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Complexes of Polylysine with Polyuridylic Acid and Other Polynucleotides*

Dana Carroll†

ABSTRACT: Mixing of poly(U) with polylysine in solutions of low ionic strength resulted in conversion of the polynucleotide to its double-stranded form. The amount of poly(U) converted depended on the amount of polylysine added, complete conversion being achieved by about lysine:phosphate = 0.7. The melting temperature of the double-helical structure in complexes with high molecular weight polylysines was approximately 33°, independent of lysine:phosphate ratio and polylysine $\overline{\mathrm{DP}}$. In complexes with the reversibly bound cations pentalysine and Mg^{2+}, T_m increased with increasing cation concentration. Neither polyarginine nor protamine was effective in converting poly(U) to its ordered form. At lysine:phosphate ratios near unity, poly(U)–polylysine complexes formed aggregates with greatly enhanced circular dichroism (($\epsilon_l - \epsilon_r$) = >100). Very high molecular weight polylysines ($\overline{\mathrm{DP}}$ = 300, 500) were relatively ineffective in producing these large-magnitude spectra. At lysine:phosphate ratios well above 1.0, the complexes were once again soluble, probably due to binding of excess polylysine. In light of the fact that complexes of polylysine with both poly(U) and DNA are capable of forming aggregates with greatly enhanced circular dichroism (CD) spectra, complexes were prepared with a number of other polynucleotides of various secondary structures. Aggregates were formed in electroneutral mixtures with all polynucleotides; however, enhanced CD spectra were observed only in the cases of the double-stranded polynucleotides examined (DNA, [poly(U)]$_2$, poly(I)·poly(C), and poly(A)·poly(U)). None of the single- (poly(A), poly(C), and TMV RNA) or triple-stranded (poly(A)·2poly(U), [poly(I)]$_3$) polynucleotides gave complexes with enhanced spectra. Interestingly, though, poly(I) appeared to form aggregates with large-magnitude CD in high concentrations of NaCl, without added polylysine.

It has been found that polylysine converts poly(U) to its double-stranded form (Lipsett, 1960; Thrierr *et al.*, 1971). At lysine:phosphate ratios near 1.0, the polylysine–poly(U) complexes formed aggregates having a CD[1] spectrum of greatly increased magnitude, as has been observed for annealed polylysine–DNA complexes (Cohen and Kidson,

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* From the Department of Chemistry, University of California, Berkeley, California 94720. *Received June 14, 1971.* This work was supported by a grant from the National Institutes of Health (GM 10840) to Professor I. Tinoco, Jr. The author was a predoctoral fellow of the U. S. Public Health Service.

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[1] Abbreviations used which are not listed in *Biochemistry, 5,* 1445 (1966), are: ORD, optical rotatory dispersion; CD, circular dichroism; $\overline{\mathrm{DP}}$, average degree of polymerization; TMV, tobacco mosaic virus.

1968; Shapiro *et al.*, 1969; Haynes *et al.*, 1970; Olins and Olins, 1971; Carroll, 1972). A survey has been made of the ability of a number of polynucleotides of various secondary structures to form complexes with polylysine which are characterized by large-magnitude CD.

Experimental Section

Materials. Polyadenylic acid, polycytidylic acid, and polyinosinic acid were purchased from Miles Research Products; polyuridylic acid was from Miles or from Schwarz BioResearch. RNA isolated from tobacco mosaic virus (Mandeles and Bruening, 1968) was stored as an alchohol precipitate at −20° in the presence of bentonite, which was removed by centrifugation before use. Polynucleotide solutions were dialysed once against EDTA in buffer α, then exhaustively against buffer α. In most cases they were filtered through washed Millipore filters (0.45 μ pore size). Poly-L-lysine, poly-L-arginine, and protamine samples were the same as used previously and were handled as described (Carroll, 1972).

Common chemicals were reagent grade; solutions were prepared using water redistilled in a glass still. Buffer α is 0.005 M NaCl–0.001 M cacodylate (pH 7.0).

Methods. Extinction coefficients of the homopolynucleotides were determined by alkaline hydrolysis (0.3 N NaOH, 37°, 24–36 hr). The results, which are comparable to those reported by other authors (Sigler *et al.*, 1962; Stevens and Felsenfeld, 1964; Chamberlin, 1965; Ts'o *et al.*, 1966), are given in Table I. Solutions of poly(C) and CMP were buffered to pH 8 to avoid spectral changes caused by ionization of the base moiety. Two- and three-stranded complexes of homopolynucleotides were prepared by incubating the appropriate stoichiometric mixtures in approximately 0.3 M NaCl–0.001 M cacodylate (pH 7.0) at a concentration between 1×10^{-3} and 2×10^{-3} M (as nucleotide), overnight at 4°. The concentration of TMV RNA was determined from spectra in 0.15 M NaCl using an extinction coefficient of 7260 (Cantor *et al.*, 1966).

For preparation of complexes by direct mixing, the polynucleotide was diluted with buffer solution to within about 20% of the desired final volume. Polylysine was then added from a dilute solution (3×10^{-4} to 5×10^{-4} M, as monomer) with vigorous magnetic stirring. The polylysine solution was delivered slowly from a pipet, the tip of which was kept below the surface of the polynucleotide solution so that areas of local high concentration, even as large as that created by a single drop, were avoided. The solution was diluted to the desired polynucleotide concentration (approximately 5×10^{-5} M) by addition of a small supplementary amount of buffer. The mixing operation was carried out in an ice bath.

In the survey of complexes of polylysine with various polynucleotides, polylysines of $\overline{\text{DP}} = 26$ and 75 were used; both were effective in inducing the large-magnitude CD in complexes with DNA and poly(U). The salt gradient used in formation of the complexes was that employed for DNA–polylysine mixtures (Carroll, 1971, 1972): a linear gradient from 1.50 to 0.15 M NaCl. Two- and three-stranded polynucleotide complexes were not subsequently dialyzed into buffer α, but were kept in 0.15 M NaCl. This salt concentration is sufficient to maintain the stoichiometric complexes at room temperature and at 2.5° (Stevens and Felsenfeld, 1964; Krakauer and Sturtevant, 1968). The concentrations of the polynucleotides in these annealed complexes were not determined directly, but were assumed to be the same as in a sample containing no polylysine which was subjected to the same dialysis gradient.

TABLE I: Extinction Coefficients of Polynucleotides.[a]

Polymer	λ_{max} (mμ)	ϵ_{max}	Solvent
Poly(A)	257	10,000	Buffer α
Poly(C)	268	6,400	0.01 M Tris (pH 8.0)
Poly(I)	248	10,400	Buffer α
Poly(U)	260	9,200	Buffer α
Poly(A)·poly(U)	258	6,700	0.15 M NaCl
Poly(A)·2poly(U)	257	6,200	0.15 M NaCl
Poly(I)·poly(C)	265	5,200	0.15 M NaCl

[a] All of the above measurements were made at room temperature. The solvents for the polymer complexes were buffered to pH 7.0 with 0.001 M cacodylate.

Absorption–temperature profiles were measured with a Gilford Model 2000 multiple sample absorbance recorder, which was calibrated with respect to both temperature and absorbance. The temperature was driven up or down at a rate of about 20°/hr by circulating an ethylene glycol–water (1:1, v/v) mixture from an external bath through the sample chamber.

As before (Carroll, 1972), absorption spectra were measured on Cary Model 14 and Model 15 spectrophotometers; and ORD and CD spectra were measured on a Cary Model 60 spectropolarimeter. Since aggregates of poly(U) with polylysine were quite unstable (see Results), CD at a wavelength near the maximum was monitored after placing the sample in the instrument. The complete spectrum was measured just after the CD had stopped increasing and before it began to decrease. The necessary wait varied considerably with the nature of the sample and was sometimes as long as 45 min. Because of this instability, poly(U)–polylysine mixtures were prepared immediately prior to measurement of their CD spectra.

Results

Poly(U)–Polylysine Complexes. As expected, direct mixing of polylysine with poly(U) at low-salt concentration led to the conversion of the polynucleotide to its ordered form. This transition was reflected in both absorption and CD spectra (Figures 1 and 2). The amount of double-stranded poly(U) increased with increasing amounts of polylysine until a plateau was reached at lysine:phosphate around 0.6–0.7 (Figure 3). That a simple conversion between two forms of poly(U) is involved was indicated by the existence of isosbestic points in the CD spectra at 250.5, 239.5, and 211 mμ, and by the parallel changes at several wavelengths (Figure 3). The spectral changes induced by polylysine were essentially identical to those induced by Mg^{2+} (Figure 4) and to those induced by Cs^{+} (Thrierr *et al.*, 1971), except for the contributions of polylysine itself below 240 mμ. The total hypochromicity at 260 mμ was 32%. It was also evident (Figure 1) that the absorption in the 205-mμ band of poly(U) was reduced in the complex, despite the contribution of polylysine in this region.

Polylysines of all molecular weights tested were effective in inducing the structural conversion in poly(U) (Figure 5). For large polylysines, the degree of conversion increased linearly with lysine:phosphate until the plateau was reached. In the

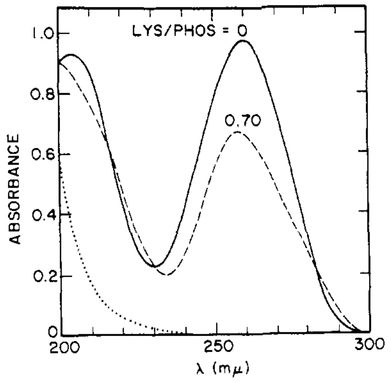

FIGURE 1: Absorption spectra of poly (U) alone and with polylysine, $\overline{DP}$ = 26, lysine:phosphate = 0.70, at poly(U) = 5.28 × 10^{-5} M; and polylysine, $\overline{DP}$ = 26, alone (·····) at 5.28 × 10^{-5} M. All spectra in buffer α, at room temperature, in 2-cm path length.

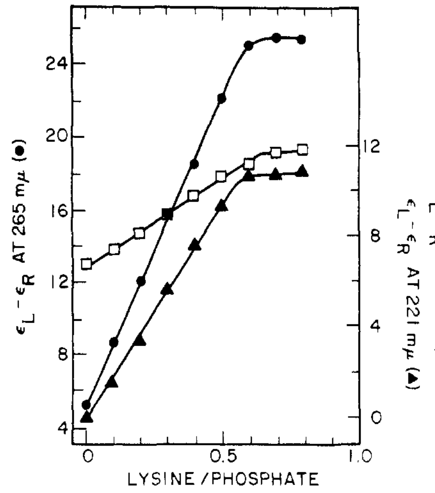

FIGURE 3: CD of poly(U)-polylysine ($\overline{DP}$ = 26) complexes as a function of lysine:phosphate ratio. In buffer α, 2.5°.

case of pentalysine, there was a slight lag at low + : − ratios, most likely reflecting the cooperative nature of the transition and the requirement of a stretch longer than a single pentalysine binding site to nucleate it. The CD at the plateau level was smaller for pentalysine than in the cases of the higher molecular weight polymers, and lower still in the case of Mg^{2+}, reflecting the incompleteness of the conversion to double-stranded poly(U) at the temperature of measurement (2.5°) by the smaller cations (see Figure 6).

Absorbance-temperature profiles for a number of poly(U)-polylysine complexes are presented in Figure 6, and the temperatures of half-melting are collected in Table II. The absorbance changes were completely reversed on returning to low temperatures, though the transitions were sometimes shifted a couple of degrees lower in temperature, under the conditions of the experiments. When the amount of polylysine in a poly(U) solution was less than sufficient to cause complete conversion to the ordered form, the amount of absorbance change in the melting was reduced by the expected factor, but the T_m remained the same. This is consistent with irreversible binding of the polypeptide and cooperative melting of the region to which a single polylysine molecule is bound. In contrast, pentalysine and Mg^{2+} bind reversibly to poly(U); and the T_m's of samples containing either of these molecules increased with increasing cation concentration.

Poly(U)-Polylysine Aggregates. At lysine:phosphate ratios near 1.0, aggregation occurred in poly(U)-polylysine mixtures; and these aggregates often showed greatly enhanced CD spectra, as in the case of DNA-polylysine complexes (Cohen and Kidson, 1968; Shapiro *et al.*, 1969; Haynes *et al.*, 1970; Olins and Olins, 1971; Carroll, 1972). Figure 7

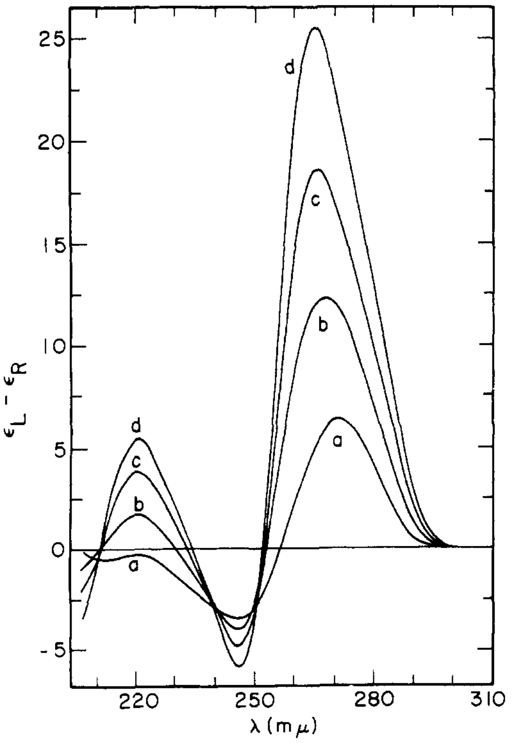

FIGURE 2: CD spectra of poly(U)-polylysine ($\overline{DP}$ = 26) mixtures at various lysine:phosphate ratios: (a) 0, (b) 0.20, (c) 0.40, and (d) 0.70. In buffer α, 2.5°.

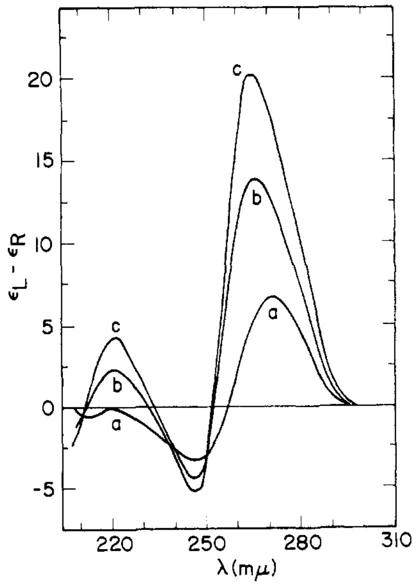

FIGURE 4: CD spectra of poly (U) in the presence of MgCl$_2$. MgCl$_2$ = (a) 1.00 × 10^{-4} M, (b) 1.00 × 10^{-3} M, and (c) 1.00 × 10^{-2} M. In buffer α, 2.5°.

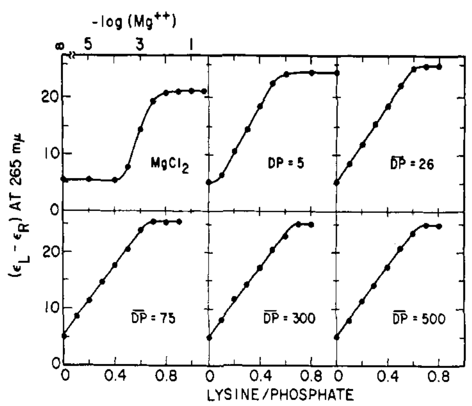

FIGURE 5: Conversion of poly(U) to its ordered form by $MgCl_2$ and polylysines of several molecular weights. Poly(U) = 5.34×10^{-5} M, in buffer α, 2.5°.

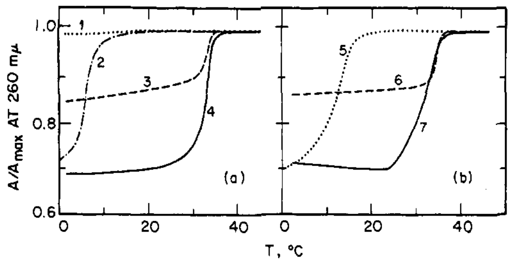

FIGURE 6: Absorption–temperature profiles for poly(U) in the presence of various cations: (a) curve 1, poly(U) alone; 2, 10^{-2} M $MgCl_2$; 3, polylysine ($\overline{DP}$ = 26), Lys:P = 0.30; 4, polylysine ($\overline{DP}$ = 26), Lys : P = 0.75. (b) Curve 5, pentalysine, Lys:P = 0.50; 6, polylysine ($\overline{DP}$ = 500), Lys:P = 0.30; 7, polylysine ($\overline{DP}$ = 300), Lys:P = 0.75. In buffer α, poly(U) = 5.29×10^{-5} M.

shows some of the variety in large-magnitude CD spectra which were observed. As was true of DNA–polylysine aggregates (Carroll, 1972), the CD of these samples did not ordinarily go to zero outside the absorption region.

The appearance of the large-magnitude circular dichroism depended on the molecular weight of the polylysine employed, as well as rather dramatically on the lysine:phosphate ratio (Figure 8). Although Mg^{2+} near 1 M caused precipitation of poly(U), this was not accompanied by enhancement of CD. With pentalysine, a broad transition of larger $\epsilon_l - \epsilon_r$ occurred between lysine:phosphate = 1.3 and 2.0; the CD remained large at ratios as high as 4.5. Polylysines of very high molecular weight were much less effective than those in an intermediate range in promoting the appearance of the large-magnitude CD.

In the cases of polylysines with DP = 26 and above, a minimum in CD was observed at lysine:phosphate = 1.00. (In fact, the locations of these minima are probably better estimates of the point at which electrostatic equivalence was achieved than are those based on measured polymer concentrations, which were not more accurate than ±4%.) As was the case with DNA–polylysine complexes (Carroll, 1972), this represents large-scale aggregation at electroneutrality, rather than a loss of structure. Both the absorption and CD spectra of these samples had the flattened and shifted maxima characteristic of large particles (Duysens, 1956; Urry and Ji, 1968); and in some cases, flaky particles could be discerned by eye. It seems reasonable to suggest that if the content of structure responsible for the enhanced CD could be measured directly, it would be found to reach a peak at electroneutrality and to decrease on either side of that point.

TABLE II: Melting Temperatures of Poly(U) in the Presence of Various Cations.[a]

Cation	T_m (°C)
1×10^{-2} M $MgCl_2$	5.8
1×10^{-1} M $MgCl_2$	7.5
Pentalysine ($+:- = 0.20$)	5.5
Pentalysine ($+:- = 0.50$)	11.8
Pentalysine ($+:- = 0.90$)	16.6
Polylysine, $\overline{DP}$ = 26 ($+:- = 0.30$)	33.0
Polylysine, $\overline{DP}$ = 26 ($+:- = 0.75$)	32.6
Polylysine, $\overline{DP}$ = 75 ($+:- = 0.30$)	32.0
Polylysine, $\overline{DP}$ = 75 ($+:- = 0.75$)	31.3
Polylysine, $\overline{DP}$ = 300 ($+:- = 0.30$)	35.0
Polylysine, $\overline{DP}$ = 300 ($+:- = 0.70$)	32.7†
Polylysine, $\overline{DP}$ = 300 ($+:- = 0.75$)	31.5
Polylysine, $\overline{DP}$ = 300 ($+:- = 0.80$)	33.0††
Polylysine, $\overline{DP}$ = 500 ($+:- = 0.30$)	34.5
Polylysine, $\overline{DP}$ = 500 ($+:- = 0.75$)	31.8

[a] All melting temperatures were determined in buffer α, except those labeled †, which was measured in 0.01 M Tris (pH 8.5) and ††, which was determined from the temperature dependence of ORD spectra in 0.001 M cacodylate–10^{-4} M EDTA (pH 7).

The aggregates were redissolved at higher lysine to phosphate ratios; the scattering in the solutions disappeared, and the spectra reverted to those characteristic of double-stranded poly(U) (Figures 7 and 8). Complexes with $+:- = >1.5$ showed somewhat sharper absorbance–temperature profiles and lower T_m's than those with $+:- = <0.8$ (Table III).

The poly(U)–polylysine aggregates with enhanced CD were found to be quite unstable with respect to time, agitation, and temperature. The general aspects of the instability were universal, while the specifics varied considerably among samples.

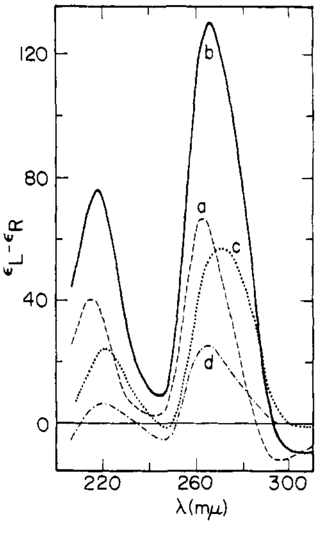

FIGURE 7: Some CD spectra of poly(U)–polylysine ($\overline{DP}$ = 26) aggregates; in buffer α, 2.5°. Lys:P = (a) 0.90, (b) 0.92, (c) 1.00, and (d) 2.00.

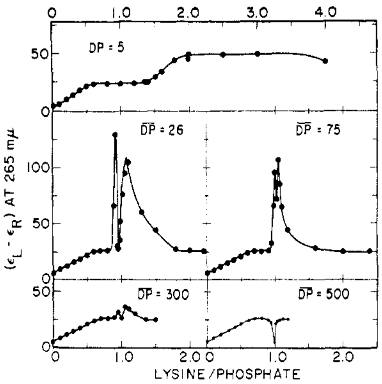

FIGURE 8: Dependence of CD of poly(U)-polylysine complexes on lysine:phosphate ratio for polylysines of various molecular weights; in buffer α, 2.5°.

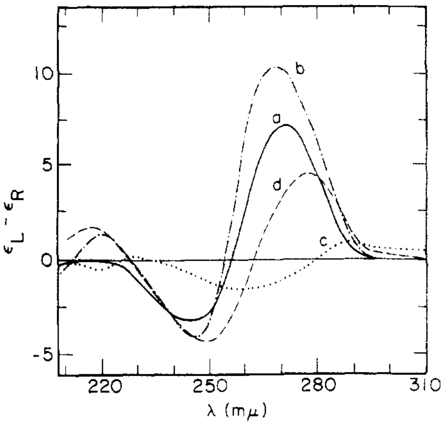

FIGURE 9: CD spectra of poly(U)-polyarginine complexes; in buffer α, 2.5°. Arginine:phosphate = (a) 0, (b) 0.50, (c) 1.00, and (d) 1.50.

On standing in the sample compartment of the Cary 60 at 2.5°, the magnitude of the CD spectrum of the aggregates decreased slowly over a period of hours. (One sample had decreased to 15% of its maximum value in 5 hr.) This decay was accompanied by a broadening and shift to longer wavelengths of the CD maximum, indicating larger particles in the older samples. Agitation of the aggregates, simply by passing the sample through a Pasteur pipet, caused the CD spectrum to disappear entirely. The spectrum reappeared, over a period of minutes for most samples. Since agitated samples still scattered light extensively, this sensitivity of the CD spectrum indicates that the anomalous optical effects are due to a specific, disruptable structure of the aggregates.

The enhanced CD was also quite sensitive to changes in temperature, substantial reductions in magnitude being observed at temperatures only as high as 10°. Absorption–temperature profiles of poly(U)–polylysine aggregates showed somewhat ragged transitions with small absorbance changes around 10° (Table III).

Complexes of Poly(U) with Polyarginine and Protamine. Surprisingly, neither polyarginine nor protamine was effective in converting poly(U) to its double-stranded form. Both of these polypeptides caused a small extent of conversion, as evidenced by an increase in the magnitude and shift to shorter wavelength of the first maximum in the CD spectrum (Figure 9). The maximum extent of conversion in both cases occurred at lysine:phosphate near 0.5, decreasing for both higher and lower ratios; and the largest spectrum obtained corresponded to about 20% of the maximal conversion induced by polylysine. Precipitation did occur near electroneutrality, with some redissolution on addition of more polypeptide. However, there was no appearance of the very large-magnitude CD. As in the case of DNA–polypeptide complexes (Carroll, 1972), the effects of polylysine on binding to polynucleotides are not shared by all basic polypeptides.

TABLE III: Melting Temperatures of Poly(U)–Polylysine Complexes at + : − = >1.0.[a]

Polylysine $\overline{DP}$	+ : −	T_m (°C)
	a. Redissolved Complexes	
26	2.20	22.1
75	2.20	18.8
300	1.55	21.8
500	1.60	19.8
	b. Aggregates	
5	2.50	10.7 (260 mμ) 10.9 (320 mμ)
26	1.05	10.0 (260 mμ) 9.8 (320 mμ)
300	1.05	10.0 (260 mμ)
500	1.05	11.1 (260 mμ) 10.5 (320 mμ)

[a] All melting temperatures were determined in buffer α.

Complexes of Polylysine with Some Other Polynucleotides. As polylysine complexes with both DNA and poly(U) were found to be capable of forming aggregates with greatly enhanced CD spectra, a survey was made of the effects of polylysine on the spectra of a number of other polynucleotides, to determine the influence of polynucleotide structure on the characteristics of the aggregates. In complexes formed by direct mixing and by salt gradient dialysis, complexes were judged to assume structures generally similar to those observed with DNA and poly(U) if their CD spectra were increased greatly in magnitude compared to the isolated polynucleotide. It is possible that in some of these cases definite complexes were formed having reduced optical activity, such as described by Davidson and Fasman (1969) for poly(A)–polylysine complexes. However, only complexes showing greatly enhanced CD were noted in this study.

In agreement with Davidson and Fasman (1969), direct mixing of poly(A) and polylysine at low salt concentration led to reduction of the magnitude of the CD spectrum (Figure 10A). In contrast to these authors, however, a small *hyper*chromism in the absorption band of poly(A) was observed at low inputs of polylysine. There was no evidence of aggregation up to a lysine:phosphate ratio of 0.74; and when aggregation did occur near electrostatic equivalence, it was still accompanied by decreased CD.

Poly(A) is precipitated at 1.5 M NaCl, the starting salt concentration in the gradient dialysis procedure. At the conclusion of the annealing process, complexes were contained in very large aggregates, which showed essentially zero circular dichroism and had an apparent stoichiometry of lysine:phosphate = 0.7. At an initial input of lysine:phosphate = 0.35, precisely half of the poly(A) was included in the precipitate; at lysine:phosphate = 0.70, none of the polynucleotide was left free in solution.

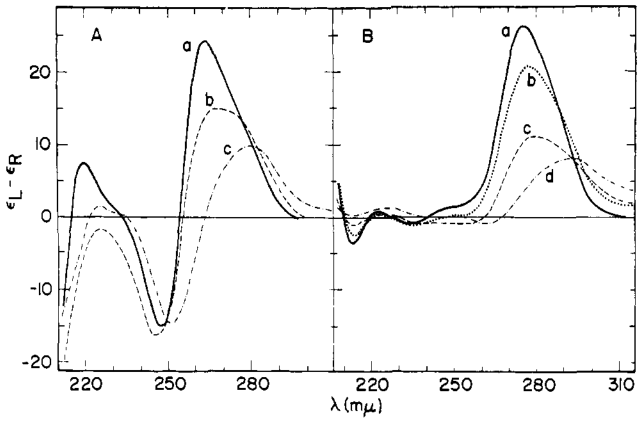

FIGURE 10: CD spectra. (A) Of poly(A)–polylysine ($\overline{\text{DP}}$ = 26) complexes formed by direct mixing in buffer α, 2.5°. Lysine:phosphate = (a) 0, (b) 0.70, and (c) 1.00. (B) Of poly(C)–polylysine ($\overline{\text{DP}}$ = 26) complexes formed by salt gradient dialysis; in buffer α, 2.5°. Lysine: phosphate = (a) 0, (b) 0.35, (c) 0.70, and (d) 1.00.

In direct mixtures of polylysine and poly(C) in 0.01 M Tris (pH 8.0) there was little or no change in absorption or CD spectrum until precipitation occurred at electrostatic equivalence. This precipitation was accompanied by a decrease in CD. Salt gradient annealing of poly(C)–polylysine mixtures also resulted in the formation of aggregates with reduced CD spectra, the degree of reduction depending on the lysine: phosphate ratio in the mixture (Figure 10B).

TMV RNA was also unable to form complexes with enhanced CD spectrum. In both directly mixed and annealed complexes, no change in spectrum was seen until precipitation occurred at lysine:phosphate = 0.85 in direct mixtures and around 0.7 in annealed complexes. These aggregates had spectra reduced in magnitude compared to the RNA alone. A similar result has been reported for annealed mixtures of polylysine and rRNA (Haynes *et al.*, 1970).

Annealed complexes of polylysine with both poly(I)·poly(C) and poly(A)·poly(U) showed greatly enhanced CD spectra (Figures 11 and 12). In the case of poly(I)·poly(C), no aggregation occurred in mixtures with lysine:phosphate as high as 0.8; and this behavior, which stands in contrast to the precipitation of DNA and poly(A)·poly(U) at low ratios in annealed mixtures, may be related to the greater solubility of poly(I)·poly(C) in the presence of oligolysines (Latt and Sober, 1967) and to the A-T preference of polylysine. The large-magnitude spectra of aggregates formed from mixtures with lysine:phosphate near unity were quite similar to those observed by Haynes *et al.* (1970). The fact that these authors found aggregates formed at lysine:phosphate = 0.5 may reflect differences, *e.g.*, in polymer or salt concentrations, between our conditions of measurement.

Haynes *et al.* (1970) have reported enhanced CD spectra for annealed poly(A)·poly(U)–polylysine complexes which are opposite in sign to those shown in Figure 12. And they have evidence that the polynucleotide had, in their samples, rearranged to give poly(A)·2poly(U) and poly(A), the former of which was involved in the complexes. That such a rearrangement had not taken place in the complexes examined here was demonstrated by the fact that the polynucleotide remaining in the supernate of a lysine:phosphate = 0.55 sample after removal of the aggregates by centrifugation was poly(A)·poly(U), not poly(A), as judged by its CD spectrum. In addition, it was found that aggregates formed on annealing of genuine poly(A)·2poly(U)–polylysine mixtures showed reduced, not enhanced, CD spectra. Enhanced spectra were not observed in directly mixed complexes of poly(A)·poly(U) and polylysine.

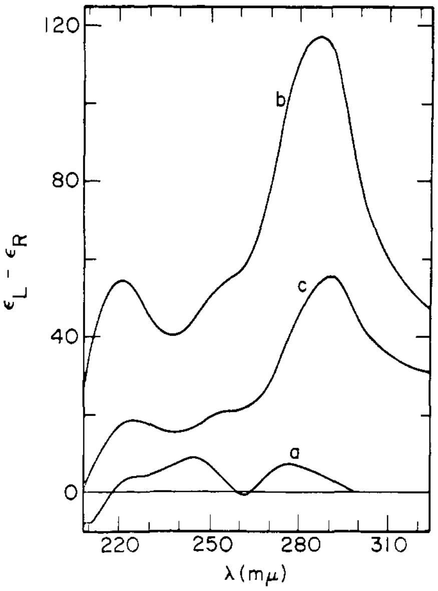

FIGURE 11: CD spectra of annealed poly(I)·poly(C)–polylysine ($\overline{\text{DP}}$ = 75) complexes, in 0.15 M NaCl–0.001 M cacodylate (pH 7.0), 2.5°. Lysine:phosphate = (a) 0, (b) 1.00, and (c) 1.20.

At moderately high salt concentrations, poly(I) is converted to an ordered form, which is characterized by hypochromism in the absorption spectrum, an increase in magnitude of the ORD spectrum and a sharp melting at elevated temperatures (Rich, 1958; Doty *et al.*, 1959; Sarkar and Yang, 1965). X-Ray diffraction (Rich, 1958) and hydrodynamic studies (Haselkorn, 1959) have indicated that the ordered form is triple stranded.

The effect of salt concentration on the spectral properties of poly(I) is reinvestigated in Figure 13. Two transitions in $A_{248\ \text{m}\mu}$ were observed at about 0.2–0.5 M NaCl and near 0.9 M NaCl. There was some aggregation associated with both these transitions, and above 1.25 M NaCl, even larger particles were formed. These same transitions were reflected in CD

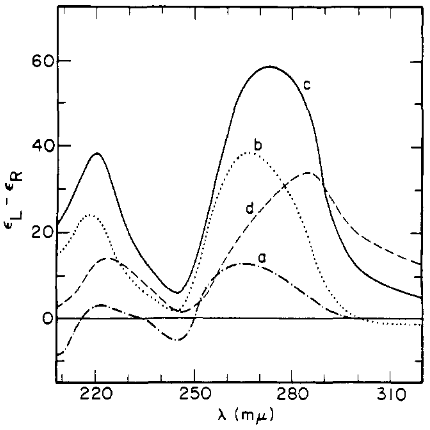

FIGURE 12: CD spectra of annealed poly(A)·poly(U)–polylysine ($\overline{\text{DP}}$ = 75) complexes, in 0.15 M NaCl–0.001 M cacodylate (pH 7.0), 2.5°. Lysine:phosphate = (a) 0, (b) 0.55, (c) 0.65, and (d) 0.85.

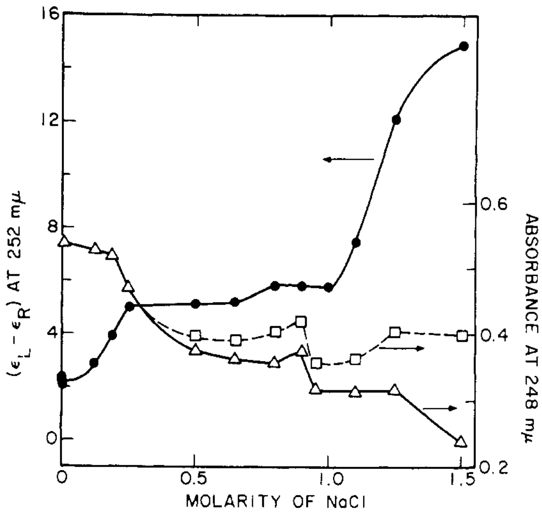

FIGURE 13: Dependence of CD and absorption of poly(I) on salt concentration. CD measurements (○) were made at 2.5°; absorption spectra with (△) and without (□) a scattering correction (made by linear extrapolation of scattering optical density into the absorption region) were measured at room temperature; in 0.001 M cacodylate (pH 7.0).

spectra (Figures 13 and 14); but since the measurements were made at lower temperature, the profile was shifted to lower salt concentrations. It was also seen that the higher order aggregation at high-salt concentrations was accompanied by a large increase in CD. This may represent the packing of individual triple-stranded helices into larger ordered arrays.

Neither magnesium ion nor polylysine ($\overline{\text{DP}}$ = 26) on direct mixing was effective in converting poly(I) to any of its ordered forms. A small amount of hypochromism was seen in both cases, but CD spectra of the samples were very little altered compared to poly(I) at low ionic strength. Precipitation without enhancement of optical activity occurred by 10^{-2} M $MgCl_2$ and at electrostatic equilvalence in the case of polylysine. Salt gradient annealed poly(I)–polylysine complexes having lysine:phosphate = 0.50 showed a CD spectrum similar in shape to that of poly(I) in 0.5 M NaCl, but differing by being smaller in magnitude and shifted to longer wavelengths (Figure 14). Thus, although an ordered form of poly(I) existed at the initial salt concentration of the gradient (1.50 M NaCl), its existence was not preserved by the presence of polylysine. Precipitates with reduced magnitude spectra were observed in annealed mixtures having lysine:phosphate = 0.75 and 1.00.

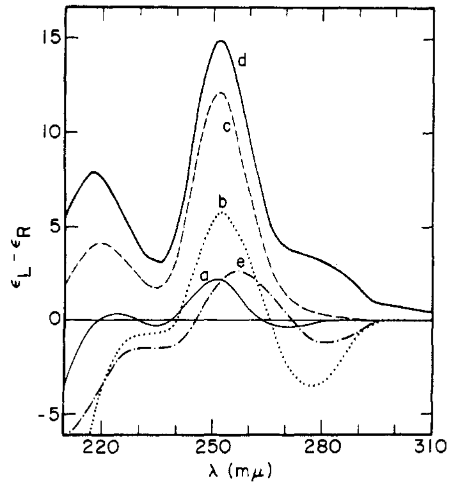

FIGURE 14: CD spectra of poly(I) at several salt concentrations: (a) 0.005 M, (b) 0.80 M, (c) 1.25 M, (d) 1.50 M NaCl; and in an annealed complex with polylysine ($\overline{\text{DP}}$ = 26), (e) lysine: phosphate = 0.50, in buffer α. All spectra measured at 2.5°.

TABLE IV: Effect of Polynucleotide Structure on the CD of Polylysine–Polynucleotide Complexes.[a]

	$(\epsilon_l - \epsilon_r)_{max}$		
Polynucleotide	Alone	Polylysine Complex	Transformation?
	Single Stranded		
Poly(A)	24.2	9.7	No
Poly(C)	26.2	8.2	No
TMV RNA	8.3	1.0	No
	Double Stranded		
DNA	2.5	−28.0	Yes
$[\text{Poly(U)}]_2$	25.5	159	Yes
Poly(A)·poly(U)	12.9	58.8	Yes
Poly(I)·poly(C)	7.0	117	Yes
	Triple Stranded		
Poly(A)·2poly(U)	10.1	4.8	No
$[\text{Poly(I)}]_3$	2.2	2.7	No

[a] Summary of the effects of complex formation with polylysine on the CD spectra of a variety of polynucleotides. Except for poly(A) and $[\text{poly(U)}]_2$, salt gradient annealed complexes are referred to in each case. The entry in the right-handed column refers to whether or not the CD spectrum of the polynucleotide was transformed, by complexing with polylysine, to one of much greater magnitude.

Discussion

Poly(U)-Polylysine Complexes. The addition of polylysine to a solution of poly(U) clearly converts the polynucleotide to its double-stranded form. The ordered form is more stable in the presence of this highly charged polycation than in the presence of divalent metal ions or oligoamines (Szer, 1966a,b).

If the binding of polylysine to double-stranded poly(U) is of the type and stoichiometry envisaged for DNA–polylysine complexes (Tsuboi, 1967), it is not clear why the conversion to the ordered form is complete at such low lysine:phosphate ratios (Figures 3 and 5). Possibly the necessity of forming hairpin loops restricts the proportion of the uracil bases which can participate in double-stranded formation. Alternatively, the double-stranded sections may extend somewhat beyond the regions bound by polylysine. And it is conceivable, of course, that due to differences in polynucleotide geometry, this complex differs in its binding stoichiometry from DNA–polylysine complexes.

The aggregates formed near electroneutrality show greatly enhanced CD spectra, as is the case for annealed DNA–polylysine complexes. The fact that high molecular weight polylysines were relatively ineffective in producing the enhanced spectra in both of these situations (Figure 8 and Carroll, 1972) may reflect interference of intermolecular cross-linking with good order formation in the aggregates.

The redissolution of the aggregates at higher lysine:phosphate ratios is very likely due to binding of a greater than equivalent amount of polylysine to the poly(U). The excess positive charge would separate the complexes, just as they were kept apart by excess negative charge at lysine:phosphate ratios less than 0.9. This charge repulsion would also explain the lowered melting temperatures of the redissolved complexes.

Polyarginine and protamine were ineffective in producing either double-stranded structures or aggregates with enhanced CD when mixed with poly(U). This resembles the difference between these polymers and polylysine in complexes with DNA (Inoue and Ando, 1968, 1970; Carroll, 1972). If these differences are not simply the result of formation of the complexes under nonoptimal conditions for the arginine-containing polypeptides, they may be worth pursuing for their implications concerning nucleohistone structure.

Requirements for Ordered Aggregate Formation. Compilation of the results of the survey of a variety of polylysine–polynucleotide complexes (Table IV) demonstrates that it is only with fully double-stranded polynucleotides that aggregates with enhanced CD spectra are formed. It is only with such structures that polylysine can form regular, electroneutral complexes. The phosphates on a single polynucleotide chain are too far apart to permit binding of all the polylysine ϵ-amino groups without cross-linking of separate molecules or parts of the same molecule. This cross-linking must have a disorganizing effect on polynucleotide structure and on aggregates that are formed at electroneutrality. Although TMV RNA is about 50% double-stranded in buffer α at 2.5° and is capable of attaining a maximum of 70% double strandedness (McMullen *et al.*, 1967), the remaining single-stranded regions would necessarily be cross-linked by polylysine.

The same is true of the third strand of triple-stranded polynucleotides. After neutralization of two strands by polylysine molecules, binding to the third strand must involve cross-linking. It is possible that at higher salt concentrations, the third strand could be neutralized by monovalent cations, thereby allowing ordered aggregates to form; and indeed, enhanced CD spectra have been reported for polylysine–poly(A)·2 poly(U) complexes (Haynes *et al.*, 1970). In addition, poly(I) triple helices appear to be able to aggregate in a specific fashion at high-salt concentrations in the absence of polylysine (Figures 13 and 14).

Enhanced CD spectra have been observed only when aggregation of the complexes has occurred and, except for the case of poly(U), only when the aggregates were formed by an annealing procedure. Under the conditions of complex formation, the poly(U) double helix is only about 30° below its melting temperature; so the annealing in this case may be within the polynucleotide structure itself, which is in register for all linear arrangements of the two strands.

Acknowledgments

I thank Professor I. Tinoco, Jr., for support during the period of this research; Drs. D. E. and A. L. Olins for communication of results in advance of publication; and them and Dr. Gary Felsenfeld for helpful discussions. I am grateful to Barbara Dengler for providing the sample of TMV RNA.

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