Optical Properties of Deoxyribonucleic Acid–Polylysine Complexes*

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ABSTRACT: In a mixture of DNA and polylysine at very high ionic strength (1.50 M NaCl) there appeared to be little or no interaction between the oppositely charged polymers. As the salt concentration was gradually lowered, in a linear dialysis gradient, aggregated complexes were formed which had a circular dichroism spectrum much larger in magnitude than and much different in shape from the sum of the spectra of the individual components. The region of salt concentrations in which the aggregate formation occurred varied with the molecular weight of the polylysine used and with the lysine:phosphate ratio in the mixture, but was generally in the range 0.7–1.0 M NaCl. Up to lysine:phosphate ratios around 0.75, addition of more polylysine led to the incorporation of more of the DNA into the aggregates with anomalous circular dichroism (CD) spectra. Beyond this point, although additional complex may still have been formed, the size of the aggregates became very large and the magnitude of the CD spectrum was reduced due to "shadowing" of the optically active material. It was found that very long polylysines ($\overline{DP}$ = 300, 500) formed aggregated complexes having lower magnitude CD spectra than those formed with intermediate sizes ($\overline{DP}$ = 26, 75). Annealed complexes of DNA with polyarginine and protamine had only slightly altered circular dichroism spectra, never approaching the magnitude of those characteristic of DNA–polylysine complexes.

Since synthetic poly-α-amino acids became readily available about 20 years ago (Katchalsky, 1951), these relatively simple polymers have been widely used as models for proteins. Poly-L-lysine has been of interest as a model for histones and protamines, two classes of naturally occurring basic proteins which are found in association with DNA. Investigators have been encouraged in pursuing the analogy with histones by the observations that basic residues are frequently clustered in the amino acid sequences of the histones (Smith *et al.*, 1970). In addition, polylysine shares with histones the ability to completely inhibit *in vitro* transcription of DNA (Shih and Bonner, 1970a,b) and a preference for precipitating (A + T)-rich DNA (Spitnik *et al.*, 1955; Leng and Felsenfeld, 1966; Brown and Watson, 1953; Crampton *et al.*, 1954; Lucy and Butler, 1955; Johns and Butler, 1964).

Investigations of physical properties of polylysine–DNA complexes have thus been undertaken in several laboratories, in the hope that uncovering details of their structure will provide insights into histone function. When complexes are formed by salt gradient dialysis, the binding of the polypeptide is cooperative and aggregates form (Olins, *et al.*, 1967; Cohen and Kidson, 1968; Shih and Bonner, 1970b). These aggregates have optical rotatory dispersion (ORD)[1] and circular dichroism (CD) spectra which are quite unlike the sum of the spectra of the separate polymers, both in shape and in magnitude (Cohen and Kidson, 1968; Shapiro *et al.*, 1969; Haynes *et al.*, 1970; Olins and Olins, 1971). It has recently been demonstrated that similar spectra can be obtained for aggregated DNA–lysine-rich histone complexes, formed under some conditions (Fasman *et al.*, 1970; Olins and Olins, 1971).

Reported here are the results of a study of the conditions necessary to formation of DNA–polylysine complexes with altered CD spectra, and the origin of the large-magnitude anomaly is discussed.

Experimental Section

Materials. Calf thymus DNA was purchased from Calbiochem (A grade) and was spooled once from ethanol before use. Stock solutions were dialyzed once against EDTA in buffer α, exhaustively against buffer α, and filtered through a medium porosity sintered-glass filter. Concentrations of DNA solutions were determined spectrophotometrically, assuming a molar extinction coefficient at 258 mμ of 6500 (Hirschman and Felsenfeld, 1966) at all salt concentrations from 0.005 to 3 M NaCl. DNA concentrations in solutions containing aggregated complexes were determined by dissolving the aggregates with an equal volume of 4 M NaCl–0.001 M cacodylate (pH 7.0) and measuring the absorption spectra of the resulting, nonscattering solutions.

Penta-α-L-lysine hexaacetate was purchased from Fox Chemical Co. It was shown to give a single ninhydrin-positive spot on thin-layer chromatography in 1-butanol–acetic acid–water–pyridine (30:6:24:20, v/v) (Waley and Watson, 1953) and to be the pentamer by chromatography of partial acid hydrolysates. Samples of poly-L-lysine·HBr of several molecular weights were obtained from the following sources: $\overline{DP}$ = 26 (lot LY98) and $\overline{DP}$ = 300 (lot LY104) from Miles Research Products; $\overline{MW}$ = 10,000–20,000 ($\overline{DP}$ = 75) (lot F1621) from Fox Chemical Co.; and $\overline{MW}$ = 105,000 ($\overline{DP}$ = 500) (lot L-67) from Pilot Chemical Co. Stock solutions of these polymers were dialyzed exhaustively against buffer α and filtered through washed Millipore filters (0.45 μ pore size). On dialysis, a moderate amount of (low molecular

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[1] Abbreviations used which are not listed in *Biochemistry*, 5, 1445 (1966), are: ORD, optical rotatory dispersion; CD, circular dichroism; $\overline{DP}$, average degree of polymerization; MW, molecular weight; OD, optical density.

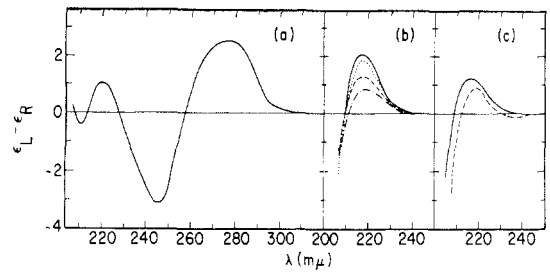

FIGURE 1: CD spectra of individual polymers, in buffer α, 2.5°. (a) DNA; (b) poly-L-lysines of various molecular weights: - · - · - · -, $\overline{DP}$ = 5; - - - -, $\overline{DP}$ = 26; · · · · · · ·, $\overline{DP}$ = 75; ——, $\overline{DP}$ = 500; (c) ——, poly-L-arginine ($\overline{DP}$ = 70) and - - - -, protamine.

weight) material was lost from the $\overline{DP}$ = 26 samples,[2] so the working DP was probably somewhat higher. Poly-L-arginine·HCl (Miles, lot AR39, $\overline{DP}$ = 70) solutions were prepared in the same manner. Salmon protamine sulfate was a product of Merck, Sharpe and Dohme Research Laboratories and was kindly provided by Dr. W. R. Carroll, who has characterized the material (Callanan *et al.*, 1957). Protamine chloride was prepared from a water solution of the sulfate (Carroll *et al.*, 1959) by passing it through Dowex 1-X2 in the chloride form. The protamine chloride was lyophilized and dissolved in buffer α. Polypeptide concentrations were determined, after hydrolysis for 48 hr in 6 N HCl, using the ninhydrin method (Moore and Stein, 1948), by comparison to L-lysine·HCl or L-arginine·HCl standards. For protamine solutions, the relative color yields for the various amino acids taken from Moore and Stein (1948) and the amino acid composition from Callanan *et al.* (1957) were used in calculation of the concentration by comparison to an L-arginine standard. Polypeptide solutions were handled with and stored in plastic ware to as great an extent as possible (Nevo *et al.*, 1955).

Ordinary cellulose dialysis tubing was boiled for half an hour in 5% $NaHCO_3$ plus a pinch of EDTA, then once or twice in distilled water, the last time immediately before use. Common chemicals were reagent grade; solutions were prepared in water redistilled in a glass still. Buffer α is 0.005 M NaCl–0.001 M sodium cacodylate (pH 7.0).

Gradient Dialysis. Samples were prepared for gradient dialysis by mixing in the desired proportions and in the order given: 4 M NaCl–0.001 M cacodylate (pH 7.0), DNA in buffer α, polypeptide in buffer α, and buffer α to the desired final volume. Final concentrations in these mixtures were: NaCl, 1.50 M; DNA, 6.8×10^{-5} M, as nucleotide. The lysine:phosphate ratios reported are those in the original mixtures before dialysis. In fact, the small decreases (10–15%) in DNA concentration in these samples can be largely accounted for by dilution up to lysine:phosphate ratios of 0.8. Beyond this point, larger aggregates formed and losses often became substantial; and it is difficult to assure oneself that the remaining material was representative of the total.

Exploratory work with dialysis gradients disclosed the following: (1) with stepwise gradients (Huang *et al.*, 1964; Olins *et al.*, 1967; Cohen and Kidson, 1968), the magnitude of the CD spectra of the resulting complexes was a function of the number of steps between 2.0 and 0.005 M NaCl, being larger for more gradual gradients; (2) linear and exponential continuous flow gradients (Carroll, 1971) gave very similar results, the CD spectral magnitudes being slightly larger for complexes formed in a linear gradient; (3) in continuous flow linear gradients, starting at 1.5 M rather than 2.0 M NaCl did not affect the results; (4) continuous flow linear gradients developed over periods of 8 and 24 hr gave identical results. Thus a continuous flow linear gradient from 1.50 to 0.15 M NaCl, developed over 8–8.5 hr at 4°, was routinely employed. The samples were subsequently dialysed for two periods of at least 4 hr against 100 volumes of buffer α. When determining the effect of salt concentration in the gradient on the properties of the complexes, the salt concentrations of samples removed at different points in the gradient were measured by refractometry.

Spectrometry. Absorption spectra were measured using a Cary Model 14 or Model 15 spectrophotometer. Circular dichroism spectra were measured with a Cary Model 60 spectropolarimeter, equipped with the Model 6001 circular dichroism accessory. Small volume cylindrical quartz cells of 1- and 2-cm path length were employed. Data were smoothed and reduced with the aid of a PDP 8/S computer (Digital Equipment Corp.) and programs written by Drs. B. L. Tomlinson and M. S. Itzkowitz (Tomlinson, 1968). The temperature of the samples was controlled and maintained in an electronically thermostatted cell holder designed by Dr. D. M. Gray. Unless otherwise indicated, CD spectra were measured at 2.5° and absorption spectra at room temperature. Circular dichroism is expressed as $\epsilon_l - \epsilon_r = \theta/33(lc)$, where θ is the measured ellipticity in degrees, l the path length in centimeters, and c the molar concentration. For polymers it is reported per mole of monomer; and for DNA–polypeptide complexes it is given per mole of nucleotide.

Results

For purposes of comparison, the CD spectra of the individual polymers used in this study are presented in Figure 1. Only DNA shows spectral features of any magnitude above 240 mμ; the 218-mμ peak in the spectrum of polylysine is seen to increase in magnitude with increasing chain length.

Effect of Dialysis Gradient on the Complexes. Direct mixing of dilute solutions of polylysine and DNA at low ionic strength, with vigorous stirring and at lysine:phosphate ratios less than 1.0, resulted in the formation of complexes with both absorption and CD spectra very little altered compared to the sum of the component spectra. This is consistent with the observations of Tsuboi *et al.* (1966) and indicates that the simple association of the polymers causes no drastic alteration in the structure of DNA. Near electrostatic equivalence, precipitation occurred which was not similar to the aggregation found on annealing, as it resulted in a large decrease in magnitude of the CD spectrum. With some polylysine samples, the spectra of these precipitates bore a vague resemblance in shape to those of annealed aggregates, but were much smaller in magnitude. In agreement with Shapiro *et al.* (1969), it was found that direct mixing in salt concentrations in the range 0.7 –1.0 M NaCl did not lead to complexes with anomalous CD spectra.

The effect of salt concentration in a linear dialysis gradient on a polylysine–DNA mixture is shown in Figures 2 and 3. Initially, the CD spectrum was characteristic of DNA at high salt concentrations (Permogorov *et al.*, 1970). As the salt concentration was lowered, both absorption and CD

[2] Up to 40% of the polylysine was lost from this sample on dialysis. That low molecular weight material was lost was deduced from the disappearance of material which moved away from the origin on thin-layer chromatography. All $\overline{DP}$'s given are those supplied by the manufacturers.

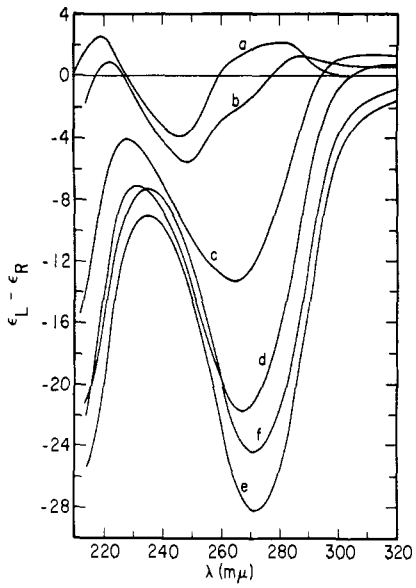

FIGURE 2: CD spectra of DNA–polylysine mixtures in a linear dialysis gradient. Polylysine $\overline{DP}$ = 75, lysine:phosphate = 0.75, 2.5°; NaCl = (a) 1.06 M, (b) 0.94 M, (c) 0.84 M, (d) 0.76 M, (e) 0.50 M, and (f) 0.007 M.

spectra were converted to those characteristic of the annealed aggregates, the transition occurring between 1.0 and 0.7 M NaCl. The minimum in $\epsilon_l - \epsilon_r$ and the maximum in $A_{260\ m\mu}$ represent not a solubility minimum as is observed for chromatin (Oth and Desreux, 1957; Ohlenbusch *et al.*, 1967; Jensen and Chalkley, 1968), but a balance between order in the aggregate particles and particle size. Below 0.4 M NaCl, the extrema in both absorption and CD spectra became broader and were shifted to longer wavelengths, indicating "shadowing" (Duysens, 1956; Urry and Ji, 1968) of the absorbing, optically active complexes in large particles of increasing size. Very similar results were obtained with all polylysine samples, the midpoint of the transition occurring at slightly higher salt concentration for greater lysine:phosphate ratios and for polylysines of larger molecular weight.

Dependence of CD Spectra on Lysine:Phosphate Ratio and Polylysine Molecular Weight. As greater amounts of polylysine were included in the linear gradient dialyzed mixtures, a larger portion of the DNA circular dichroism spectrum was converted to that characteristic of the aggregated complexes (Figure 4). This was paralled by changes in the absorption

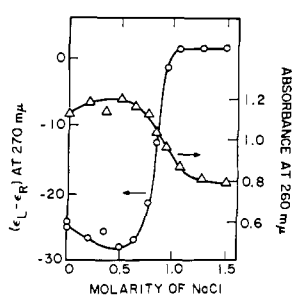

FIGURE 3: Dependence of absorption and CD of a DNA–polylysine mixture on salt concentration in a linear dialysis gradient. Polylysine $\overline{DP}$ = 75, lysine:phosphate = 0.75. Absorption spectra were measured at room temperature in 2-cm path length and are normalized to the same DNA concentration. CD spectra were measured at 2.5°.

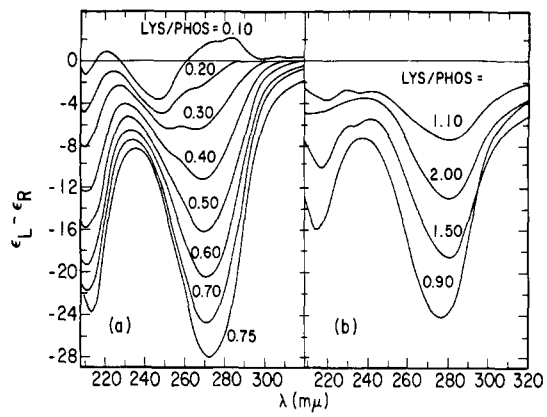

FIGURE 4: CD spectra of annealed DNA–polylysine ($\overline{DP}$ = 75) complexes at several lysine:phosphate ratios, in buffer α, 2.5°. (a) Lysine:phosphate = <0.8, (b) lysine:phosphate = >0.8.

spectrum (Figure 5). The effectiveness of different polylysine samples in producing the anomalous spectrum was a function of their molecular weights, larger polylysines ($\overline{DP}$ = 300, 500) being rather ineffective (Figure 6).

Focusing on the case of the most effective polylysine ($\overline{DP}$ = 75) (Figures 4 and 6), the cooperative nature of aggregate formation was reflected in the less rapid decrease in $\epsilon_l - \epsilon_r$ at very low lysine:phosphate ratios. Beyond this, the magnitude of the CD at 270 mμ increased approximately linearly with increasing polylysine concentration, until a maximum was reached at about lysine:phosphate = 0.75. The flattening of extrema and red shifts in both absorption and CD spectra indicate that the decrease in magnitude at ratios greater than 0.75 represents "shadowing" (Duysens, 1956; Urry and Ji, 1968) due to increasing size of the aggregates, not a loss of structure. Indeed, in some solutions having lysine:phosphate $\geq$ 1.0, individual particles were large enough to be distinguished by the naked eye. Spectra of samples in this range of lysine:phosphate were also quite variable, as might be expected where such a high level of unregulated aggregation occurs.

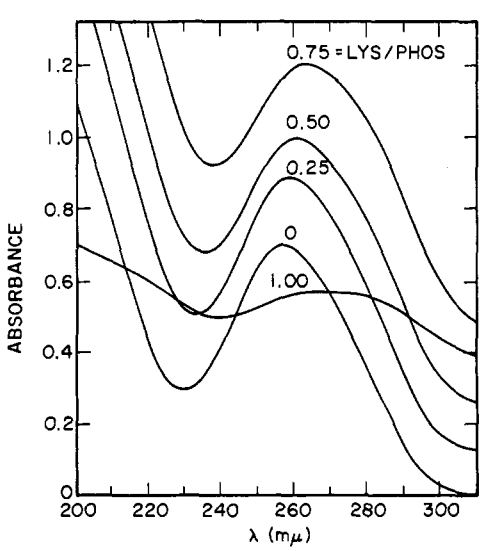

FIGURE 5: Absorption spectra of annealed DNA–polylysine ($\overline{DP}$ = 75) complexes at several lysine:phosphate ratios; in buffer α, room temperature, 2-cm path length.

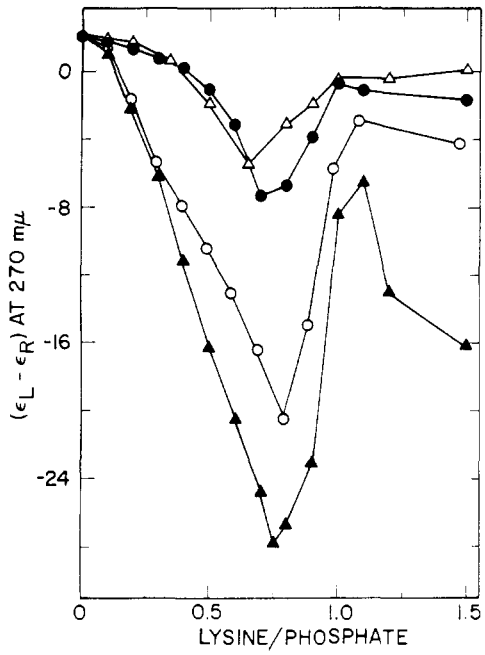

FIGURE 6: Dependence of CD at 270 mμ of annealed complexes on lysine:phosphate ratio for polylysines of various molecular weights: and (○) $\overline{DP}$ = 26, (▲) $\overline{DP}$ = 75, (●) $\overline{DP}$ = 300 (△) $\overline{DP}$ = 500. In buffer α, 2.5°.

Some Other Properties of Annealed Complexes. In general, the CD of the annealed complexes did not return to zero outside the DNA absorption region. The spectrum of a DNA–polylsyine ($\overline{DP}$ = 75) sample having lysine:phosphate = 0.75 crossed the base line at about 365 mμ and remained positive and nearly flat, with a magnitude around $\epsilon_l - \epsilon_r = 3$, to wavelengths as high as 500 mμ. Quite possibly this represents unequal scattering of right and left circularly polarized light (Urry and Krivacic, 1970) and is related to the structure of the aggregates.

When the aggregates were removed from suspension by sedimentation at 28,000*g* for 30 min, the supernatant solutions showed absorption and CD spectra characteristic of free DNA. In mixtures containing less than an equivalent amount of polylysine, the distribution of DNA between pellet and supernatant was consistent with previous findings (Leng and Felsenfeld, 1966; Shapiro *et al.*, 1969) that the aggregates contain all of the added polypeptide and, at low salt concentrations, a slightly greater than equivalent amount of DNA. The aggregates were easily resuspended in buffer α

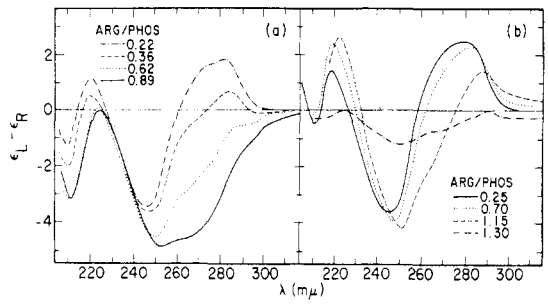

FIGURE 7: CD spectra of annealed (a) DNA–protamine and (b) DNA–polyarginine complexes at several arginine:phosphate ratios, in buffer α, 2.5°.

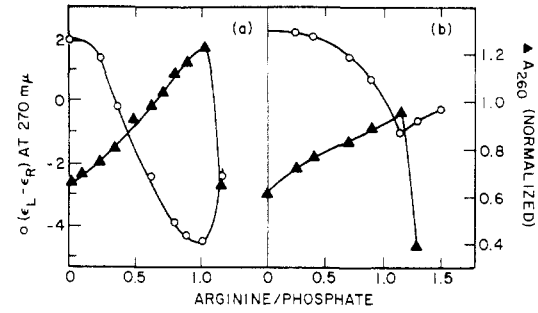

FIGURE 8: Dependence of absorption and CD of annealed (a) DNA–protamine and (b) DNA–polyarginine complexes on arginine:phosphate ratio; in buffer α, CD at 2.5°, A_{260} at room temperature in 2-cm path length.

and shown to possess the characteristic CD spectrum. There was no evidence for further aggregation on centrifugation, as the average of pellet plus supernatant spectra very nicely reproduced the spectrum of the original sample.

Increasing the DNA concentration in the annealing mixture to 2.4 × 10^{-4} M (from the usual 6.8 × 10^{-5} M) did not alter the CD spectrum of the resulting complexes. The magnitude of the CD spectrum of the aggregates was decreased slightly but reversibly by heating to 80°; no sharp melting was observed. The melting of DNA in such complexes occurs at approximately 100° (Olins *et al.*, 1967).

Complexes of DNA with Protamine and Polyarginine. CD spectra of salt gradient annealed DNA–protamine complexes are displayed in Figure 7a. While there is in these spectra a negative band near 270 mμ which increases in magnitude with increasing arginine:phosphate ratio and this is accompanied by increasing scattering in the solutions (Figure 8a), the magnitude of the CD never approaches that attained by polylysine–DNA aggregates. It might be thought that the nonbasic amino acids in the protamine sequence interfere with good order formation; however, polyarginine also failed to produce the large-magnitude CD on complexing with DNA (Figures 7b and 8b).

Discussion

The work reported here confirms that salt gradient annealed mixtures of DNA and polylysine form aggregated complexes with dramatically altered optical activity spectra (Cohen and Kidson, 1968; Shapiro *et al.*, 1969; Haynes *et al.*, 1970; Olins and Olins, 1971). Such spectral changes can be achieved to some extent on direct mixing of solutions of highly sonicated DNA and polylysine at lysine:phosphate ratios near unity (Inoue and Ando, 1968, 1970). Detailed comparison of spectra of the complexes obtained in different laboratories is of little value because of the large dependence of their magnitude on lysine:phosphate ratio, the steepness of the salt gradient used in their formation, the salt concentration at which measurement is made and the molecular weight of the polylysine. However, consistent use of a continuous flow linear salt gradient dialysis procedure has made it possible to reproduce CD spectra of complexes within 5–10% at all wavelengths over a considerable period of time.

A plausible description of the formation of DNA–polylysine aggregates is as follows. Initially separated at high salt concentrations, the DNA and polylysine molecules begin to associate as the ionic strength is lowered. The association is of the type described by Wilkins (1956) for protamine–DNA

complexes, with the extended polypeptide occupying one groove of the double helix. In a range where bound and unbound polylysine are in equilibrium with one another, polylysine molecules bind cooperatively to DNA; this cooperativity may be due to slight structural alterations in the DNA which extend beyond the actual region to which the polypeptide is bound, or perhaps to the subsequent formation of a separate coacervate phase. The electrostatically neutral DNA–polylysine complexes aggregate and pack themselves into the aggregates in such a manner as to give rise to the characteristic CD spectrum. At lysine : phosphate ratios below unity, the size of the particles is limited by a small residual negative charge due to a slight excess of DNA in the complexes; but as this is neutralized near lysine : phosphate = 1.0, the particle size becomes much larger.

The appearance of the large-magnitude CD spectrum, in complexes of polylysine with DNA and other polynucleotides (Haynes *et al.*, 1970; Carroll, 1972), is invariably accompanied by turbidity; while formation of complexes by direct mixing under conditions in which no aggregation occurs leads to little or no enhancement of CD. In two systems where large-magnitude CD spectra, very similar to those for annealed DNA–polylysine complexes, have been reported for DNA in the absence of polylysine—*i.e.*, in concentrated DNA films (Tunis-Schneider and Maestre, 1970) and in the presence of polyethylene oxide (Lerman *et al.*, 1970)—aggregation is also observed.

As discussed by previous workers (Shapiro *et al.*, 1969; Haynes *et al.*, 1970), there are two general ways in which inclusion in the aggregates might lead to the characteristic CD. First, individual polylysine–DNA complexes could be closely packed and ordered in an asymmetric fashion. Possibly the inability of high molecular weight polylysines to produce the large-magnitude spectra (Figure 6) is due to cross-linking which would restrict the mutual spacial orientation of the complexes.

Alternatively, a dramatic alteration in the secondary structure of DNA may occur in the environment provided by the aggregate, possibly due to exclusion of solvent. This would have to be more drastic than that accompanying supercoiling of DNA, which does lead to measurable but small changes in CD spectrum (Maestre and Wang, 1971). Neither do the spectral changes observed on complex formation correspond to a transition from B- to A- or C-form double helices (Tunis-Schneider and Maestre, 1970). Haynes *et al.* (1970) have reported that DNA retains its B form in the aggregates; however, it is difficult to be quantitative in statements of this sort on the basis of X-ray diffraction data. It is possible that the DNA in the aggregates is partitioned between two forms: a portion of it being highly distorted, giving rise to the anomalous spectrum, the remainder being undistorted and displaying the familiar reflections. Indeed, at low salt concentrations, the amount of DNA included in the complexes is somewhat in excess of the amount polylysine added (Shapiro *et al.*, 1969; this work).

Under conditions of moderate ionic strength, salt gradient annealed DNA–f1 histone complexes can also give rise to large-magnitude CD spectra (Fasman *et al.*, 1970; Olins and Olins, 1971). However, there is no clear relevance of the structures responsible for the large-magnitude CD for biological control mechanisms, since such spectra are not observed for isolated chromatin (Oriel, 1966; Tuan and Bonner, 1969; Permogorov *et al.*, 1970; Shih and Fasman, 1970; Simpson and Sober, 1970), nuclei (D. E. Olins, personal communication), or metaphase chromosomes (Cantor and Hearst, 1970). The chromosomes do share with DNA–polylysine aggregates a non-zero CD at wavelengths above 330 mμ (K. P. Cantor, personal communication), possibly due in both cases to asymmetric scattering (Urry and Krivacic, 1970).

Annealed complexes of protamine and polyarginine with DNA show much less dramatically altered CD spectra than are characteristic of DNA–polylysine complexes. This has also been observed in directly mixed complexes by Inoue and Ando (1968, 1970); and arginine-rich histones have been shown to produce less enhanced CD spectra than do lysine-rich histones (Olins and Olins, 1971; Shih and Fasman, 1971). This reason for difference between lysine- and arginine-containing polypeptides may lie either in their modes of binding to DNA or in the aggregation properties of the complexes once formed.

Protamine and polyarginine do appear to be quite similar in their effects on DNA, suggesting that this analogy may be a fruitful one to pursue. The primary functions of protamines, in their replacement of histones during spermatogenesis, appear to be a complete shutoff of transcription and compacting of DNA for packaging into a sperm head (Dixon and Smith, 1968). No high degree of control is necessary in these roles, and it is not inconceivable that an essentially polyarginine-like binding would achieve the purpose.

Acknowledgments

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Complexes of Polylysine with Polyuridylic Acid and Other Polynucleotides*

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