reflects the onset of the aggregative phase (some sample opalescence was discernible at this ratio). In addition, the assumption that all of the added poly-L-lysine is converted to complex is an oversimplification. There could be some significant error in the computed concentrations of the various system components. However, the calculated $[m]_\lambda$ values show no monotonic variation with increasing residue ratios, indicating that aggregation is probably the better explanation.

[1] Abbreviation used is: K_{comb}, the equilibrium constant for the primary combination of poly A and poly-L-lysine.

TABLE II: The Primary and Secondary Complex of Poly A and Poly-L-lysine. Residue Ratio and Thermal Stability.[a]

Lysyl/ Adenyl	Temp (°C)	$\alpha_{obsd,p\text{-}t}$[b] (mdeg)	λ_{trough} (mμ)	Reversibility[c] (%)
0.67	9	15.0	258	
	21	14.3	258	
	40	10.0	258	
	56	5.6	258.5	
	77	4.6	259	
	56–21 (cool)	14.3	258	100
	77–21 (cool)	14.3	258	100
1.1	22	20	260	
	35	13.5	260	
	50	9.5	260	
	65	6.5		
	77	4.0	259	
	96	3.0	259	
	96–35 (cool)	11	259	76
	35–22 (cool)	16	259	76
1.3[d]	16	17	259	
	31	12	260	
	45	8.5	260	
	60	5.0	262	
	78	3.0	262	
	78–30 (cool)	7.0	260	45
1.3[e]	10	14.2	266	
	22.5	9.2	266	
	22–10 (cool)	10.2		0
1.4	6	10.2	275	
	20	6.4		
	40	4.2		
	40 to 6 (cool)	4.2		0
1.5	10	12.5	270	
	21	9.0	278	
	21–10 (cool)	9.2		0

[a] pH 7.2, 0.01 M phosphate buffer. [b] Amplitude of Cotton effect, 245-mμ peak to ~260 mμ trough. [c] Per cent reversibility = $\Delta\alpha_{obsd}$ cool $(T_2-T_1)/\Delta\alpha_{obsd}$ heat $(T_1-T_2) \times 100$. [d] Mixed at 3°. [e] Mixed at 10°.

The Effect of Temperature on Complex Formation. The rate of formation of the [poly A + poly-L-lysine] complex is temperature dependent. At lysyl/adenyl ratios of less than 1.0, primary complex formation occurred within 30 sec of mixing at 22°, but required 15 min for completion at 6°. The final optical rotatory dispersion spectrum was the same in both cases. However, when the ratio exceeded 1.4, the optical rotatory dispersion of the secondary complex was found within 1 min at 22° and 30 min at 6°.

Thermal Stability. An attempt was made to disrupt the [poly A–poly-L-lysine] complex by means of heat and to restore it by subsequent cooling. The experiments are summarized in Table II. The Cotton effect of the complex is described upon heating and cooling, both with respect to observed (peak to trough) rotation and to spectral position of the optical rotatory dispersion trough. In all cases, increased temperatures result in rotational decreases but only the optical rotatory dispersion spectra having λ_{trough} around 260 mμ display any recovery of rotation upon cooling.

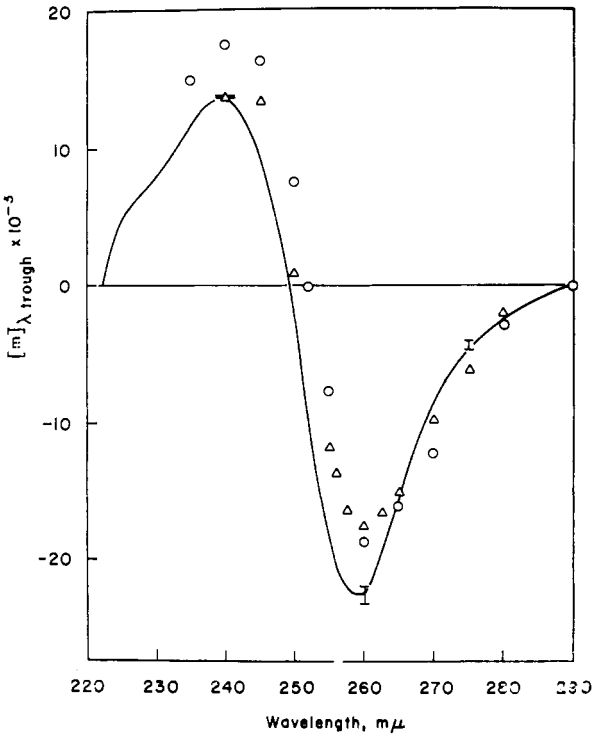

FIGURE 9: The optical rotatory dispersion of the [poly A–poly-L-lysine] complex. Solid line: computed averaged [*m*] values, using the experimental data of Figure 1, as described in the Methods and Results section; Δ, optical rotatory dispersion of the 1:1 complex, no excess poly A or poly-L-lysine (curve 7, Figure 1); ○, 1:1 complex in the presence of a tenfold residue excess of poly-L-lysine (curve 4, Figure. 2). The error bar at 240 mμ is drawn as =.

The limitation of partial reversibility to complexes having one type of optical rotatory dispersion spectrum (λ_{trough} 260 mμ) suggests that the red-shifted ($\lambda_{trough} \geq 265$ mμ) optical rotatory dispersion spectra represent a different molecular species of the [poly A–poly-L-lysine] complex, probably an aggregate. Heating would simply cause precipitation; cooling the now-insoluble material would have no effect.

Table II also shows that with complexes formed at ratios of 1.3 or less (λ_{trough} 260 mμ) reversibility is observed. As the ratio is lowered, the reversibility increases, and at a ratio of 0.67 it is 100%. The red-shifted optical rotatory dispersion form of the complex is more likely to be formed at lysyl/adenyl ratios between 1.3 and 1.5 and is not temperature reversible. At ratios between 1.1 and 1.5, the type of complex formed, *i.e.*, primary or secondary, depends upon the temperature of mixing, rate of mixing, and ionic strength. As seen in Table II, at a ratio of 1.3, mixing at 3° produced a predominately primary complex (λ_{trough} 259 mμ), which was 45% temperature reversible, while mixing at 10° resulted in formation of the secondary complex (λ_{trough} 266 mμ), which was totally temperature nonreversible. The λ_{trough} 260 mμ form is again present as the residue ratio further increases from 2 to 41. The latter data were not included in the table, as they were measured at 22° only.

The Specificity of Complex Formation. There is no observable change in the optical rotatory dispersion spectrum of poly A upon addition of lysine-HCl monomer, diglycyl methyl ester, or tetraglycine (pH 7.2, 0.01 M phosphate buffer). Conversely, AMP has no effect on the optical rotatory dispersion spectrum of poly-L-lysine. Poly-L-histidine, however, does interact with poly A at a histidyl/adenyl ratio of 1.2, pH 6.04, in 0.16 M NaF. Under these conditions, the polypeptide is positively charged and the polynucleotide is single stranded. The change induced in the poly A optical rotatory dispersion spectrum is only about 10% less than that induced by an equivalent mole residue amount of poly-L-lysine.

The above results indicate that both the nucleotide and the amino acid components must be polymerized in order to form a complex of detectably different conformation from that of the components. Some positive charge is also necessary, at least for short oligopeptides. The result with poly-L-histidine suggests that the secondary structure of poly A will probably be affected by any positively charged polypeptide.

Discussion

This work was originally planned as an initial step in investigating the proposal that the binding of histones to DNA may result in conformational alterations which in turn lead to alterations in the DNA replicative properties. Poly A and poly-L-lysine were chosen with the expectation that if these well-characterized polymers exhibit any conformational changes upon association, they should be readily detected by the optical methods chosen.

In the single-stranded form of poly A, the nucleotide bases are stacked such that their π–π^* transitions interact in an exciton system, resulting in an optical rotatory dispersion spectrum consisting in part of two Cotton effects of opposite sign and nearly equal rotational strengths (Tinoco *et al.*, 1963; Warshaw *et al.*, 1965). When this form of poly A is mixed with poly-L-lysine, a new species is formed, having optical rotatory dispersion and ultraviolet spectra centered at somewhat higher wavelengths. The optical rotatory dispersion spectrum of the 1:1 complex is simpler than that of single-stranded poly A. It appears to be a single negative Cotton effect centered at about 251 mμ, with $[m]_{260\,m\mu} \simeq -22{,}000$. The analogous optical rotatory dispersion parameters for poly A are λ crossover 244 mμ; $[m]_{256\,m\mu} = -78{,}500$. The retention of an optical rotatory dispersion spectrum of significant magnitude indicates that the conformation of the 1:1 complex still has some order (compare with the optical rotatory dispersion spectrum of AMP (Holcomb and Tinoco, 1965)). Nonetheless, the stacking of the nucleotide bases is disrupted sufficiently to diminish exciton interactions in the system. This conclusion is reached because of the failure, within experimental limits, to discern the positive limb of the first (positive) Cotton effect belonging to the split transition.

With further addition of poly-L-lysine (residue ratio lysyl/adenyl = 1.1 and 1.2) and also with time, the optical rotatory dispersion spectrum of the complex shifts further to higher wavelengths and diminishes. The Cotton effect is now centered at 255 mμ with the trough at 265–270 mμ, much the same as for AMP but still having a much greater molar rotation than the mononucleotide. Very similar optical rotatory dispersion results have recently been reported for systems of poly-L-lysine and highly polymerized DNA (Cohen and Kidson, 1968). These authors describe a red shift and a conversion from a positive to a negative Cotton effect in the optical rotatory dispersion spectrum of DNA upon association with poly-L-lysine. Upon centrifugal resolution of the complex into a "soluble" and an "insoluble" fraction, they find that the optical rotatory dispersion of the insoluble material is red shifted with respect to that of the soluble complex. The latter is in turn red shifted with respect to DNA. These two optical rotatory dispersion spectra for the complex are analogous to the primary and secondary complexes discussed here.

The ultraviolet spectral data also suggest the occurrence of a new species (complex) having a somewhat different ultraviolet spectrum from that of poly A. The new spectrum is shifted to higher wavelength by about 6 mμ and appears hypochromic with respect to single-stranded poly A. However, it must be noted that the decrease in absorbance at λ_{max} was small (0.03 in Figure 4 and 0.035 in Figure 3). In addition, the ultraviolet spectra are in some sense "derived" in that they have been corrected for light scattering effects. These factors add uncertainty to any conclusions regarding the hypochromic effect of complex formation. Nonetheless, this phenomenon is observed to the same extent whether poly-L-lysine is added to poly A (Figure 3) or poly A to poly-L-lysine (Figure 4), even though the scatter corrections are different in each instance. The ϵ_{max} of the complex remains constant at $\sim 9.4 \times 10^3$, as calculated from the experiment of Figure 4, assuming that the concentration of complex = concentration of added poly A (concentration of free poly A = 0). These results show that there is no significant precipitation of complex and that the scattering absorbance has been correctly subtracted. It is concluded that the observed decrease in ϵ_{max} is probably real. This is of interest since single-stranded poly A itself (Holcomb and Tinoco, 1965) exhibits hyperchromicity as the secondary structure loses order (ϵ_{max} 10.1×10^3 at 22° and 12.9×10^3 at 83°; ϵ_{max} adenylic acid (2′,3′) 15.4×10^3 at 25°). The double-stranded form of the polynucleotide, which has greater order than the single-stranded form, has ϵ_{max} 8.7×10^3 at 22°. Although the molar rotation and probably exciton interactions are diminished upon poly A–poly-L-lysine association, the resultant complex does not necessarily have diminished order. The ultraviolet data suggest that it may even be more rigidly structured than either of the components, but with different spatial relationships of the nucleotide bases.

It is suggested that complex formation between poly-L-lysine and poly A is a biphasic process. The initial stage is the formation of a "primary complex": the binding of positively charged poly-L-lysine molecules to a larger chain of poly A until most of the negatively charged nucleotide sites have been covered by lysyl residues. As suitable lengths of polynucleotide sequence become increasingly unavailable, additional poly-L-lysine chains cross-link the "primary" complex, resulting in an aggregated "secondary" complex. The intercomplex interactions might involve lysyl–lysyl, lysyl–phosphate, or lysyl–adenyl interchain associations. The properties of the final product differ from those of the initial complex in several ways. Its optical rotatory dispersion spectrum is red shifted with respect to the initial complex and can no longer be reversibly diminished by heating. Further, the material becomes increasingly insoluble with time. The latter two properties indicate that the product of the final phase is an aggregate. Additional evidence for this view is the observation that rapid combination of the two polymer compounds at near-equiv-

alent residue concentrations invariably results in turbidity and occurrence of the red-shifted optical rotatory dispersion spectrum. Under conditions of gradual combination (*e.g.*, combination of small increments of one polymer to another or mixing at decreased temperatures), excessive formation of the aggregated product can be avoided, even at residue equivalence. Increased insolubility has been associated with a transition from reversible to irreversible complex formation in similar systems (Leng and Felsenfeld, 1966; Tsuboi, 1967; Latt and Sober, 1967). Leng and Felsenfeld (1966) have also noted that precipitation is somewhat inhibited at 0°.

The complexing process is not exclusively an electrostatic phenomenon. The primary interaction appears to be insensitive to ionic strength, over a range 0–0.2 M NaF. Moreover, association occurs over a poly-L-lysine ionization range of $\alpha = 0$ to $\alpha = 0.8$ (Figure 6). It seems reasonable to conclude that some form of nonionic lysyl–adenyl interaction contributes to the stability of the primary complex.

The secondary (aggregative) interaction apparently has some structural preferences. It occurs most readily when the poly-L-lysine resembles a flexible coil rather than a charged extended rod, as determined by the hydrodynamic measurements of Applequist and Doty (1962). Thus, the aggregation is spread over a wider ionization range in salt than in H_2O and occurs maximally when the polypeptide is in a half-helical, half-random conformation (Figures 6–8). Conditions for maximal aggregation probably represent the best compromise between optimal charge relationships and greatest flexibility. As the poly-L-lysine is approximately 70% un-ionized at maximum aggregation, perhaps the charged portion associates with the phosphates of poly A, while the uncharged side chains associate intermolecularly, causing maximum aggregation. In this connection, it is of interest to note that Leng and Felsenfeld (1966) found that poly-L-lysine forms a precipitated aggregate more readily with denatured than with native DNA, while Akinrimisi *et al.* (1965) report that at low histone or poly-L-lysine to DNA ratios (1:10) a soluble complex is formed, with a slight preference for native DNA.

The primary complex appears to be formed with an equilibrium constant sufficiently large that at 25° all added polymer is promptly converted to complex. This conclusion is supported by the data of Figures 5a and 9. In Figure 5a it is seen that each equal increment of poly-L-lysine added to poly A results in the same rotational decrease, *i.e.*, the system shows no saturation behavior. This linearity would be observed if K_{comb}, the equilibrium constant for complex formation, were either very large or very small. The data of Figure 9 show that K_{comb} is large. Optical rotatory dispersion spectra of the complex have been computed at several input ratios of lysyl to adenyl residues, by making the assumption that the concentration of poly-L-lysine added equals the concentration of complex formed (K_{comb} is very large). The computed optical rotatory dispersion spectra are not substantially different from each other, supporting the above assumption. Further, a 1:1 complex formed by addition of poly A to a tenfold excess of poly-L-lysine has the same optical rotatory dispersion spectrum as that formed by adding equivalent mole residues of poly-L-lysine to poly A (Figure 9); this finding rules out a small K_{comb}. The observed quantitative binding of poly-L-lysine to poly A is in agreement with results obtained for similar systems under similar conditions (Tsuboi *et al.*, 1966; Olins *et al.*, 1967, 1968; Leng and Felsenfeld, 1966).

The results of this study have shown that at pH 7 a highly stable complex is formed between poly A and poly-L-lysine, which is structurally well defined and different from poly A alone. This complex formation causes a rearrangement in the base stacking and other associations, holding the poly A in its characteristic single-stranded helical structure. The exact nature of base rearrangement in the complex is not known, but the changes in optical rotatory dispersion and ultraviolet parameters strongly indicate that a conformational change has been brought about. The possible biological significance of these findings is that they demonstrate that it is physically feasible for separated single-stranded portions of the genome to be combined with basic nuclear proteins, resulting in a highly stable but conformationally altered DNA.

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Studies on the Catalytic Mechanism of *Escherichia coli* Succinic Thiokinase*

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ABSTRACT: The coenzyme A analog, desulfocoenzyme A, does not significantly affect the rate of succinyl phosphate formation from phosphorylated succinic thiokinase (succinate:coenzyme A ligase (adenosine diphosphate), EC 6.2.1.5).

The effector action of desulfocoenzyme A seems to require the presence of adenosine triphosphate. Phosphoryl-enzyme is shown to be an intermediate in succinyl phosphate formation from adenosine triphosphate and succinate, and additional evidence is presented supporting the intermediary role of phosphoryl-enzyme in the over-all catalytic mechanism of the enzyme.

Succinic thiokinase catalyzes the formation of succinyl phosphate from ATP and succinate (Nishimura and Meister, 1965). This reaction is stimulated by the coenzyme A analog, desulfo-CoA[1] (Grinnell and Nishimura, 1969b). Since the formation of succinyl phosphate from phosphoryl-STK[1] and succinate was also known to occur (Nishimura, 1967), it was of interest to investigate the influence of the CoA analog on reactions involving phosphoryl-STK. We have studied the effect of desulfo-CoA on the rate of succinate phosphorylation by STK-P at various succinate concentrations, and measured the rate of STK-P $\rightleftarrows$ ATP exchange in the presence and absence of desulfo-CoA. Additional studies on the formation of succinyl phosphate from ATP and succinate are also reported. Possible mechanisms of stimulation of succinyl phosphate formation by desulfo-CoA are discussed.

Further evidence for the participation of STK-P in the catalytic mechanism of succinic thiokinase is also presented. In the presence of all substrates, the initial rates of the reactions, STK-P $\rightarrow$ P_i and ATP $\rightarrow$ P_i, have been measured simultaneously by dual-radioisotope experiments using [^{32}P]-STK-P and [γ-^{33}P]ATP.

Experimental Section

Materials. [2,3-^{14}C]Succinic acid, [^{32}P]P_i, and [^{33}P]P_i were purchased from New England Nuclear Corp. [γ-^{32}P]ATP and [γ-^{33}P]ATP were synthesized enzymatically (Glynn and Chappell, 1964). All labeled ATP and P_i used in this investigation was purified by DEAE-cellulose chromatography (Wehrli *et al.*, 1965).

Enzyme. Succinic thiokinase was isolated and assayed as described previously (Grinnell and Nishimura, 1969a).

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[1] Abbreviations used are: CoAH, desulfocoenzyme A; STK, succinic thiokinase; STK-P, phosphorylsuccinic thiokinase.