THE PREFERENTIAL INTERACTIONS OF POLYLYSINE AND POLYARGININE WITH SPECIFIC BASE SEQUENCES IN DNA

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The interaction of nucleic acids with basic proteins and polypeptides has been the subject of numerous studies[1-9] motivated in large part by the desire to understand the nature of the complexes formed between nucleic acids and histones, the basic proteins which Stedman and Stedman[10] first suggested might play a role in regulation of the biological activity of DNA.

A major question which arises in any discussion of the biological role of histones is whether or not certain histone fractions are capable of interacting preferentially with regions of DNA possessing a special base sequence. From the point of view of the physical chemist, the most relevant observations may be those of Chargaff and his collaborators[1, 2] and Lucy and Butler.[8] These workers found that precipitates formed by the reaction of DNA with histones or polylysine could be redissolved in NaCl solutions of concentrations 1–2 *M*, and that under these conditions guanine-cytosine (G-C)-rich DNA was eluted more readily than was A-T-rich DNA.

In this paper, we describe an investigation of the interaction between DNA's of varying base composition and the synthetic polypeptides, polylysine, and polyarginine. We have attempted to carry out the reactions in a manner which would permit us to compare the stabilities of the various complexes, i.e., we have attempted to study the reactions under reversible conditions. At the same time, we have chosen conditions under which the binding forces are relatively weak and differences in selectivity are likely to be enhanced. We find that in solutions about 1 *M* in NaCl concentration, polylysine of degree of polymerization $\sim$ 100 exhibits an almost perfect selectivity for interaction (as judged by preferential precipitation) with A-T-rich DNA. Polyarginine interacts about equally with DNA's of base compositions in the range 40–60 per cent GC, but exhibits a slight preference for a DNA of composition 72 per cent GC, and a marked preference for the homopolymer, poly dG : dC.

Methods and Materials.—Materials: DNA from bacterial sources was prepared either according to methods described previously,[11] or by the method of Berns and Thomas,[12] modified by addition of RNase treatment, Sevag and phenol extraction, and isopropanol precipitation. DNA labeled with P^{32} was obtained by growth of bacteria in tryptone containing 0.1% yeast extract and 0.5–1 mc of P^{32} (as phosphate) per liter of culture. *S. marcescens* DNA labeled with C^{14} was obtained by growth on media containing 0.022 *M* KH_2PO_4, 0.042 *M* NaH_2PO_4, 0.018 *M* NH_4Cl, 2×10^{-3} *M* $MgCl_2$, 1.6×10^{-4} *M* Na_2SO_4, 6×10^{-7} *M* $FeCl_3$, 0.2% glucose, 0.3% yeast extract, and 5 mc C^{14}-glucose per liter of culture. Typical values of the median sedimentation coefficients ($S_{20,w}$) for such preparations were 26.3 and 23.4 Svedberg units for P^{32}-labeled *P. mirabilis* DNA and *S. marcescens* DNA, respectively. Poly-L-lysine hydrochloride samples [average degree of polymerization ($\overline{D.P.}$) $\sim$ 100, 200, and 1500 determined by viscosity measurement] were obtained from New England Nuclear Corp. (Yeda); samples of $\overline{D.P.} \sim 7$ were a gift of Dr. Herbert A. Sober. All of these samples were polydisperse. The samples of $\overline{D.P.} \sim 100$ and 200 behaved identically in the titration experiments described below. One portion of the $\overline{D.P.}$ 200 sample was fractionated on a Bio-Rad P150 polyacrylamide gel column, and the fraction of intermediate mobility isolated. Another portion of the $\overline{D.P.}$ 200 sample was fractionated by ethanol precipitation. The least soluble fraction was chromatographed on carboxymethyl cellu-

lose in the laboratory of Dr. H. A. Sober; it revealed no material of $\overline{D.P.}$ less than 15. Both this fraction and the fraction of intermediate size from the polyacrylamide gel column behaved like the other polylysine samples of this size range. Poly-L-arginine sulfate ($\overline{D.P.} \sim 160$) was obtained from New England Nuclear Corp. Polyarginine of smaller molecular weight was obtained by hydrolysis (70 hr at 25°, 6 *N* HCl) followed by dialysis for 3 hr against 1 *M* NaCl, 0.01 cacodylate, pH 7. The approximate number average degree of polymerization of this sample was 13 ± 3, as determined by hydrogen ion titration of α-amino groups.

Sodium perchlorate was obtained from Fisher Scientific Co. Thermal denaturation studies were carried out in 4 *M* $NaClO_4$, 0.01 *M* cacodylate, pH 7, 0.0001 *M* ethylene diamine tetraacetate. Synthetic poly dA:dT and poly dG:dC were a gift of Dr. F. Bollum; poly dAT was a gift of Dr. R. Baldwin. Base composition analysis of the poly dG:dC sample (personal communication, Dr. Bollum) gave 51% G, 49% C. The median sedimentation coefficient of the poly dG:dC was 7.2 Svedberg units ($S_{20,w}$). Thermal denaturation of poly dG:dC in 0.005 *M* NaCl, 0.001 *M* cacodylate, 10^{-4} *M* EDTA, pH 6.8, gave identical T_m values of 77° at 250, 260, and 278 mμ. The value of (Δabs/starting absorbance) was 0.72 at 278 mμ.

Methods: Spectral analyses and thermal denaturation experiments were carried out in a Cary model 14 spectrophotometer, equipped for thermal denaturation studies as described earlier.[13] The methods of analysis for base composition and concentration have also been described.[14] Analytical centrifugation of DNA was carried out in a Spinco model E centrifuge with ultraviolet absorption optics.

Radioactivity of DNA samples was determined by pipetting 200-μl aliquots of solution onto strips of Whatman 3 MM filter paper, drying and cutting into 4 × 4-cm squares, which were then further cut or pleated and counted in vials filled with "PPO-POPOP-toluene" scintillation fluid. Analyses of polymer concentration were made by direct determination of nitrogen content in the National Institutes of Health analytical laboratory. Polylysine concentrations were sometimes determined by the ninhydrin method on unhydrolyzed samples, using polylysine solutions of known concentrations as standard. The amount of color developed was linear with the polylysine concentration in the range of concentrations measured, and appeared to be only a slowly varying function of molecular weight for the larger molecular weight polymers.

All additions of polypeptide solutions to DNA solutions were made slowly, with vigorous mixing on a "Vortex" agitator. The polypeptide solution was usually not more than twice as concentrated (in monomoles/liter) as the DNA solution, and the solvents for both solutions were identical in any given experiment. In typical experiments, mixtures were allowed to stand 5–30 min before centrifuging. All experiments, unless otherwise noted, were carried out at room temperature (23–25°C). All solutions contained 0.01 *M* cacodylate buffer, pH 7, in addition to NaCl.

Results.—Polylysine: If polylysine ($\overline{D.P.} \sim 7$ or $\overline{D.P.} \sim 100$) is added to DNA in a solvent containing 0.5 *M* NaCl under conditions of dropwise addition with vigorous agitation, an aggregate will form. Though a precipitate may not readily be apparent, centrifugation at 25,000 × *g* for 20–30 min results in removal of an appreciable portion of the DNA from solution, while further centrifugation at this speed has little additional effect. At higher NaCl concentrations, a smaller amount of DNA is precipitated (Fig. 1*a*). The results depend somewhat upon the size of the polylysine used, but for a given polylysine sample in the range $\overline{D.P.}$ 7–200 the fraction of DNA precipitated depends only upon the DNA:polylysine concentration ratio over the range of DNA concentrations from 2×10^{-5} to 7×10^{-4} *M*. A typical titration curve in 1 *M* NaCl is shown in Figure 1*b*. The results are nearly independent of the source or base composition of the DNA in the molecular weight range 4–30 million.

At any point in the titration, most of the polylysine is found in the precipitate. When 44 per cent of a calf thymus DNA sample is precipitated, 85 per cent of the polylysine added is also precipitated, as determined by ninhydrin analysis (see *Methods*). If the supernate, containing both the polylysine and DNA, is distrib-

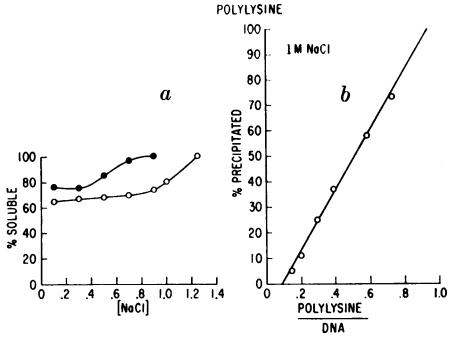

FIG. 1.—(*a*) Solubility of DNA in a mixture of polylysine and calf thymus DNA as a function of NaCl concentration (moles/liter). *Closed circles*, polylysine $\overline{D.P.}$ = 7, polylysine:DNA ratio (monomoles) = 0.45. *Open circles*, polylysine $\overline{D.P.}$ = 100, polylysine:DNA = 0.27. (*b*) Titration of DNA with polylysine (100-mer) in 1 *M* NaCl. Per cent DNA precipitated is plotted vs. the ratio polylysine:DNA in monomoles. DNA concentration 1.5 × 10^{-4} *M* in (*a*) and (*b*).

uted uniformly through a gradient of 5–20 per cent in sucrose concentration and centrifuged, the DNA boundary sediments in a manner identical to that of a control containing no polylysine; furthermore, there is only a small difference in polylysine concentrations on either side of the boundary.

If a sample of calf thymus DNA is partially precipitated with polylysine in 1 *M* NaCl, the base composition of the precipitate is richer is adenine-thymine (A-T) content than is the supernate. The base composition of the DNA fractions was obtained by spectral analysis of thermal denaturation in 4 *M* $NaClO_4$, using the value of T_m,[15] the hyperchromic spectrum, or the spectrum of the denatured DNA.[14] Density gradient centrifugation in CsCl was also used.[16] Control experiments showed that at these concentrations of $NaClO_4$ and CsCl, polylysine has no effect upon the properties of DNA. A summary of a typical experiment is given in Table 1. The less easily precipitated DNA fraction was indistinguishable from unfractionated DNA in sedimentation velocity experiments in the analytical ultracentrifuge.

Further experiments were carried out with a pair of bacterial DNA's, possessing identical precipitation properties with polylysine, isolated from *S. marcescens* (SDNA, 42% AT) and *P. mirabilis* (PDNA, 62% AT). If SDNA is mixed in equal concentration with the synthetic polynucleotide poly dA:dT, titrated with polylysine (100-mer), and the supernates are heat-denatured in 4 *M* $NaClO_4$, the results shown in Figure 2 are obtained. It is evident that essentially all of the poly dA:dT is precipitated before any of the bacterial DNA is precipitated. Similar results are obtained with competition between PDNA and the alternating synthetic polymer, poly dAT (Fig. 2). If PDNA and SDNA are mixed in equal quantities and titrated with polylysine (100-mer), examination of the supernate by CsCl density gradient centrifugation reveals that the band containing PDNA is almost completely eliminated before any of the SDNA is removed from solution.

TABLE 1

PRECIPITATION OF CALF THYMUS DNA BY POLYLYSINE

	Mole Fraction at		
	Denatured spectrum	Hyperchromic spectrum	CsCl gradient
First fraction precipitated (21% of DNA)	0.67	0.64	0.63
Fraction remaining in solution after 72% of DNA is precipitated	0.56	0.57	0.55

* Distribution of DNA in the band was slightly asymmetric; the median density was used in calculating base composition.

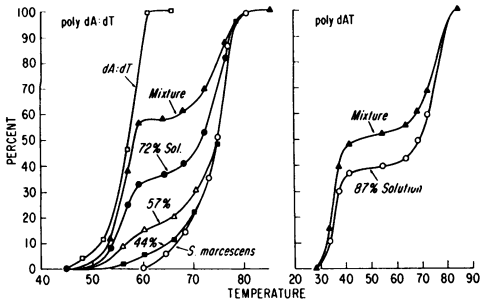

FIG. 2.—Thermal denaturation curves of mixtures of *S. marcescens* DNA with poly dA:dT (*a*) and poly dAT (*b*), after partial precipitation with polylysine in 1 *M* NaCl. Denaturation was carried out in 4 *M* $NaClO_4$, 0.01 *M* cacodylate, 10^{-4} *M* EDTA, pH 7. Original DNA concentration about 3×10^{-4} *M*.

We have also used C^{14}-labeled SDNA and P^{32}-labeled PDNA to study these reactions. In one class of experiments, both DNA's were labeled and their concentrations determined by liquid scintillation counting. Table 2 shows the result of a double-label experiment of this sort using large (100-mer) polylysine, which confirms that AT-rich DNA is selectively precipitated in 1 *M* NaCl. If small polylysine ($\sim$ 7-mer) is used in 0.5 *M* NaCl as solvent, the selection of PDNA is somewhat reduced. Results similar to those described are obtained with mixtures of radioactive PDNA or SDNA and DNA isolated from *M. lysodeikticus* (28% AT). Polylysine precipitates either PDNA or SDNA in preference to *M. lysodeikticus* DNA.

The reactions of polylysine described above are reversible. If sufficient polylysine is added to P^{32}-labeled PDNA to precipitate 30 per cent of the DNA (total DNA concentration 2×10^{-5} *M*), and a 15-fold excess of unlabeled PDNA is added at any time between 0.5 and 20 min later, but before centrifugation, 96 per cent of the total P^{32} is found in the supernate. At a higher lysine:DNA ratio sufficient to precipitate all of the DNA, only about 25 per cent of the P^{32} is solubilized by addition of cold DNA, presumably because of formation of larger, less easily dissolved aggregates after the initial reaction.

It must be emphasized that the results presented here depend upon the choice of conditions. Thus, we find that the use of quite large polylysine ($\bar{D}.P. \sim 1500$) results in a marked decrease in the amount of precipitation observed at a given lysine:nucleotide ratio. Also, very high molecular weight DNA, such as that from T4 phage, is somewhat more readily precipitated by polylysine (100-mer) than is the DNA shown in Figure 1*b*. Finally, the formation of precipitate is somewhat inhibited if the reaction is carried out at 0°. However, results obtained at 37° are identical to those shown in Figure 1*b*. The choice of ionic conditions is also important. All of the selective effects are abolished if the reactions are carried out in 0.1 *M* NaCl, rather than 1 *M* NaCl, while in 1 *M* tetramethyl ammonium

TABLE 2

PRECIPITATION OF MIXTURES OF *P. mirabilis* AND *S. marcescens* DNA BY POLYLYSINE

Lysine/DNA (monomoles)	Fraction of all DNA pptd.	Fraction of *Proteus* DNA pptd.	Fraction of *Serratia* DNA pptd.
0.48	0.48	0.83	0.12
0.40	0.37	0.68	0.06
0.32	0.31	0.51	0.11
0.24	0.23	0.34	0.11
0.16	0.13	0.16	0.09

Total DNA concentration = 9.2×10^{-5} *M* (equal concentrations of P^{32}-PDNA and C^{14}-SDNA).

chloride the selectivity of polylysine is reversed, so that GC-rich DNA is precipitated preferentially.

Polylysine will precipitate denatured DNA in preference to native DNA, regardless of base composition. Thus, in a mixture of native PDNA and heat-denatured SDNA, addition of polylysine results in precipitation of SDNA first despite the usual preference for AT-rich material.

TABLE 3

PRECIPITATION OF BACTERIAL DNA BY POLYARGININE IN 1 *M* NaCl*

P^{32}-SDNA + PDNA + Polyarginine (160-mer)	
DNA pptd. (%)	SDNA/PDNA in ppt.
20	1.0
29	1.3
33	1.2
58	1.2

* Each solution initially contained 0.5 absorbance units (260 mμ) of each DNA in a final volume of 1.5 ml. Total precipitate was measured by decrease of absorbance at 260 mμ in the supernate. Radioactivity measurement gave the fraction of precipitate which was SDNA.

Polyarginine: All of the experiments with polylysine described above have also been carried out with polyarginine. The precipitation curves are somewhat like those shown in Figure 1*a*, but displaced to higher salt concentrations; in 1 *M* NaCl about 0.6 equivalent of polyarginine is required to precipitate one equivalent of bacterial DNA, whereas the stoichiometry of the polylysine precipitation is close to 1:1. Like polylysine, polyarginine will precipitate denatured DNA preferentially.

If polyarginine ($\sim$ 160-mer) is added to a mixture of P^{32}-labeled SDNA and unlabeled PDNA, both DNA's are precipitated in approximately equal amounts (Table 3). Similar results are obtained using calf thymus DNA and SDNA, with either large or small molecular weight polyarginine. Unlike polylysine, the binding of polyarginine to DNA persists at quite high salt concentrations, even though no precipitation is observed. Thus, polyarginine raises the denaturation temperature of DNA in 4 *M* $NaClO_4$, and addition of polyarginine to DNA in a CsCl solution of density 1.7 results in disappearance of absorbing material from the band of DNA produced by subsequent CsCl density gradient centrifugation.

We have studied the binding competition between the DNA of *M. lysodeikticus* (MDNA, 72% GC) and SDNA. In a mixture of equal concentrations of MDNA and P^{32}-SDNA, the first 14 per cent of the DNA to be precipitated by polyarginine (160-mer) contains about six times more MDNA than SDNA, i.e., about 24 per cent of the MDNA is precipitated. Control experiments with polylysine indicate that a maximum of 12 per cent of the MDNA competes successfully with DNA richer in AT. Even assuming that all of the polylysine-precipitable MDNA is partly denatured (and therefore also precipitable by polyarginine), there appears to be a small amount of MDNA which is preferentially precipitated by polyarginine, but not by polylysine.

We have carried out similar experiments using mixtures of the synthetic polynucleotide, poly dG:dC, and either PDNA or SDNA. Results are shown in Table 4. There is a preferential precipitation of poly dG:dC relative to PDNA or SDNA. [The precipitation curve of dG:dC alone with 160-mer polyarginine is almost exactly like that shown in Fig. 1*b* for polylysine-DNA precipitates, with a stoichiometry of about 1:1. Thus, dG:dC alone is somewhat less readily precipitated by 160-mer polyarginine than are PDNA or SDNA alone. The preferential precipitation of dG:dC in mixtures with DNA manifests itself despite this. The amount of polymer (either DNA or dG:dC) precipitated in all of these experiments at a given ratio of arginine or lysine to nucleotide is, within the limits of

TABLE 4

PRECIPITATION OF MIXTURES* OF dG:dC AND DNA IN 1 *M* NaCl BY POLYARGININE AND POLYLYSINE

Absorbance precipitated, %	Radioactivity precipitated, %	dG:dC:DNA ratio (monomoles) in precipitate divided by ratio in starting solution
(*a*) dG:dC + P^{32}-SDNA + polyarginine (13-mer)		
6	3	3.0
12	5.5	3.3
23.5	11.5	3.1
33.5	13	4.1
(*b*) dG:dC + P^{32}-PDNA + polyarginine (13-mer)		
10.5	8.3	1.5
15.5	10.3	2.0
28.5	16	2.6
36	19.1	2.7
(*c*) dG:dC + P^{32}-SDNA + polyarginine (160-mer)		
3	0	>10
10.5	0	>10
15.5	8.5	2.7
21	11	2.8
(*d*) dG:dC + P^{32}-PDNA + polyarginine (160-mer)		
8	0	>10
15	5.1	4.8
24	12.5	2.8
(*e*) dG:dC + P^{32}-SDNA + polylysine (100-mer)		
16.5	28	0.18
27.5	67.5	0.19
(*f*) dG:dC + P^{32}-PDNA + polylysine (100-mer)		
18	35.5	0.01
32	54	0.18
45	74	0.22

* Mixtures contained 0.5 absorbance unit (260 mμ) each of DNA and poly dG:dC in 1.5 ml final volume of solution. The molar extinction of poly dG:dC at 260 mμ is 7.95×10^3 (ref. 17). The last column is corrected for the inequality of DNA and poly dGdC molar extinction.

error, the amount expected for that species from precipitation experiments with DNA or dG:dC alone.] These results must be compared with the effect of adding polylysine to the same mixtures (Table 4); the preference of polylysine for either PDNA or SDNA, relative to poly dG:dC, is marked.

The polyarginine-DNA reaction in 1 *M* NaCl appears to be reversible. If sufficient polyarginine is added to P^{32}-labeled PDNA to precipitate 31 per cent of the DNA, and a 15-fold excess of cold PDNA added after waiting about 10 min, 82 per cent of the P^{32} which would otherwise be precipitated is rendered soluble.

Discussion.—The reaction between basic polypeptides and DNA appears to be a cooperative phenomenon under some solvent conditions, as suggested by previous workers.[9] In 1 *M* NaCl, addition of polylysine results in creation of two classes of DNA molecules: those which are combined with most of the polylysine and precipitated, and those free in solution, which our sucrose gradient experiments reveal are not complexed with the small amount of polylysine present. Tsuboi *et al.*[3] have shown that it is possible to produce nonuniform binding of polylysine to DNA molecules by working under conditions where both irreversible reaction and a local excess polylysine concentration occur. Under conditions used in our experiments, though the larger aggregates formed after some time may be slow to redissolve, the initial reaction between DNA and polylysine appears to be reversible, as judged both by experiments on the solubilization of radioactive DNA with cold DNA, and by the fact of selectivity for AT-rich DNA. The nonuniform distribu-

tion of polylysine on DNA means that the addition of more polylysine to a DNA molecule partly reacted with polylysine is energetically more favorable than the initiation of a complex with DNA completely free of polylysine, i.e., the reaction is cooperative. (Our results are different from, but not inconsistent with, those of Sober *et al.*,[7] who studied RNA-polylysine interaction at low lysine:nucleotide ratios and low ionic strength, and found noncooperative binding. In the case of small polylysine, these workers found selective protection of regions of the RNA rich in G and C as judged by resistance to enzymic attack.)

It is important to realize that selective precipitation of AT-rich DNA by polylysine (Table 2) does not merely represent a difference in solubility between the complexes of polylysine with PDNA and SDNA; the precipitation curves, analogous to those in Figure 1, for PDNA or SDNA reacted separately with polylysine are identical. We conclude that the free energy of formation of polylysine-DNA complexes varies with the base composition of the DNA, and is more favorable for AT-rich DNA.

The lack of selectivity of polyarginine for DNA's in the range 40–60 per cent AT might be thought to arise from some irreversible step in the reaction; the polyarginine might not dissociate once combined with DNA, and a potential selective mechanism would be unable to operate. The isotope exchange experiments in 1 *M* NaCl show that this is not the case. The selectivity of polyarginine manifests itself only with DNA quite rich in GC content. The result with *M. lysodeikticus* DNA would not, in itself, be particularly convincing evidence for preferential interaction. The results with poly dG:dC is more striking. The fact that polylysine acts in a manner quite opposite to polyarginine when added to DNA-poly dG:dC mixtures rules out the possibility of selection of "denatured" regions in poly dG:dC, since polylysine and polyarginine would behave identically in such a case. (It must be kept in mind that the helical structure of poly dG:dC is not necessarily like that of DNA, and that the polymer is not yet very well characterized. It is possible, therefore, that the preference of polyarginine is actually for small regions of one of the homopolymer strands which are not base-paired.) We conclude that polyarginine has a significantly greater free energy of binding to regions of DNA containing rather long sequences of GC pairs.

It is probably too early to assign a biological role to such interactions, especially since the ionic conditions necessary to demonstrate selectivity are not like those ordinarily found in biological systems. A model of histone specificity in which lysine residues interact with AT pairs and arginine residues interact with GC pairs is seductively attractive, but there is no reason to believe that the other amino acids of the histones play a neutral part in their biological selectivity, if it exists. A number of investigators[18, 19] have suggested that there may be a preferential interaction between each amino acid and its codon; it may be entirely accidental, however, that the codon for lysine is AAA, and that for arginine involves C and G in the first two positions. [The most active triplets in binding of arginine SRNA to ribosomes are CGA and CGU (*E. coli*) and CGA, CGG, CGU (guinea pig). In *Xenopus*, however, AGG is also quite active (Dr. M. Nirenberg, personal communication).] That the preferential interactions we report are perhaps somewhat arbitrary is demonstrated by the reversed selectivity of polylysine observed in 1 *M* tetramethyl ammonium chloride, and by the rather limited manifestation of

selectivity by polyarginine. Clearly, the effects of counterions and of hydrophobic environments both in the solvent and within the polypeptide chain will have to be investigated. We are presently carrying out such studies, and extending our investigation to the histones.

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