Deoxyribonucleic Acid–Polylysine Complexes. Structure and Nucleotide Specificity*

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ABSTRACT: The reversible interaction of polylysine with deoxyribonucleic acid has been examined. It is found that under appropriate conditions the reaction is stoichiometric (one lysine residue per nucleotide), cooperative, and with deoxyribonucleic acid samples heterogeneous in base composition, almost completely selective for the A·T-rich fraction. Although preference for A·T-rich deoxyribonucleic acid persists under a variety of conditions, it can be reversed in the presence of tetramethylammonium ion, resulting in selective interaction of polylysine with G·C-rich deoxyribonucleic acid. Under conditions of reversible and selective complex formation, the polylysine–deoxyribonucleic acid complex exists in a separate aggregated phase. The properties of this phase have been studied by sedimentation velocity and light-scattering methods. The particles containing the complex are highly solvated and remarkably uniform in size with an average radius of about 1700 Å, varying little with the size of deoxyribonucleic acid used in the molecular weight range 10^7–1.2×10^8. The optical rotatory properties of the complex are unusual. Under optimal conditions, the observed residue rotations in the region 250–290 nm are between 50 and 100 times as large as those observed for deoxyribonucleic acid alone, and the observed circular dichroism and optical rotatory dispersion curves are quite different from those of deoxyribonucleic acid. Similar results are obtained whether poly-D-lysine or poly-L-lysine is used in forming the complex. It appears likely that these changes arise either from a perturbation of the deoxyribonucleic acid secondary structure which accompanies complex formation, or from the formation within the particle of a structure with long-range order.

When poly-L-lysine is added to DNA in 0.9–1 M NaCl, a separate aggregated phase is formed that can be centrifuged out of solution. This phase contains both polylysine and DNA in a constant ratio of lysine residue to nucleotide (1:1). If the DNA is heterogeneous in base composition and present in excess, then the DNA found in the aggregate is the fraction richest in A·T pairs (Leng and Felsenfeld, 1966). This ability of polylysine to precipitate the A·T-rich fraction selectivity is manifested not only in preparations of mammalian DNA, but also in mixtures of bacterial DNAs differing in base composition, and in mixtures of bacterial DNA with synthetic polymers containing only dA:T base pairs. The selective interaction also can be detected by studying the effect of polylysine upon the thermal denaturation of DNA (Olins *et al.*, 1967).

It has been pointed out earlier (Tsuboi *et al.*, 1966; Raukas, 1965; Leng and Felsenfeld, 1966; Olins and von Hippel, 1966) that the reaction of polylysine with DNA in 1 M NaCl is a cooperative process: when 0.5 equiv of lysine is added per equiv of nucleotide, half of the DNA and all of the polylysine are found in the aggregated phase. It has also been shown that the formation of the separate phase is a reversible reaction: radioactively labeled DNA in this phase can exchange freely with DNA in the external solution (Leng and Felsenfeld, 1966).

The purpose of this paper is to examine the polylysine–DNA interaction in detail and to try to answer some of the questions raised by the earlier work. We have studied how the selectivity of polylysine for A·T-rich DNA is altered by variation in solvent conditions, polymer chain length, and the stereochemistry of the polylysine chain. We will show that under optimal conditions the selective process results in almost complete exclusion from the aggregated phase of DNA richer in G·C pairs. All of the results can be explained in terms of a well-ordered, reversibly formed complex between polylysine and DNA. Since the formation of an aggregated phase is sometimes associated with irreversible processes, and the reversible behavior might therefore seem to be inconsistent with formation of aggregates, we have studied the physical properties of the aggregated phase in some detail. We will show that the DNA–polylysine complex tends to form highly hydrated micelle-like particles of remarkably uniform size, with an average radius of 1700 Å estimated from light-scattering data. The large amount of solvent included in this particle probably accounts for the ability of components of the complex to enter and leave the particle rapidly. We have also examined the optical rotatory properties of the complex. When polylysine–DNA complexes are formed under optimal conditions, we observe residue rotations between 50 and 100 times as large in magnitude as those observed in DNA in the region 250–290 nm. The rotatory changes probably arise either from a perturbation in the secondary structure of the DNA which accompanies the formation of the complex or from the presence within the large particles of a structure with long-range order.

Materials and Methods

Materials

DNA from bacterial sources was prepared according to previously described methods (Leng and Felsenfeld, 1966) modified as described in the following paper (Shapiro *et al.*,

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TABLE I: Types of DNA Used.

Source	Abbreviation	% A·T
Bacterial		
P. mirabilis	PM	62
E. coli	EC	50
S. marcescens	SM	42
M. lysodeikticus	ML	28
Mammalian		
Calf thymus	CT	
Phage		
PBS-2[a]	PBS-2	72[b]
T4	T4	65

[a] This DNA has all the thymidylic acid replaced by deoxyuridylic acid. [b] Indicates A–dU composition (see text).

1969). *Proteus mirabilis* DNA and *Serratia marcescens* DNA labeled with ^{32}P and *S. marcescens* DNA labeled with ^{14}C were prepared by techniques described by Leng and Felsenfeld (1966). CT DNA was obtained from CalBiochem and Sigma Chemical Co. and purified by treatment with pronase (0.1 mg/ml), chloroform–octanol, and phenol extraction, and ethanol and isopropyl alcohol precipitation, in a manner similar to that described for bacterial DNAs. DNA from the *Bacillus subtilis* transducing phage PBS-2 (Takahashi and Marmur, 1963) was given to us by Dr. Julius Marmur. Median sedimentation coefficient values in 0.15 M NaCl–0.015 M sodium citrate for all the bacterial DNAs were 19–22 S units, corresponding to a molecular weight of about 1×10^7 (Eigner and Doty, 1965). Sonicated DNA had a molecular weight of about 5×10^5.

Table I shows all of the DNAs used in the experiments discussed in this paper, their base composition, and the abbreviations used in referring to them. Unless specifically mentioned, all of the bacterial DNAs have a molecular weight of about ten million. The T4 phage DNA had a size of about 1.2×10^8 and was a gift of Dr. Philip Ross.

Poly-L-lysine hydrochloride and hydrobromide came from New England Nuclear Co. and Pilot Chemical Co.; for the different samples used, the average degrees of polymerization, $\overline{DP}$, were approximately 16, 50, 100, and 300, determined by viscosity measurements on the blocked precursors reported by the manufacturers or by sedimentation equilibrium in our laboratory. The samples of $\overline{DP} \sim 100$–300 behaved identically in the titration experiments described below. Poly-D-lysine hydrochloride with $\overline{DP}$ between 40 and 70 was obtained from Mann Chemical Co. Poly-DL-lysine hydrobromide (a copolymer of the L and D monomers) was obtained from New England Nuclear Co., Yeda; from sedimentation equilibrium experiments its $\overline{DP}$ was about 50. The concentrations of polypeptide solutions used were determined by nitrogen analysis performed by the National Institutes of Health analytical laboratory.

N-α-Acetyl-L-lysine methyl ester was obtained from Cyclo Chemicals Co. Thin-layer chromatography in butanol–acetic acid–H_2O (80:20:20, v/v) and in methanol–chloroform 75:25, v/v) showed only one spot with R_F 0.329 and 0.507, respectively; lysine in these systems has R_F 0.03 and 0.06, respectively.

Tetra-L-lysine hydrochloride was obtained from Cyclo Chemical Co. ^3H-labeled tetra-L-lysine was synthesized by condensing trilysine made by Dr. C. B. Anfinsen, with the lysine derivative [^3H]*N*-α-(*t*-butyloxycarbonyl)*N*-ε-trifluoroacetyl-L-lysine[1] prepared in this laboratory. The solid-phase system described by Merrifield (1965) was used. We are indebted to Dr. Anfinsen for providing technical advice and equipment. Nonradioactive *N*-α-(*t*-butyloxycarbonyl)*N*-ε-trifluoroacetyl)-L-lysine was obtained from Cyclo Chemical Co. and used as a standard for testing that the radioactive (*t*-butyloxycarbonyl)trifluoroacetyllysine preparation was successful. The [^3H]tetralysine hydrobromide was chromatographed by Dr. H. A. Sober and found to be 95% tetramer. The concentrations of [^3H]tetralysine solutions used were determined by the biuret reaction, using commercial tetralysine to prepare the standard curves.

The quaternary amine salts used in the precipitation experiments described below came mostly from Eastman Organic Chemicals Co. and were purified by recrystallization before use.

Actinomycin D was a gift of Merck Sharpe and Dohme Research Laboratories.

Methods

Precipitation and Competition. In precipitation experiments, polylysine was added in varying amounts to a series of tubes containing identical amounts of a single kind of DNA. After incubation at 25° for 10–30 min, the tubes were centrifuged at 25,000*g* for 25 min and the optical density or radioactivity of the supernatant was determined. The process of removing aggregates from solution by centrifugation will be referred to as "precipitation" throughout the text although the aggregate particles are usually small enough in size to remain in suspension for some days if not centrifuged. Polylysine solutions were always added to DNA solutions dropwise with continuous vigorous mixing on a "Vortex" agitator. The polylysine solution was usually about the same concentration (in monomoles per liter) as the DNA solution, and the solvents for both were identical in any given experiment. Final DNA concentrations varied between 2×10^{-5} and 5×10^{-4} M.

Competition experiments were performed in a manner similar to precipitation experiments, except that the DNA solutions consisted of a mixture of equal concentrations of DNA from two sources, differing in base composition. After addition of polylysine, incubation, and centrifugation, the supernatant was analyzed for the concentration of both DNA components. In some experiments both DNA samples were radioactively labeled (one with ^{14}C, the other with ^{32}P) and the concentrations were determined by two-channel liquid scintillation counting (see below). In other experiments only one of the two DNAs was labeled; their individual concentrations were determined by measuring the optical absorbance at 260 mμ and the radioactivity. All experiments were carried out at

[1] This derivative is prepared from L-[^3H]lysine, uniformly labeled. The trifluoroacetyl group protects the ε-amino group of lysine; the *t*-butyloxycarbonyl group reacts with active trilysine attached to the Merrifield resin.

room temperature (~25°), and all solutions contained 0.01 or 0.001 M cacodylate buffer (pH 7) besides the principal salt.

In other competition experiments, neither DNA sample was radioactive. The concentrations of the two kinds of DNA in the supernatant were determined by banding in CsCl in the analytical ultracentrifuge; the bands formed by the two DNAs were well separated, and their concentrations were determined from the areas of the bands. Small amounts of residual polylysine that might be present in the samples would have no effect upon the banding of the DNA, since polylysine is completely dissociated.

Spectral analyses were carried out in a Cary Model 14 spectrophotometer. Thermal denaturation experiments were done with a Zeiss spectrophotometer with a thermostatic cell holder. The methods of analysis for base composition and concentration have been described (Hirschman and Felsenfeld, 1966). Analytical centrifugation of DNA was carried out in a Spinco Model E ultracentrifuge with ultraviolet absorption optics. Early experiments were done with photographic recording; later, the Beckman scanner apparatus (Lamers *et al.*, 1963) was used. Interference optics were used for sedimentation equilibrium of polypeptide solutions.

Radioactivity of DNA and [^{3}H]tetralysine samples was determined by pipetting a known quantity of the solution onto a Whatman GF glass filter, drying, and counting in 2,5-diphenyloxazole–1,4-bis[2-(5-phenyloxazoly)]benzene–toluene scintillation fluid in a Nuclear-Chicago Mark I scintillation counter.

Light-scattering measurements were carried out in a light-scattering photometer (Wippler and Scheibling, 1954) manufactured by Sofica, Paris. Since the particles of DNA–polylysine complex were too large to permit direct clarification of the solutions by filtration or centrifugation, the following procedure was used: 18 ml of solvent was filtered through a Millipore filter (Grade HA) directly into a clean scattering cell. The scattering was measured. A small quantity (usually 1 ml or less) of unfiltered solution containing the polylysine–DNA complex was added, the solution was mixed using a small magnetic stirrer, and the scattering was measured again. Finally, 2 ml of 5 M NaCl, passed through a Millipore filter, was added to dissociate the complex and the scattering was measured a third time. The latter results were assumed to represent the background scattering and were subtracted from the scattering of the complex; the scattering from the complex was between three and ten times larger than the assumed background scattering.

Optical rotatory dispersion and *circular dichroism measurements* were carried out in a Cary Model 60 spectropolarimeter equipped for circular dichroism measurement. Except for experiments in which the cell path length was purposely varied, cells of 2.5-cm path length were used.

Samples for use in optical rotatory density–circular dichroism studies and in light scattering were made by mixing polylysine and DNA in 1.5 M NaCl, then dialyzing against a large volume of salt solution at the desired final concentration.

Turbidity. Direct measurements of the turbidity of solutions of particles of DNA–polylysine complex were made on the Cary Model 14 spectrophotometer in the wavelength region 4500–3600 Å, where DNA and polylysine separately do not absorb. The method of Doty and Steiner (1950) was used to analyze the results.

Turbid solutions of particles were prepared from mixtures of calf thymus DNA and poly-L-lysine $\overline{DP} \sim 50$ by dialysis into approximately 1 M NaCl. The concentration of residual DNA (DNA not complexed with poly-L-lysine in the particles) was determined by centrifuging to remove the particles and measuring the absorbance of the supernatant at 2600 Å. Although large excesses of polylysine were added, it was often not possible to eliminate the residual DNA completely under these conditions. This is probably the result of working at NaCl concentrations slightly higher than 1 M where the complex tends to be partly dissociated. Concentration of DNA in the particles was taken to be the original DNA concentration less the residual DNA. In view of previous results (Leng and Felsenfeld, 1966), it was reasonable to assume that in the particles polylysine was bound to DNA mole for mole; the total concentration of particles in milligrams per milliliter could then be calculated.

Flow birefringence experiments were done using a Rao flow birefringence instrument, described by Edsall *et al.* (1952). This model had two sizes of stationary inner cylinder, giving a gap of either 0.5 or 1.0 mm; the 0.5-mm gap was generally used. The solutions of DNA–polylysine complexes were prepared as described for turbidity measurements, except that the concentrations were in some cases higher. The speed of the rotating cylinder was determined with a stroboscope. Temperature was monitored continuously, and extinction angle and birefringence were measured at several speeds with both forward and reverse direction of rotation. Frequently the same solutions were used for both birefringence and turbidity measurements. Approximate particle concentration was monitored by the amount of scattering observed at 3200 Å. It was very difficult to control the size of the particles formed in a given solution at the high DNA concentrations necessary for these measurements. Examination of the particle solutions in the flow birefringence instrument was also difficult, since a solution containing many particles scattered so much light that very little could be observed.

Results

Precipitation and Competition. In earlier experiments (Leng and Felsenfeld, 1966), it was shown that addition of poly-L-lysine ($\overline{DP}$ 100) to DNA in 1 M NaCl results in precipitation of an equivalent of nucleotide for each equivalent of lysine residues added, and that all of the polylysine added is in the precipitate. The reaction is thus stoichiometric in two senses: a complex is formed in which one lysine residue combines with each nucleotide, and the precipitation reaction itself follows the same stoichiometry. If, under similar conditions, a mixture of PM DNA (62% A·T) and SM DNA (42% A·T) is titrated in a competition experiment (see Methods), the PM DNA is precipitated in considerable excess of the SM DNA during the first part of the titration.

We have extended the competition experiment to include DNA from other sources. In Table II are shown the results of a competition experiment in which polylysine ($\overline{DP}$ 300) has been added in varying amounts to three tubes containing a 1:1 mixture of ML DNA and EC DNA. The amount of each DNA remaining in the supernatant after centrifugation was determined by banding in a CsCl density gradient (see Methods). The results show that EC DNA is almost completely eliminated from solution, while the ML DNA is nearly unchanged in concentration. We have also carried out competition experi-

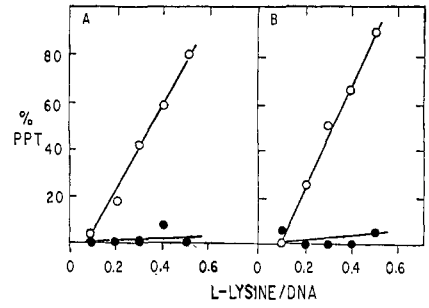

FIGURE 1: Selective precipitation of bacterial DNAs with poly-L-lysine. Per cent DNA precipitated is plotted *vs.* total lysine/DNA ratio (amino acid residues/nucleotide). $\overline{DP} \sim 300$ in 1.0 M NaCl. (A) (○) PM DNA and (●) ML DNA. (B) (○) PM DNA and (●) SM DNA.

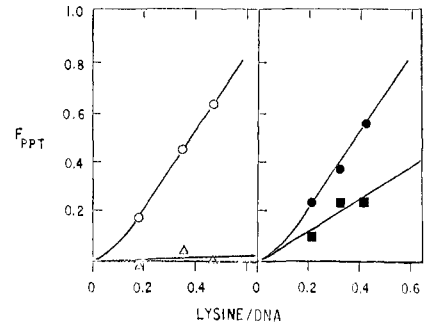

FIGURE 2: Fraction DNA precipitated *vs.* total lysine/DNA ratio. (a, left) ^{32}P-labeled PM DNA, sonicated (○) in competition with equal concentration of unlabeled ML DNA, sonicated (△). Total DNA concentration, 7.2×10^{-5} M. (b, right) ^{32}P-labeled PM DNA, sonicated (●) in competition with ^{14}C-labeled SM DNA, unsonicated (■). Total DNA concentration, 7.9×10^{-5} M. Solvent was 1 M NaCl.

ments with mixtures of ML DNA, with PM DNA, and of SM DNA with PM DNA. In both of these experiments one or both of the DNAs was radioactively labeled. Results are shown in Figure 1. The A·T-rich DNA shows a strong preferential interaction with polylysine in both cases.

The preferential interaction with A·T-rich DNA persists if both the competing DNAs are reduced to a molecular weight of about 5×10^5 by sonication. Figure 2a gives the results of a competition experiment between ML DNA and PM DNA, both sonicated. The curves are quite similar to those of Figure 1A for the same DNAs in the unsonicated form. In Figure 2b are given the results of an experiment in which only the A·T-rich DNA was sonicated. By comparison with Figure 1B, in which the equivalent pair of unsonicated DNAs (PM and SM) compete for polylysine, it is clear that the ability of PM DNA to compete effectively is somewhat reduced, but by no means abolished, by sonication of one of the two DNAs.

In an effort to understand what role electrostatic and solvent effects may play in the selective interaction of polylysine with A·T-rich DNA, we have examined the effects of varying salt and pH upon the selective properties. The salt series LiCl, NaCl, and CsCl was studied using poly-L-lysine of $\overline{DP} = 50$. A set of precipitation experiments (see Methods) was carried out for each salt to determine the salt concentration at which 1 equiv of lysine residues precipitated 1 equiv of nucleotides. Competition experiments were carried out at that salt concentration, because under those conditions, the electrostatic interactions are suppressed as much as possible without destroying completely the strong interaction between polyanion and polycation. At salt concentrations above the 1:1 precipitation point, the interaction is weakened sufficiently so that an excess of polylysine is required to drive the reaction between polylysine and DNA to completion. As the salt concentration is reduced below that necessary to give 1:1 precipitation, the ratio of lysine:nucleotide necessary to produce complete precipitation decreases slightly below 1:1, probably as a result of cross-linking which is "irreversible" at the lower ionic strength. The salt concentration at which 1 equiv of lysine residues precipitates 1 equiv of nucleotide is thus the optimal condition for competition experiments. Using polylysine of $\overline{DP} \sim 50$, we find that 1:1 precipitation occurs in 0.7 M LiCl, 0.9 M NaCl, or 1.1 M CsCl. Note that the NaCl concentration is slightly lower than the 1.0 M NaCl concentration required to give 1:1 precipitation with polylysine in the size range $\overline{DP}$ = 100–300. Poly-L-lysine ($\overline{DP} = 50$) preferentially interacts with A·T-rich DNA in all three of these salts, as determined by competition experiments similar to those described above. The competitive advantage of A·T-rich DNA is also preserved in sodium perchlorate (0.4–0.5 M).

High pH appears to improve the efficiency of selection. In 1 M Tris (pH 10.8) and also in 10^{-2} M NaCl–10^{-2} M lysine (pH 10.8), poly-L-lysine ($\overline{DP} = 100$) will precipitate most of the PM DNA in a mixture of PM and SM DNAs without removing detectable quantities of SM DNA.

The effect of TMA^+ upon selectivity is quite different from that of Na^+, Cs^+, or Li^+. In 2 M TMA-Cl, the appropriate concentration for 1:1 precipitation, polylysine selectively precipi-

TABLE II: Precipitation of Mixtures of ML and EC DNA by Poly-L-lysine.

Lysine/DNA (Monomoles)	Fraction of ML DNA Pptd	Fraction of EC DNA Pptd
0.50	0.06	1.00
0.25	0.06	0.51
0.00	0.00	0.00

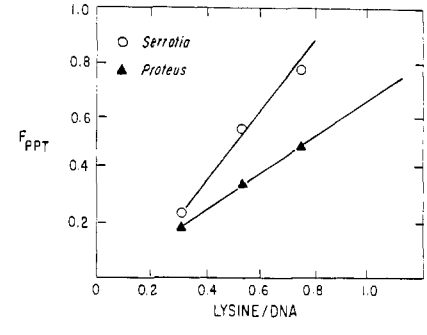

FIGURE 3: DNA competition in TMA^+. Total DNA concentration, 4.9×10^{-5} M. Fraction of DNA precipitated *vs.* total lysine/DNA ratio in 2 M TMA^+-Cl^-, ^{14}C-labeled SM DNA; ^{32}P-labeled PM DNA.

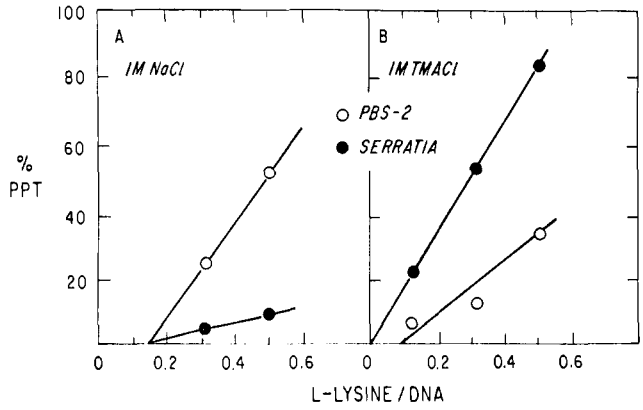

FIGURE 4: Selective precipitation of mixtures of PBS-2 DNA and SM DNA with poly-L-lysine $\overline{DP}$ 100 in 1 M NaCl and 1 M TMA-Cl. Total DNA concentration, 8.5×10^{-5} M.

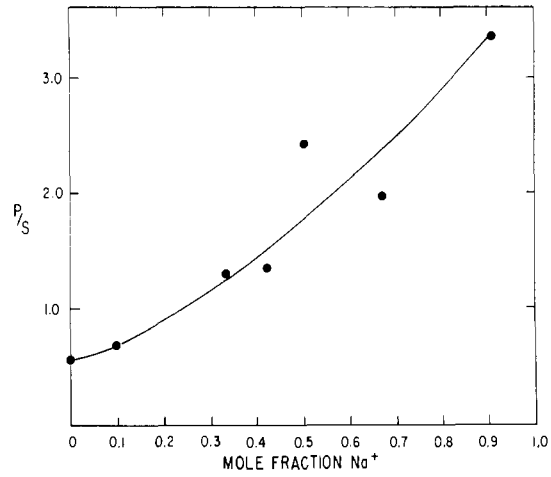

FIGURE 5: Ratio of ^{32}P-labeled PM DNA to ^{14}C-labeled SM DNA precipitated in mixtures of NaCl and TMA^+-Cl, as a function of the mole fraction of Na^+. Experimental details are described in the text.

tates G·C-rich DNA, thus reversing the behavior observed in all other salts we have studied. The results of a competition experiment are shown in Figure 3. A summary of results obtained with other tetraalkylammonium salts is given in Table III. The reversed selectivity is observed in the presence of all the quaternary amine salts having at least one methyl group, as well as with tetraethylammonium chloride. Tetrabutylammonium chloride and ethyltripropylammonium chloride do not behave in this way; in these salts A·T-rich DNA is precipitated in preference to G·C-rich DNA, just as it is in Na^+ Li^+, or Cs^+.

It seems possible that the selectivity of poly-L-lysine and its reversal in TMA-Cl might be due to a strong preferential interaction of the aliphatic lysine side chain and tetramethylammonium ion with the methyl group of thymine. To test this hypothesis we used DNA from the *B. subtilis* transducing phage PBS-2 in competition experiments. PBS-2 DNA has all of its thymidylic acid residues replaced by deoxyuridylic acid residues (Takahashi and Marmur, 1963). Mixtures of PBS-2 DNA (28% G·C) and SM DNA (58% G·C) were precipitated with poly-L-lysine; the results are shown in Figure 4. In 1 M NaCl, poly-L-lysine selects the PBS-2 DNA, while in 1 M TMA-Cl the selectivity is reversed as usual, with poly-L-lysine selecting the G·C-rich SM DNA. The substitution of dU for T thus has no effect on competitive behavior.

We have studied the effects of mixed Na^+–TMA^+ solvents upon selectivity. Precipitation experiments with solvents of fixed Na^+/TMA^+ ratio and varying ionic strength were used to determine the conditions necessary to obtain 1:1 precipitation. Competition experiments were carried out at this ionic strength. Since the graphs of DNA precipitated *vs.* lysine added in some cases were not linear, we used the point in competition experiments at which the ratio of the two kinds of

TABLE III: Effect of Quaternary Amine Salts on Selectivity of Poly-L-lysine for A·T-Rich DNA.

Salt[a]	PM DNA/SM DNA in Ppt[b]
NaCl (for reference)	4.7
Tetramethylammonium chloride (or bromide)	0.52
Trimethylphenylammonium chloride	0.47
Methyltriethylammonium chloride	0.42
Tetraethylammonium chloride	0.22
Ethyltripropylammonium nitrate	1.46
Tetrabutylammonium chloride	1.20
NaCl–50% methanol	1.07

[a] Concentration = 1 M for all salts except tetrabutylammonium chloride, which was 1.1 M. [b] Ratio calculated at added lysine:total DNA = 0.3. Poly-L-lysine used was $\overline{DP} \sim 100$.

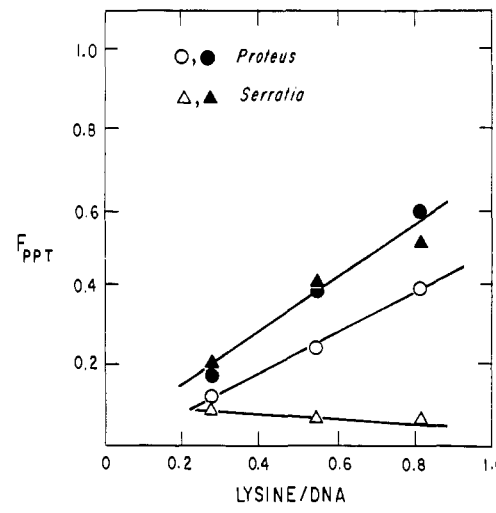

FIGURE 6: Effect of actinomycin D on selectivity. Fraction of DNA precipitated *vs.* total lysine/DNA ratio. O, Δ: PM, SM DNA in 1 M NaCl, ●, ▲: PM, SM DNA in 2 M TMA^+-Cl^-. Actinomycin concentration = 5.4×10^{-6} M, total DNA concentration = 8.5×10^{-5} M.

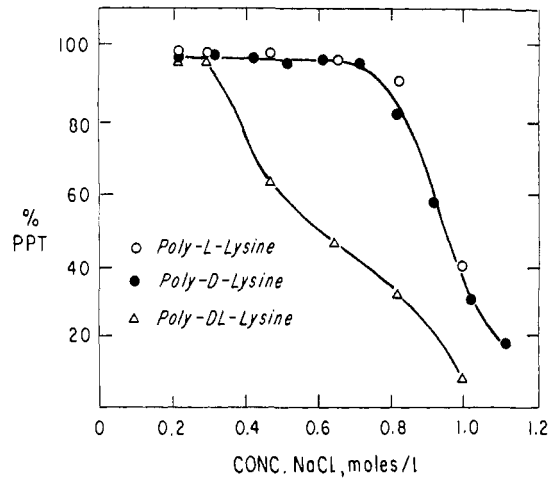

FIGURE 7: Precipitation of bacterial DNA with poly-L-lysine, poly-D-lysine, and poly-DL-lysine, all $\overline{DP} \sim 50$, in solvents of varying NaCl concentration. Lysine/DNA (monomoles) = 0.7.

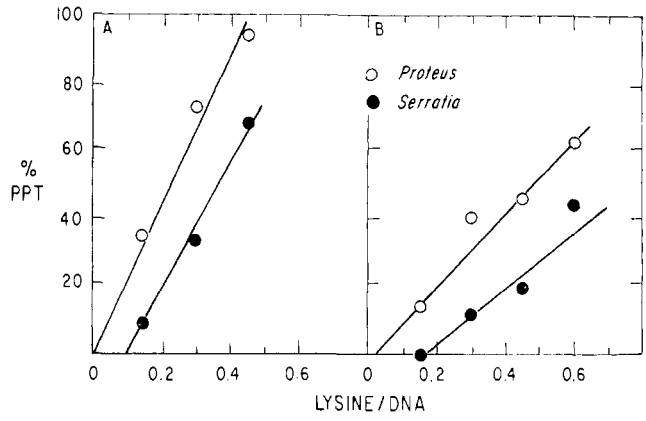

FIGURE 9: Selective precipitation of mixtures of PM DNA and SM DNA in 0.5 M NaCl. (A) Poly-L-lysine, $\overline{DP} \sim 50$. Total DNA concentration, 1.13×10^{-5} M. (B) Poly-DL-lysine. $\overline{DP} \sim 50$. Total DNA concentration, 7.6×10^{-5} M.

DNA precipitated was a maximum as a rough indication of the properties of the solvent. Similar experiments were carried out at varying Na^+/TMA^+ ratios. In Figure 5, the Na^+/TMA^+ ratio is plotted against the maximum ratio of DNAs precipitated at that value of Na^+/TMA^+. The point at which the ratio of DNAs precipitated is equal to one is the point at which there is no selectivity; at this point ($Na^+/TMA^+ \sim 0.2$–0.3) the opposing effects of Na^+ and TMA^+ cancel.

We have also studied the effect of actinomycin upon selectivity. Addition of actinomycin to PM–SM DNA mixtures in NaCl has no effect upon polylysine selectivity measured by standard competition experiments. Addition to mixtures containing TMA^+ results in complete abolition of the G·C selective effect (Figure 6). In both cases actinomycin raises the polylysine/DNA ratio required for precipitation.

Precipitation and competition experiments were also carried out with polymers of the optical isomers of lysine, using poly-L-lysine, poly-D-lysine, and poly-DL-lysine all of $\overline{DP} \sim 50$, in NaCl. Poly-L- and poly-D-lysine are homopolymers, while poly-DL-lysine is a copolymer of the two stereoisomers. Figure 7 shows the ability of these three polypeptides to precipitate a bacterial DNA and the variation of precipitation with salt concentration. As the graph shows, poly-L-lysine and poly-D-lysine act alike, precipitating DNA stoichiometrically in about 0.9 M NaCl, while poly-DL-lysine is less effective in precipitating DNA, with a stoichiometric point at 0.45 M NaCl. In 0.90–1.0 M NaCl, mixtures of poly-L-lysine and poly-DL-lysine precipitate an amount of DNA stoichiometrically equivalent to the amount of poly-L-lysine present, even when the poly-DL-lysine to poly-L-lysine ratio is 2:1. This suggests either that the poly-DL-lysine is not bound to the DNA at this salt concentration, or that the poly-L-lysine displaces it.

The ability of the optical isomers of polylysine to precipitate A·T-rich DNAs preferentially is shown in Figures 8 and 9. Figure 8 shows the competition of mixtures of PM DNA and SM DNA for poly-D-lysine of $\overline{DP} \sim 50$, in 1 M NaCl; the ratio of PM DNA to SM DNA precipitated is similar to that found with poly-L-lysine of $\overline{DP} \sim 50$. In Figure 9 are compared the selective activities of poly-L-lysine and poly-DL-lysine both of $\overline{DP} \sim 50$. In 0.5 M NaCl both of these polypeptides select A·T-rich DNA, but for neither is the selectivity as pronounced as for poly-L-lysine of $\overline{DP} \sim 100$ in 1 M NaCl.

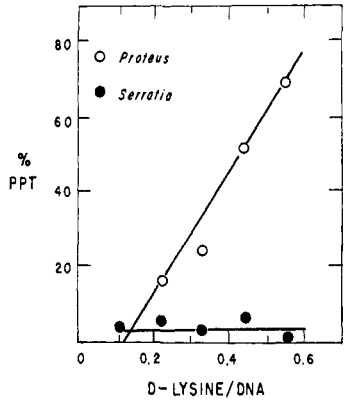

FIGURE 8: Selective precipitation of mixtures of PM DNA and SM DNA in 1 M NaCl with poly-D-lysine, $\overline{DP} \sim 50$. Total DNA concentration, 1.23×10^{-4} M.

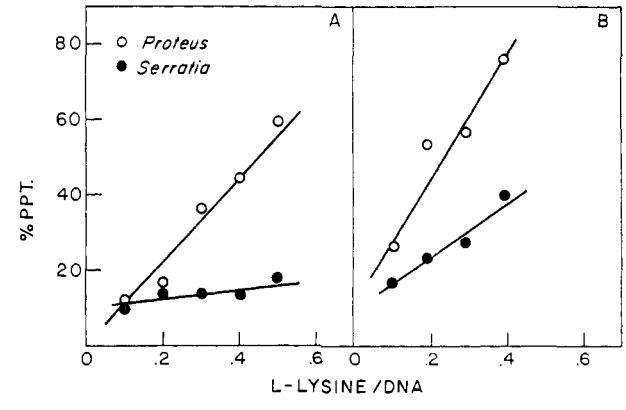

FIGURE 10: Selective precipitation of mixtures of PM DNA and SM DNA with poly-L-lysine, $\overline{DP} \sim 300$. (A) 0.40 M lysine. Total DNA concentration, 9.6×10^{-5} M. (B) 0.61 M N-α-acetyl-L-lysine methyl ester hydrochloride. Total DNA concentration, 6.9×10^{-5} M.

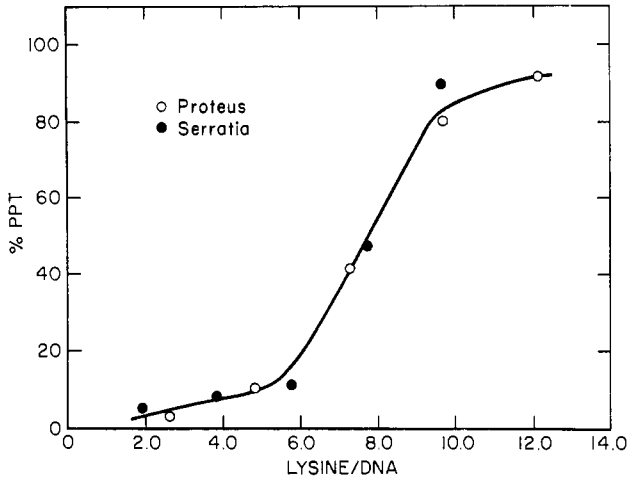

FIGURE 11: Precipitation of individual bacterial DNAs with tetralysine in 0.05 M NaCl. The two DNAs were titrated in separate experiments, not in mixtures. PM concentration 5.7×10^{-5} M; SM concentration, 7.2×10^{-5} M.

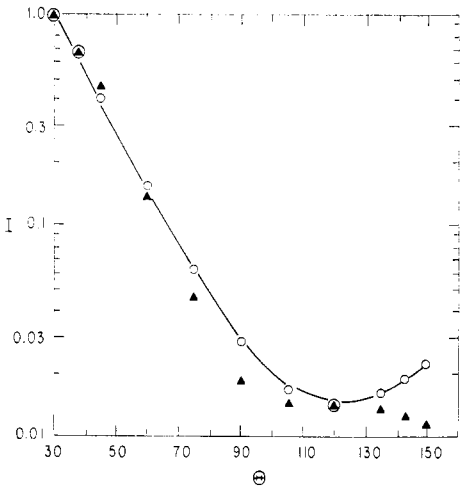

FIGURE 12: Total scattered light I as a function of angle θ, for a polylysine CT DNA complex in 0.85 M NaCl. I has been corrected by the factor $\sin \theta$. (○) Experimental points. (▲) Theoretical values predicted from the simple model described in the text.

If the ability of poly-L-lysine to select A·T-rich DNAs in NaCl solutions reflects a preference exhibited at the monomer level, perhaps the polypeptide selectivity could be obliterated by performing the experiments in lysine solutions, or, to simulate more closely the monomer unit of poly-L-lysine, in solutions of *N*-α-acetyl-L-lysine methyl ester hydrochloride. In the latter, the α-amino and COOH groups are blocked as they are in the polymer structure, while the ε-amino group is free. Figure 10 shows that in either of these salts, poly-L-lysine of $\overline{DP} \sim 300$ is still able to precipitate A·T-rich DNA selectively. In these experiments the lysine and *N*-α-acetyl-L-lysine methyl ester hydrochloride concentrations were chosen to give stoichiometric precipitation.

Previous work (Leng and Felsenfeld, 1966) has shown that poly-L-lysine, $\overline{DP} \sim 7$, functions in the same way as the higher molecular weight polymers, except that the salt concentration at which stoichiometric precipitation occurs is lower. We have studied a smaller oligolysine, tetra-L-lysine; it behaves quite differently from heptalysine. Figure 11 shows a typical precipitation curve of bacterial DNAs with tetralysine in 0.05 M NaCl; this salt concentration is the highest at which good binding of tetralysine to DNA occurs. The amount of tetralysine necessary to induce precipitation of both bacterial and mammalian (CT) DNAs is at least a factor of 10 greater than the amount necessary to precipitate DNA with larger poly-L-lysine samples. The shape of the precipitation curve is quite different; there is a region at the beginning of the curve in which no DNA can be centrifuged out of solution. At lysine/DNA (monomoles) > 6, precipitation begins, and selectivity for A·T-rich DNA is exhibited.

Structure of the Complex Phase

Light Scattering. If DNA–polylysine complexes are prepared by mixing in 1.5 M NaCl (see Methods), followed by dialysis into 1 or 0.85 M NaCl, particles of relatively uniform size are formed. The results of a typical light-scattering experiment using calf thymus DNA and poly-L-lysine of $\overline{DP} = 50$ are shown in Figure 12. In this case the DNA–polylysine (1:1) complex (about 10^{-4} M in nucleotides), formed by dialysis into 0.85 NaCl, has been diluted about 20-fold into 0.85 M NaCl to obtain concentrations suitable for the measurements. Figure 12 shows the angular dependence of the scattered light intensity. There is a shallow minimum in the scattered intensity at about 120°. The results of a typical sedimentation velocity experiment are shown in Figure 13. The median sedimentation coefficient of the complex varies between about 5,000 and 10,000 units, depending upon salt conditions and complex concentration. The shape of the boundary in Figure 13 indicates that most of the DNA is contained in particles that do not vary by more than a factor of 2 in sedimentation rate. If the particles are spheres of the same density and impenetrable to solvent, this corresponds to a variation of a factor of $1/\sqrt{2}$ in radius.

The angular dependence of light scattering observed for these particles can be explained readily if it is assumed that the particles are highly solvated spheres. If n is the refractive index of the particle, n_0 that of the solvent, a the radius of the sphere, and λ the wavelength of light in the solution, then provided that $(n/n_0 - 1)4\pi a/\lambda \ll 1$, the scattered intensity will be given (Van de Hulst, 1957) by

$$I = KI_0G^2(u)(1 + \cos^2 \theta) \qquad (1)$$

where $u = (2\pi a/\lambda) \sin \theta/2$, and $G(u) = 3/u^3 (\sin u - u \cos u)$. K contains angle-independent constants. The restriction on the product $(n/n_0 - 1)4\pi a/\lambda$ means that if the particle is large compared with the wavelength of light the difference between

FIGURE 13: Sedimentation velocity experiment on polylysine–DNA complex. This scanner tracing was taken about 12 min after reaching a rotor speed of 2600 rpm. Temperature was about 22°.

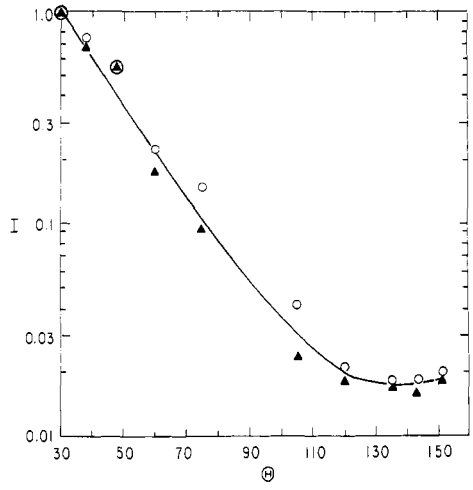

FIGURE 14: Scattered light intensity, I, as a function of angle θ for the DNA–polylysine complex in 0.85 M NaCl. (▲) Vertically polarized light, (○) horizontally polarized light, intensity divided by $\cos^2\theta$ (see text). Both components have been multiplied by $\sin\theta$ to correct for variation in scattering volume with angle. Results are normalized to 1.0 at 30°; at this angle the magnitudes before normalization were within 1.5% of each other.

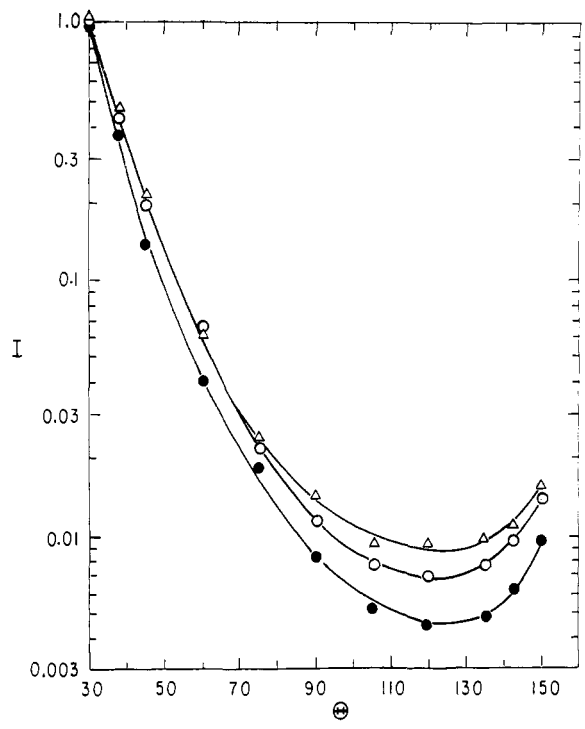

FIGURE 15: Angular dependence of scattering of DNA–polylysine complexes under varying conditions. (○) Polylysine–CT DNA in 1 M NaCl; (●) same complex in 10^{-3} M NaCl; (△) polylysine–T4 phage DNA in 1 M NaCl.

n and n_0 must be small. The requirement will be met if sufficient solvent is included in the particle, and the scattering will then be given by the Rayleigh–Gans equation above, even though a is of the same size as λ. From the shape of the curve of Figure 12 the mean radius of the particles may be estimated. If the particles were all spheres of radius a, the intensity function, I, given in eq 1 would vanish at $u = 4.49$. The fact that the intensity curve merely goes through a rather broad nonzero minimum near 120° means that there is some heterogeneity of particle size. Quite good agreement between theory and experiment may be obtained by assuming the simplest model of heterogeneity. The triangular points in Figure 12 are calculated from eq 1 by assuming a mixture of spheres of radii 1300 and 2100 Å in the number ratio 0.45:0.55. The average particle size is thus about 1700 Å.

The above analysis depends upon the assumption that the particles have a refractive index close to that of the solvent, so that eq 1 holds. In part this assumption is substantiated by the angular dependence of scattering of horizontally and vertically polarized light, shown in Figure 14. Within the limits of error, the scattering of the horizontal and vertical components is identical (except for the factor $\cos^2\theta$), a necessary condition for Rayleigh–Gans scattering which is however not generally fulfilled if the product $(n/n_0 - 1)4\pi a/\lambda$ is large (Van de Hulst, 1957). The solvation of the particles may also be demonstrated more directly by examining the absolute intensity of the scattered light. If the functional dependence upon angle given in eq 1 is used, the scattered intensity can readily be extrapolated to 0°. (The intensity per unit mass does not vary with concentration, *i.e.*, the scattering is not concentration dependent.) The estimation of the mass per particle now depends upon the assumption of some value for dn/dc, the refractive index increment. We used a value of 0.175 cc/g for dn/dc, calculated from a weighted means of values for DNA and protein. For the purposes of this rough calculation, any conceivable error in dn/dc is insignificant. We calculate that the dry mass of nucleoprotein per particle is 1.6×10^{-15} g, so that in order to have a radius of 1700 Å the particles would have to be about 95% water by weight.

The size of the complex particles does not depend to any great extent upon the size of polylysine or DNA, or upon the ionic strength, provided that the complex itself is stable. The angular dependence of scattering for a calf thymus DNA–polylysine mixture diluted into 1 or 10^{-3} M NaCl is shown in Figure 15, and compared with the results for a polylysine complex with T4 phage DNA. The differences among the curves are small. In other experiments, we find that there is also no significant difference between results obtained with polylysine of $\overline{DP} = 40$ and $\overline{DP} = 300$.

Turbidity. Doty and Steiner have discussed a method for determining particle demensions from measurements of solution turbidity *vs.* wavelength. If τ = turbidity, λ = wavelength in solution, and Q = particle dissipation factor, which is the correction term for the increase in transmission of a solution due to internal interference, then eq 2 enables us to deter-

$$\frac{-\mathrm{d}(\log \tau)}{\mathrm{d}(\log \lambda)} = 4 - \frac{\mathrm{d}(\log Q)}{\mathrm{d}(\log \lambda)} = 4 - \beta \qquad (2)$$

mine $\beta = \mathrm{d}(\log Q)/\mathrm{d}(\log \lambda)$. The value of β at a given wavelength for a spherical particle depends upon only the radius a. Therefore a determination of β establishes a certain value of a.

We have measured turbidity as a function of wavelength for solutions of polylysine–DNA complex at wavelengths between 3600 and 4500 Å, where DNA does not absorb. A direct log–log plot of these data yields an average value of β at 4000 Å. The variation of β with particle concentration is shown in

TABLE IV: Variation of β with Particle Concentration.

Particle Concn (mg/ml)	β
0.050	1.55
0.154	1.48
0.176	1.35
0.251	1.72

Table IV. We have used the values of β at the three lowest concentrations to estimate the value $\beta = 1.65$ at zero concentration. The graph given by Doty and Steiner (1950) relating β to $2a/\lambda$ does not extend to sufficiently large values of $2a/\lambda$. However, the graph may be extended using a table given by Van de Hulst (1957) that relates the total scattering to $2a/\lambda$ for large values of the latter. Unfortunately, the value of $2a/\lambda$ is a very sensitive function of β in the region $\beta = 1.65$, and there is considerable uncertainty in evaluating a. We estimate a mean particle size of about 1200 Å by this method. This is considerably smaller than the value obtained from light scatttering, though it is also consistent with the existence of a highly hydrated particle. As pointed out by Doty and Steiner, direct light-scattering measurements are more reliable than turbidity measurements, and we believe that the particle dimensions obtained by the former method are more accurate. It should also be noted that the concentrations at which turbidity measurements were made are much greater than those used for light-scattering studies. The observed concentration dependence of β may reflect not only nonideality, but a real change in particle size at high concentration. At DNA concentrations larger than those shown in Table IV, it is difficult to obtain stable suspensions of aggregates; flocculent precipitates tend to form instead.

Flow Birefringence. The birefringence of solutions of various concentrations of calf thymus (CT) DNA alone were examined in preliminary experiments. DNA concentration was varied between 1×10^{-4} and 5×10^{-4} M; at shear rates between 600 and 5600 sec^{-1}, the extinction angle, χ, remained between 10 and 12°. There was no observable trend in χ with DNA concentration. Birefringence increased linearly with DNA concentration; the sign of the birefringence was negative. Salt concentration in the DNA solutions was varied between 1 and 2 M NaCl; χ was independent of salt concentration, but the observed birefringence decreased somewhat in 2 M NaCl.

The particle suspensions showed the same extinction angle as pure DNA solutions, and their negative birefringence corresponded approximately[2] to the birefringence which would be observed in DNA solutions of the same concentration as the residual DNA in the particle suspension. On the basis of the experiments, we conclude that the particles exhibit no birefringence, since it is to be expected on the basis of flow birefringence theory that a mixture of birefringent DNA and nonbirefringent particles will display the extinction angle of the DNA, with birefringence of a magnitude corresponding to the DNA concentration.

[2] It was observed, however, that birefringence of particle solutions of DNA–polylysine complexes was generally slightly lower than expected according to residual DNA content. This was also true of solutions in which the particles had been formed in 1 M NaCl and then dissolved by bringing the NaCl concentration to 2 M NaCl; χ remained unchanged, but the birefringence was slightly lower than for pure DNA of the same concentration in 2 M NaCl. This is not an effect of the presence of poly-L-lysine, since solutions of DNA and poly-L-lysine in 2 M salt in which particles (or complexes) had never formed showed the expected birefringence.

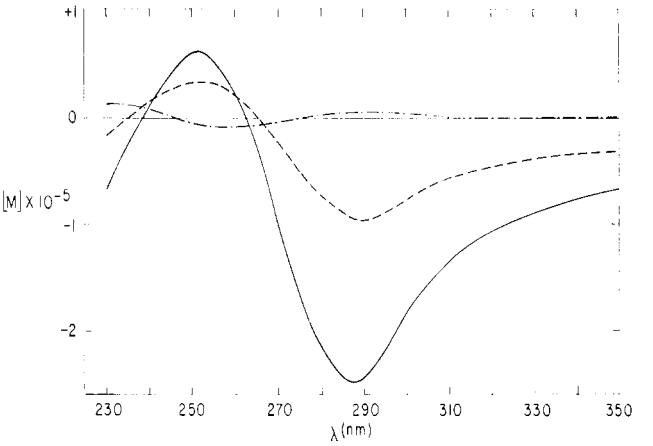

FIGURE 16: Mean residue rotation, M (based on nucleotide concentration), of DNA–polylysine complexes as a function of wavelength. (————) Calf thymus DNA + poly-D-lysine ($\overline{DP}$ 70) in 1 M NaCl; (– – – –) calf thymus DNA + poly-L-lysine ($\overline{DP}$ 50) in 1 M NaCl; (—·—·—) calf thymus DNA alone.

Optical Rotation. If the polylysine–DNA complex is formed by dialysis into 0.85–1.0 M NaCl, the optical rotatory dispersion spectrum of the complex is strikingly different from that of DNA alone (Figure 16). The maximum rotation (observed at about 290 nm) in the experiment shown is 50 times greater than that observed for DNA alone and of opposite sign to the rotation of DNA at 290 nm. Although the shape of the optical rotatory dispersion curve is about the same for all preparations, the magnitude of the rotation is not very reproducible. This presumably reflects the necessity for a high and not easily attainable degree of ordering to produce the rotatory changes. If the polylysine and DNA are merely mixed, qualitatively similar changes in optical rotatory dispersion are observed, but of considerably smaller magnitude than those shown here. Similar results have been reported for polylysine–DNA complexes by Cohen and Kidson (1968).

The curves in Figure 16 show the largest rotations that we have observed. We think that the difference in magnitude of rotation for the poly-D-lysine and poly-L-lysine complexes is fortuitous; the significant fact is that the two polypeptides produce curves of similar shape and sign.

Since we have shown that the polypeptide–DNA complex formed by dialysis is composed of rather large particles in a turbid suspension, we were quite concerned that the observed effect upon rotation might be an artifact arising from scattered light. A large part of our effort was therefore devoted to eliminating this possibility. We have carried out a series of optical rotatory dispersion measurements on the same sample in cells of varying path length, and of samples of varying concentration in a single cell. In all cases the magnitude of rotation was proportional to the cell path length and the concentration. Furthermore, samples of DNA to which sufficient spermine tetrahydrochloride had been added to produce about the same turbidity as that observed for polylysine–DNA complexes

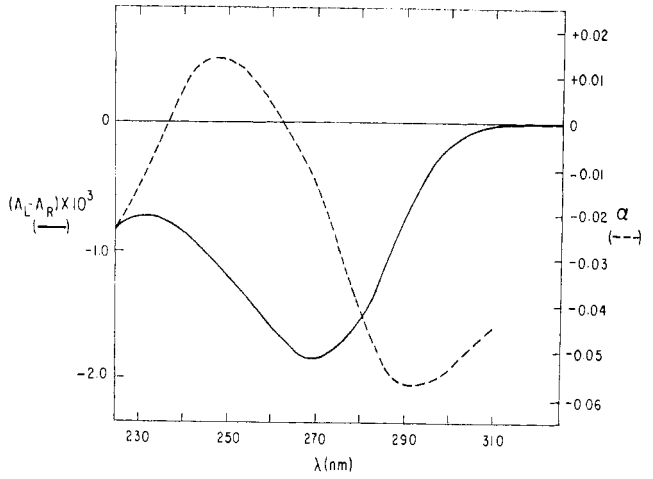

FIGURE 17: Observed optical rotatory dispersion curve (———) and circular dichroism curve (———) of a poly-L-lysine–CT DNA complex in 0.85 M NaCl. The DNA concentration was 10^{-5} M, polylysine concentration was 1.1×10^{-5} M, and the path length 2.5 cm. A_L and A_R are the absorbance of left and right polarized light.

gave optical rotatory dispersion spectra quite similar to that of DNA alone. In another experiment, we added excess DNA to a solution containing an already formed polylysine–DNA complex. The resulting optical rotatory dispersion curve was the sum of the complex spectrum and the DNA spectrum; the turbidity had no effect upon the spectrum of the added DNA. We have not been able to separate the changes in rotation from the formation of turbid systems. Addition of salt in small quantities, or of small amounts of trypsin, results in destruction of the complex. We do not observe intermediate states possessing unusual rotatory properties and little turbidity.

We have measured the optical rotatory dispersion of polylysine complexes with a variety of DNA samples under a variety of salt and mixing conditions. In all cases the shape of the spectrum was about the same. The mean residue rotation at 290 nm ranged between that shown in Figure 16 for poly-D-lysine and values one-tenth as great. Where there was extreme turbidity, the minimum at 290 nm was often broadened and shifted to slightly longer wavelengths; this is probably an artifact arising from light scattering. We have not been able to discover any regular variation in the magnitude of rotation with the molecular weight or base composition of the DNA used. This is not surprising considering the variability in rotational magnitude that we obtain with different complex preparations made from identical materials. Curves similar in shape to those shown in Figure 16 are obtained with DNA isolated from T4 phage, SM, or PM. Poly-L-lysine of $\overline{DP} = 300$ is also effective in producing the optical rotatory dispersion changes.

A comparison of the optical rotatory dispersion spectrum and circular dichroism spectrum of a polylysine–DNA complex is shown in Figure 17. The circular dichroism curve shows that there is a strong negative rotatory transition centered on about 269 nm. The optical rotatory dispersion curve crosses zero at about 263 nm, quite close to the position of the circular dichroism minimum as required if a single transition is dominant. The shape of the circular dichroism spectrum at lower wavelengths indicates that there is a second negative rotatory transition below 230 nm. At wavelengths below 225 nm, however, the turbidity becomes quite large and measurements are unreliable.

These results should be compared with the circular dichroism spectrum of DNA in ethanol–water mixtures obtained by Brahms and Mommaerts (1964). These workers find that in 80% ethanol solutions, a positive circular dichroic band appears, centered on 273 nm, with a rotational strength about three times the apparent strength of the transition observed for DNA in aqueous solution. The shape of the circular dichroism curve of DNA in 80% ethanol is like that of the DNA–polylysine complex, but of opposite sign.

Discussion

The results presented in the first part of this paper are consistent with a model of polylysine–DNA interaction having the following characteristics: reversibility, fairly well-defined stoichiometry, cooperativeness, and selectivity with regard to DNA base composition. We emphasize the reversibility of the reaction, since there is a natural uneasiness about dealing quantitatively with reactions that lead to formation of a "precipitate," a term which has connotations of irreversibility. The DNA–polylysine complex should not be thought of in this way; the complex forms a separate phase, a "complex coacervate," which is in equilibrium with the solvent phase. Such systems are quite amenable to thermodynamic and statistical analyses (Veis, 1969), and have been studied extensively. As we have pointed out in an earlier paper (Leng and Felsenfeld, 1966), the DNA contained in the polylysine–DNA complex exchanges freely with excess DNA in solution, a fact in itself sufficiently to establish the reversibility of the reaction. Furthermore, the selective incorporation of A·T-rich DNA into the separate phase can only be understood in terms of a reversible reaction: if polylysine molecules stuck to the first DNA molecules they encountered there obviously could be no selectivity. Finally, the response of the selective reaction to the composition of the solvent is understandable in terms of a reversible reaction. We have shown above that the selectivity of polylysine is reversed in solutions containing TMA^+. In a subsequent paper we will show that TMA^+ interacts preferentially with A·T-rich DNA. The reversal of polylysine selectivity is thus explained as the result of competition for sites on A·T-rich DNA between lysine residues and TMA^+. The effect of actinomycin, shown in Figure 6, is also understandable in these terms. Actinomycin binds selectively to G·C sites (Reich *et al.*, 1962; Gellert *et al.*, 1965). This has no effect upon the selectivity of polylysine in NaCl solutions, but counteracts the effect of TMA^+ in reversing the selectivity. Regardless of solvent, the effect of actinomycin is to increase the lysine/nucleotide ratio at which phase separation occurs, as might be expected if actinomycin displacement must precede polylysine binding.

The most striking property of the polylysine–DNA system is its nearly perfect selectivity with regard to base composition; the results given in the first part of the paper show the effect of varying conditions upon the selective process. It is clear from these results that the selection of A·T-rich DNA is not an artifact arising from a particular choice of DNA sources in the competitive studies; PM DNA is chosen in preference to either SM or ML DNA and EC DNA in preference to ML. Selectivity is also unrelated to changes that might arise in DNA due to labeling with isotope, since the results obtained using CsCl

gradients (Table II) did not involve labeled DNA. Finally, selectivity does not arise from some accidental correlation between DNA molecular weight and base composition. All of the DNA samples used had median sedimentation coefficients in the range 19–22 S units, and there was no systematic variation in sedimentation rate with base composition. Furthermore, the results of Figure 2 show that even when there is great disparity in the molecular weights of two competing DNAs, selectivity persists. A similar conclusion might be drawn from earlier studies showing that synthetic polymers containing only A·T pairs, and of relatively small size, are selected in preference to DNA (Leng and Felsenfeld, 1966).

Our results show that there is no very critical size range of either DNA or polylysine necessary for selection. When both DNA samples are of the same molecular weight, whether 10^7 (Figure 1) or 5×10^5 (Figure 2) selection is equally efficient. If the polylysine is of the order of 7 units long (Leng and Felsenfeld, 1966), it may be necessary to work at lower salt concentration to assure tight binding, but otherwise results are the same as with polylysine of $\overline{DP} = 300$. There are limits, however, upon the size of polylysine. We find that if $\overline{DP} = 1500$, no precipitate occurs with any DNA unless nearly stoichiometric quantities of polylysine are added (although sedimentation velocity studies show the polylysine is bound) and no selectivity is observed. This is probably the result of "irreversible" attachment of the large polylysine molecules to the DNA. At the other extreme, quite small oligolysine molecules under most conditions tend not to form coacervates with DNA, as first shown by Olins *et al.* (1967). Only under a very limited range of conditions is coacervation observed (Figure 11). The critical length for such behavior is somewhere between $\overline{DP} = 4$ and 7 in aqueous solutions.

The rationale originally given (Leng and Felsenfeld, 1966) for working at salt concentrations near 1 M was that the suppression of strictly electrostatic interactions might facilitate the formation of well ordered complexes dominated by other, more specific interactions. The mixing experiments at pH 10.8 and low ionic strength described above tend to substantiate this point of view, since at this pH many of the lysine residues are no longer positively charged while in solution, and the electrostatic contributions to binding free energy are reduced. Under such conditions we observe selectivity; if the experiment is repeated at lower pH without raising the ionic strength, the selectivity is reduced.

The selective process, with one important exception, does not depend in any special way upon the choice of cation: A·T-rich DNA is selectively complexed by polylysine in NaCl, LiCl, CsCl, and the salts of lysine and *N*-acetyllysine methyl ester. We will comment on the significance of the latter results later in the discussion. The effects of the quaternary amine ions, particularly TMA^+, have been discussed briefly above and will be dealt with at greater length in the following paper. The results given in Figure 5 suggest that when TMA^+ and Na^+ are both present there is a competition between them for binding sites on DNA. It is known (Strauss *et al.*, 1967) that TMA^+ binds to DNA more weakly than does Na^+ and it is therefore not surprising that a TMA^+:Na^+ concentration ratio of 4:1 is required to abolish entirely the "normal" A·T preference of polylysine.

Cooperativeness, Stoichiometry, and Particle Formation. The titration curves of polylysine–DNA interaction reveal that the interaction is cooperative: when there is one lysine residue for each two nucleotides, the polylysine is not distributed evenly among all DNA molecules, but instead half of the DNA molecules are completely neutralized and incorporated into a separate phase, while the remaining DNA molecules are free in solution and uncombined with polylysine, of which there is in any case very little present in the solvent phase (Leng and Felsenfeld, 1966). A similar conclusion has been reached by examining the thermal denaturation profile of DNA partially titrated with polylysine or other basic polypeptides (Olins *et al.*, 1967).

Under the conditions of most of our experiments, DNA is strongly complexed to polylysine in a 1:1 ratio of residues, and all of the complex is present in the separate phase (Leng and Felsenfeld, 1966). The effect of raising the salt concentration above the point where 1:1 precipitation occurs is to weaken the lysine–nucleotide interaction. It is most likely that even under these conditions the complex in the precipitate preserves the 1:1 ratio of lysine to nucleotide. Because of the cooperative nature of the interaction, there are probably not many partially formed complex molecules free in solution. Most complex species will tend to be completely neutralized (*i.e.*, 1:1), and to become incorporated in the separate phase. When the salt is lowered below that necessary for 1:1 precipitation we find that an equivalent of lysine will often precipitate somewhat more than an equivalent of nucleotide. The deviations from equivalence are not large and are probably attributable to some "irreversible" step such as cross-linking of DNA strands by polylysine that occurs at low ionic strength.

Stoichiometric interaction is not at all unexpected from mixtures of polyions of opposite charge; the formation of a separate phase, with the components present in equivalent quantities, is a well-known property of complex coacervates (Veis, 1969). The stoichiometry and the cooperative nature of such interactions is the result of the tendency of neutral molecules to form a separate phase, and the difficulty experienced by a partly charged molecule in entering that phase. The DNA–polylysine complex is not, however, merely a nonspecific complex coacervate. Superimposed upon the coacervate behavior is a specificity that can only be explained by the formation of a well-defined chemical structure. This is probably the explanation for the reduced affinity of poly-DL-lysine for DNA, as compared with poly-L-lysine or poly-D-lysine. A model for a well-ordered complex between basic polypeptides and DNA has been proposed by Wilkins (1956); related models can be constructed using poly-L-lysine or poly-D-lysine. In both cases the formation of well-ordered complexes is facilitated by the possibility of bringing each positively charged ϵ-amino group close to a negatively charged phosphodiester group. The formation of a well-ordered complex is likely to be more difficult when D- and L-lysine residues are interspersed.

The remarkably narrow size distribution of the particles constituting the separate phase is reminiscent of the behavior of micelles. In this case, it is not clear what opposing forces are responsible for the formation of particles so uniformly close to 1700 Å in radius, independent of the size of the DNA. The average linear path that can be drawn in a sphere of this radius is of the order of the persistence length of DNA, so that the lower size limit for the particles may be set by the flexibility of the double helix. The other noteworthy feature of the "micelles" is their high degree of solvation. Since they are 95% solvent by weight, it is not so surprising that the DNA of the complex is able to exchange

freely with excess DNA in the external phase; all of the complex in the coacervate phase must be quite accessible to solvent. It should be noted that these particles are formed only in DNA solutions of concentration approximately 0.5 mg/ml or less. At higher concentrations, much larger particles occur.

The analysis of light scattering and turbidity data given in this paper requires the assumption that the particles are spherical. This seems a reasonable assumption, since the angular dependence of scattering is fit quite well by a simple spherical model; the angular dependence for other shapes is entirely different. If the particles were rodlike one might in addition expect some dependence of shape upon DNA size, but the scattering reveals no such dependence. Finally, the particles show no flow birefringence, which is perhaps not surprising for particles of such small refractive index increment relative to solvent, but is not inconsistent with the presence of spherical particles.

Optical Rotation. The most dramatic demonstration of the formation of a specific DNA–polylysine complex is provided by the optical rotatory dispersion data. The rotations we observe are in some cases almost two orders of magnitude greater than those of DNA alone. As we have pointed out earlier, Cohen and Kidson (1968) have reported similar optical rotatory dispersion changes in such complexes, but of smaller magnitude. Apparently the formation of the optically active species is a sensitive function of the method of mixing. For this reason we think that the dependence of rotation upon base composition reported by Cohen and Kidson must be interpreted with caution, since the optically active complexes are only partly formed under optimal conditions.

The optical rotatory dispersion and circular dichroism results may be summarized as follows. Combination of polylysine with DNA gives rise to a large optically active transition of negative sign, centered at about 270 nm. Whereas the circular dichroism spectrum of DNA is to a first approximation "conservative," that of the DNA–polylysine complex is "nonconservative," *i.e.*, the rotational strengths in the 260-nm region do not sum to zero. What is the origin of such marked changes in rotation? It is unlikely that they arise from interaction between the transitions of the peptide chromophore and those of the bases, since poly-D-lysine and poly-L-lysine produce complexes with the same optical rotatory dispersion patterns. The changes resemble somewhat those produced by addition of ethanol to DNA in that this also results in formation of a nonconservative band centered near 270 nm, which is, however, much smaller in magnitude and of opposite sign to the one we observe. The appearance of such nonconservative bands in polynucleotide rotatory spectra has been attributed (Yang and Samejima, 1969) to the tilting of the stacked bases relative to the helix axis. In the case of DNA in ethanol, Brahms and Mommaerts (1964) believed that they were observing the dehydrated A form of DNA, in which the bases are tilted; in the case of single-strand stacked or two-strand helical polyribonucleotides, it is believed that the bases are always tilted relative to the helix axis, and it has been suggested that the nonconservative rotational spectrum of the ordered polyribonucleotides is related to this tilting. If we invoke base tilting to explain the rotations observed in the polylysine–DNA complex, however, it must be tilting in a different direction from that associated with the structure of DNA in ethanol, to account for the differences in the shapes of the optical rotatory dispersion curves mentioned above. Perhaps the tilting is associated with the formation of DNA "supercoils;" Pardon *et al.* (1967) have interpreted the X-ray diffraction patterns of nucleohistone complexes in terms of supercoiled structures. If supercoils are formed in our complexes, it is almost certainly the indirect effect of the distortion of the backbone upon the relative orientation of neighboring base pairs that gives rise to the anomalous optical rotatory dispersion we observe.

Another possible explanation of the unusual rotatory properties of the DNA–polylysine complex is that structures with long-range order are present within the particles. Under certain conditions, many kinds of molecules, including polypeptides, can form a liquid crystalline phase with remarkably enhanced optical rotatory power; Robinson (1961) has explained this behavior in terms of a model of the liquid crystal in which birefringent sheets of molecules are stacked with their planes parallel, but with the principal axis of orientation in the plane of each sheet somewhat rotated, with respect to the sheet beneath it, about an axis perpendicular to the sheet. Such a helical array of birefringent sheets can give rise, as Robinson shows, to very large rotations, and it is possible that the polylysine–DNA complex particles contain structures of this kind. As we have shown, the particles have a high solvent content, and the DNA and polylysine concentrations within them are low, so that if the structure fills the entire particle the mean separation between DNA molecules must be large. It is therefore not easy to imagine what forces would confer the required preferred orientation between groups of molecules. On the other hand, we have never been able to make highly rotatory complexes without also making large particles, and this suggests that the formation of the separate phase is somehow coupled to the rotatory phenomena.

Two other sets of observations bear upon our optical rotatory density results. Lees and von Hippel (1968) found that the complexing of DNA with polylysine results in the conversion of 25% of the slowly exchangeable protons of DNA into rapidly exchangeable protons. The authors suggest that tilting or other pertubation of the base pairs may make some protons more accessible to solvent. The second relevant observation is that of Maestre and Tinoco (1967) who have measured the change in optical rotatory dispersion associated with transfer of a variety of phage DNAs from an aqueous environment to the interior of the phage head. The difference optical rotatory dispersion spectrum associated with this transfer bears a remarkable resemblance in shape to the spectrum of DNA–polylysine complexes. There is a peak at about 250 nm, a crossover at 260 nm, and a trough at 285 nm. Unlike the spectrum of DNA in ethanol, the new rotational band in this case has the proper (negative) sign. The magnitude of the rotation, however, is vastly smaller in the case of the phage DNA, amounting to about $^1/_{100}$ of the largest rotation observed for polylysine-DNA. Maestre and Tinoco conclude that the rotational changes they observe probably arise from the effect upon DNA structure of reduction in water activity within the phage head. They point out, as we have done above, that dehydration can lead to changes in the relative orientation of bases, and that this in turn leads to changes in the interactions that produce optical rotation. As Maestre and Tinoco show, there is a resemblance between the phage DNA optical rotatory density and that produced by transferring DNA from aqueous solution to 24% LiCl, in which water activity is reduced. Despite this resemblance, our results with DNA–polylysine suggest another interpretation of the phage DNA results. Since it now

appears possible to produce rotatory changes of the same kind 50 or 100 times larger than those observed in phage, it may be that the DNA structure within the phage is perturbed over only a small fraction of its length, perhaps where it must bend abruptly, or where it interacts with coat protein. Of course it is still possible that all of the DNA within the phage is only slightly, but uniformly, perturbed in structure. In the case of the DNA–polylysine complex, at least, it seems unlikely to us that the optical rotatory dispersion results can be explained in terms of dehydration alone, except in the sense that a polylysine molecule that forms a well-ordered complex with DNA might exclude solvent from one of the grooves of the double helix. As we have seen, the micelles are very highly hydrated. We conclude that in DNA–polylysine, and perhaps in the DNA of phages as well, DNA may exist in a configuration or may form ordered structures not previously known.

Conclusion

We have shown that the DNA–polylysine complex behaves in some ways like a classical complex coacervate, but that there are additional properties superimposed upon the classical behavior that arise from quite specific and sterically well-defined interactions between lysine residues and nucleotides. The reaction is stoichiometric and cooperative. These two properties could be explained entirely in terms of the theory of complex coacervates, but in such well-ordered complexes it may be a mistake to attribute all of this behavior to formation of coacervates. In the DNA–polylysine complex (and similar complexes involving other basic polypeptides), the possibility exists of cooperative binding within single molecules, *i.e.*, it is possible that the combination of one polylysine chain with a DNA molecule may make it easier for successive polylysine chains to combine with the same DNA molecule. A variety of mechanisms for such behavior can be invented. For example, our optical rotatory density results and the proton-exchange results of Lees and von Hippel (1968) suggest that the binding of a polylysine molecule may involve perturbation of the DNA secondary structure; if this perturbation extends somewhat beyond the segment of the DNA molecule occupied by the polylysine, the binding of a second polylysine adjacent to the first might be facilitated. The biological implications of such a model are interesting. Under certain circumstances it might be possible, for example, to initiate binding at a site on DNA particularly rich in A·T pairs; the remainder of the DNA molecule would then be covered regardless of its base composition because of the cooperative nature of the reaction. Such behavior can be generated in a model of this sort by assuming that the preferential affinity of polylysine for A·T pairs is slightly greater in the initiation site than in other sites.

We have yet not been able to tell whether cooperative behavior exists at the intramolecular level. Unfortunately the formation of complex coacervates always accompanies the formation of DNA–polylysine complexes under reversible conditions, except when oligolysines of chain length less than about 7 are used (Olins *et al.*, 1968).

Another unanswered question arising from these studies concerns the mechanism of the remarkable specificity of polylysine for A·T-rich DNA. Our experiments with PBS-2 DNA were designed to determine whether the methyl group of thymine is an important factor in recognition. Examination of helical DNA models reveals that this methyl group is a prominent and distinguishing feature of the A·T pair; it seemed possible that the aliphatic side chains of lysine residues could make hydrophobic contact with the methyl group, and that the reversal of specificity induced by TMA^+ could arise from competition for the same site. The experiments with PBS-2 DNA, in which the methyl group is missing, show that neither the lysine residues nor TMA^+ requires the methyl group for its selective function. There are two kinds of mechanism that could account for selectivity: either a ligand such as the lysine residue interacts preferentially with an A·T pair at a specific site, or the preferential interaction arises through some "group" property of an A·T-rich DNA molecule, such as its geometry or deformability, which distinguishes it from G·C-rich molecule. The first mechanism is exemplified by the thymine methyl group model discussed above. One can equally well suppose that differences in the polarizability of A·T and G·C pairs result in differences in binding affinity for lysine residues or TMA^+. The second mechanism has already been discussed above in connection with cooperative behavior. We have mentioned the possibility that binding of polylysine can result in a change in DNA secondary structure, and the ease with which this occurs within a given region of a DNA molecule might easily depend upon the base composition of the region. If it were easier to deform an A·T-rich region than a G·C-rich region, the observed binding preference would be accounted for. However, there is little evidence yet available to help us choose between this model and the site binding model.

The selectivity experiments carried out in the presence of lysine or *N*-α-acetyl-L-lysine methyl ester (Figure 10) shows that these monomers, if they are at all selectively bound to A·T pairs, do not possess as strong a preference as the lysine residues of polylysine. If they did have as strong a preference, the selectivity of polylysine would be abolished in their presence. The results with lysine and *N*-α-acetyl-L-lysine methyl ester also suggest that the selectivity of polylysine is not merely the result of preferential binding of Na^+ to G·C-rich DNA, an effect that has been proposed to explain the properties of polylysine–polyribonucleotide complexes (Latt and Sober, 1967). As we will show in the following paper, tetralysine is not bound preferentially to A·T-rich DNA under conditions where there is no coacervate formation. On the other hand, we have already shown that it is selective when conditions are adjusted to give coacervate formation. It is difficult to assess the effect upon the tetralysine results of the high density of charged end groups. The experiments suggest, however, that a cooperative phenomenon is necessary for measurable selectivity. This means that the free energy per residue of transfer of a lysine residue from a G·C to an A·T pair must be very slight. The cooperative phenomena serve to amplify this slight selectivity, so that the balance between A·T preference and G·C preference can be tipped, for example, by the relatively weak competition of bound TMA^+ for A·T sites. We do not yet know whether this delicately balanced system has its parallel in living cells. It is clear, however, that the polylysine–DNA complex is another example of the kind of well-ordered, highly specific, and energetically "well-balanced" structure that biopolymers often seem to form.

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