Drug–Biomolecule Interactions: Interactions of Mononucleotides and Polybasic Amino Acids

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Abstract □ Histones and ribosomal proteins are basic proteins that participate in gene regulation and protein synthesis, respectively. How these proteins interact with nucleic acids is not yet clear, although specificities in these interactions have been observed. Study of the interaction of mononucleotides with basic polyamino acids is one approach to understanding such interactions. The results of studies with the mononucleotides can help elucidate the normal molecular processes in biological systems and also shed light on some effects of drugs, such as puromycin and tubericidin, that are nucleotide derivatives. A review of studies on the interaction of mononucleotides and basic polyamino acids such as polylysine and polyarginine is presented. In addition, a short review of the self-associative properties of mononucleotides is given. Studies of the mononucleotide–polyamino acid interaction have involved a wide variety of techniques including equilibrium dialysis, NMR, optical rotatory dispersion, circular dichroism, and precipitate analysis.

Keyphrases □ Mononucleotides—interactions with polybasic amino acids □ Amino acids, polybasic—interactions with mononucleotides □ Drug–biomolecule interactions—mononucleotides and polybasic amino acids □ Interactions—drugs with biomolecules, symposium

Interactions of the components of nucleic acids and proteins are significant factors in the regulation of gene expression (transcription) and the control of protein synthesis (translation). These interactions are also involved in the reactions of mononucleotides and enzymes and, possibly, in the biological activity of nucleotide analog drugs such as puromycin. *In vitro* studies of the interactions of amino acids and nucleic acids and of polyamino acids and nucleic acids have shed some light on the nature of the interactions of nucleic acids and proteins *in vivo*.

There have been only a few reported studies of the interactions of amino acids and nucleic acids, but these studies indicate that there is little tendency for amino acids to bind to nucleic acids and that there is little or no specificity for the binding observed (1). However, numerous studies of polyamino acid–nucleic acid interactions revealed significant instances of specific binding. These latter studies mostly utilized polybasic amino acids such as polylysine, polyarginine, and polyornithine because of the high electrostatic affinity between these positively charged polyamino acids and the negatively charged ribose phosphate backbone of the nucleic acid.

Although charge–charge interactions are the main attractive forces between polybasic amino acids and nucleic acids, other forces are also operative, resulting in binding specificities. For example, Leng and Felsenfeld (2) found that polylysine has a preferred binding affinity for adenine–thymine-rich regions in DNA. A weaker specificity of interaction between poly-L-arginine and guanine–cytosine regions was also observed. Later, Shapiro *et al.* (3) showed that the preference of poly-L-lysine for adenine–thymine regions could be changed to guanine–cytosine regions by the addition of tetramethylammonium salts.

Furthermore, RNA polymerase has been shown to bind to a greater extent to poly dA-T than to T_4 DNA (4). The mechanism leading to such specificities is not known. Specificities in the binding of histones and nonhistone acidic proteins have also been found (5), but the mechanism behind these specific interactions is also unknown.

Studies of the binding of ribosomal proteins to ribosomal RNA recently showed that at least five ribosomal proteins of the 30S subunit of *Escherichia coli* ribosomes have specific binding sites on the 16S ribosomal RNA (6). Again, the basis for the specificity is not known.

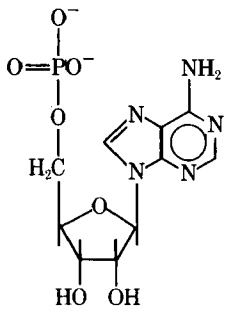
These comments are intended to place the present review of mononucleotide–polyamino acids in the proper perspective. This article is mainly a review[1] of the literature reports on mononucleotide–polybasic amino acid interactions. However, also included are some previously unreported studies from the authors' laboratory regarding the mechanism by which a lysine side chain might approach an adenine ring in a mixture of adenosine 5′-monophosphate and poly-L-lysine.

DISCUSSION

Properties of Interactants and Methods of Study—*Basic Polyamino Acids*—At pH values well below the pKa of the basic side chains, all of the side chains are positively charged and the polymers tend to assume a random coil configuration due to electrostatic repulsion. The basic polyamino acids are very soluble due to the ionic nature of the side chains. As this charge is eliminated by elevation of pH or masked by the addition of salts, the basic polyamino acid tends to go into a helical form.

The principal means for studying this transition have been optical rotatory dispersion and circular dichroism (7, 8). The random coil to helix transition normally gives rise to perturbations of the spectra in the 220–230-nm range. It has been found that poly-L-lysine and poly-L-arginine form helixes much more readily than poly-L-ornithine.

[1] Literature search was completed in August 1972.



I

Mononucleotides—The 5′-mononucleotides have several interesting properties relevant to their interaction with basic polyamino acids. In solution, the ribose-phosphate moiety is capable of freely rotating so that it might be expected to occupy randomly all possible positions relative to the plane of the organic base ring. However, NMR studies (9) indicate that the *anti*-conformation (I) is preferred over the opposite *syn*-conformation.

Another property of the mononucleotides is their propensity to self-associate so that their organic base rings are essentially parallel to each other and one above the other, forming a vertical array. This property is called base stacking and has been studied in solution using NMR. As a consequence of base stacking, signals from protons attached to the purine and pyrimidine ring systems of nucleotides are shifted upfield (to lower frequencies). The magnitude of the shift is determined by the extent of interaction of the stacking ring systems (9).

X-ray diffraction studies have confirmed the stacking tendencies of the nucleotides in the solid state (10). The tendency to self-associate by stacking is in the following order: guanosine 5′-monophosphate > adenosine 5′-monophosphate > cytidine 5′-monophosphate > uridine 5′-monophosphate.

Interaction of Mononucleotides and Basic Polyamino Acids—The work discussed here is concerned with the specificities of these interactions and with their physical–chemical basis. The effects on the interactions of changes in pH, ionic strength, length of amino acid side chain, and relative concentrations of reactants are also considered.

The history of the study of mononucleotide–polyamino acid interactions is short. The first reports in the literature came from Woese's laboratory and appeared in 1968 (11). Using equilibrium dialysis and turbidity measurements, Woese studied the interaction of the various 5′-nucleotides with poly-L-arginine. Similar experiments were already underway in the authors' laboratory. Woese found that turbidity developed when solutions of guanosine 5′-monophosphate, adenosine 5′-monophosphate, and cytidine 5′-monophosphate were mixed with solutions of poly-L-arginine. No turbidity changes were observed with uridine 5′-monophosphate. He also observed that the ease of formation of turbidity in mixtures with poly-L-arginine decreased in the order: guanosine 5′-monophosphate > adenosine 5′-monophosphate > cytidine 5′-monophosphate > uridine 5′-monophosphate. Woese pointed out that this order is the same as that observed for the base stacking tendencies of the nucleotides.

The authors found precisely the same order for the concentration dependence of turbidity changes[2] when the nucleotides were mixed with solutions of poly-L-lysine (Fig. 1) (12). These experiments were carried out in the absence of added salt to maximize the electrostatic attractions between the reactants. The addition of small amounts of sodium chloride blocked the turbidity-producing interactions. As shown in Fig. 1, the addition of nucleosides or potassium phosphate (K_2HPO_4) to solutions of poly-L-lysine did not produce significant changes in turbidity.

The changes in turbidity were due to the formation of small, translucent spheres which could be easily separated by centrifugation. Because of this property, it was possible to separate the interaction products and calculate their stoichiometry by analyzing the composition of the supernate. These data (Table I) can be interpreted in terms of a model that specifies an initial charge–charge attraction between the negative phosphate groups on the nucleotides and the positively charged ϵ-amino group of the lysine. Aggregation follows as a consequence of base stacking of bound nucleotides. This model is based on the following interpretation of the data.

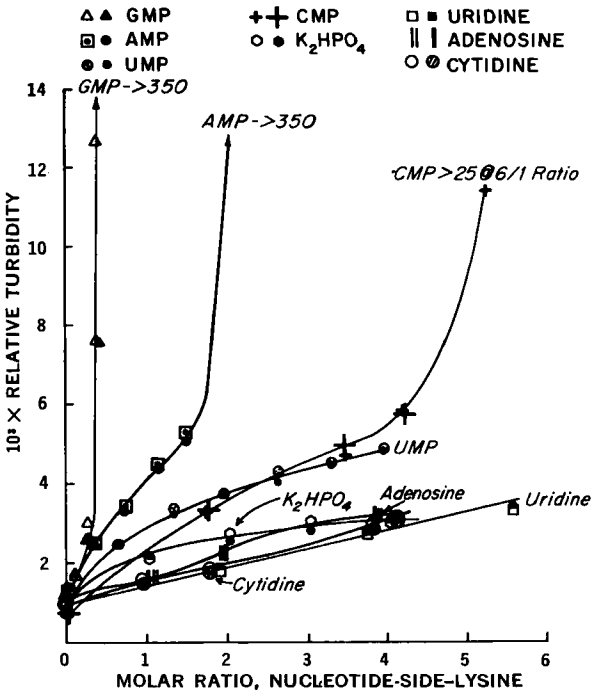


Figure 1—*Changes in relative turbidity of solutions of poly-L-lysine (100,000 mol. wt.) as solutions of 5′-mononucleotides, mononucleosides, or potassium phosphate were added at pH 8.0–9.0. The initial poly-L-lysine concentration was 1 × 10^{-3} M (lysine residue basis). Key shows symbols for duplicate samples of each additive. Numbers at top of guanosine 5′-monophosphate (GMP), adenosine 5′-monophosphate (AMP), and cytidine 5′-monophosphate (CMP) curves are extreme turbidity values reached. All turbidity values were corrected for dilution and for solvent scattering.*

At pH 8.0, all nucleotides have a net charge of −2 and the lysine residues have a charge of +1. The predicted stoichiometry for a simple charge–charge interaction would be two lysine residues for each nucleotide molecule. This is approximately the value observed for each nucleotide at pH 8.0.

At very low pH, the net negative charge on the nucleotide decreases because of protonation of both the phosphate and the purine–pyrimidine rings. At high pH, the proton is removed from the ϵ-amino group of the lysine. Thus, charge–charge interactions are different at pH extremes and the number of nucleotides bound should vary accordingly. This effect was confirmed for adenosine 5′-monophosphate. At pH 4, approximately the pKa of the nucleotide ring, adenosine 5′-monophosphate has one negative charge on the phosphate group and an average of one-half of a negative charge on the ring. The average number of bound nucleotide residues was found to increase to 1.0.

As the pH is raised, the number of bound nucleotide residues decreases. As the pH approaches the pKa of the ϵ-amino group of the lysine, the number of nucleotide residues bound decreases until no further precipitation is observed at pH 12.5.

The fact that no significant turbidity increases were found when nucleosides (lacking the phosphate group) were added to polylysine solutions further substantiates the importance of charge–charge interactions in the binding process.

The involvement of base stacking in the aggregation is suggested by the fact that the effectiveness of nucleotide precipitation of polylysine is in the order guanosine 5′-monophosphate > adenosine 5′-monophosphate > cytidine 5′-monophosphate > uridine 5′-monophosphate and that this is the same order for nucleotide base stacking tendencies.

The range of intensity of the nucleotide–polylysine aggregation reaction is very great down the series from guanosine 5′-mono-

[2] A Brice-Phoenix model 2000 light-scattering photometer was used (25°).

Table I—Stoichiometry of Binding of Nucleotides to 100,000 Molecular Weight Poly-L-lysine

Nucleotide	pH	Approximate Initial Molar Ratio Nucleotide to Lysine Residues	Molar Ratio of Nucleotide to Lysine in Residues[a]
Guanosine 5′-monophosphate	8.0	0.4	0.38
		1.0	0.40
		1.5	0.43
Adenosine 5′-monophosphate	4.0	2	No precipitate
		4	No precipitate
		8	1.0
	5.5	1	No precipitate
		2	0.60
		3	0.47
	8.0	1	No precipitate
		2	0.50
		3	0.65
		4	0.50
	11.0	1	0.16
		2	0.22
		4	0.23
	12.5	2	No precipitate
		4	No precipitate
		8	No precipitate
Cytidine 5′-monophosphate	8.0	2	No precipitate
		4	0.58
		8	0.61
Uridine 5′-monophosphate	8.0	10	0.79
		10	0.60
		10	0.89

[a] Each number is an average of duplicate analyses and was calculated by difference in concentrations before and after precipitation. Experiments were carried out at room temperature. In each experiment, the initial poly-L-lysine concentration was 1×10^{-2} *M* (lysine residue basis). The pKa of the ϵ amino group of poly-L-lysine is 10.5. The nucleotides each have a phosphate pKa at about 6.5.

phosphate to uridine 5′-monophosphate. At pH 8.0, with reactants present in equimolar amounts, guanosine 5′-monophosphate is quantitatively precipitated from polylysine solution; under the same conditions with a 10-fold molar excess of uridine 5′-monophosphate over lysine residues, only a small amount of precipitation is observed. The specificities are also clearly shown by the turbidity curves in Fig. 1.

At the same time that these experiments were being done, space-filling models were constructed to explore the stereochemical properties of the polylysine–nucleotide interactions. Around a poly-L-lysine molecule in an α-helical form, one could fit an associated helix of base-stacked mononucleotides in the *anti*-conformation (Fig. 2). This model suggested that the 2′-OH group might be an important factor in stabilizing the interaction. It also predicted a maximum binding of three nucleotides per lysine residue, a stoichiometry never observed in the laboratory.

Wagner and Arav (13) also studied the binding of mononucleotides to poly-L-lysine and poly-L-arginine, using equilibrium dialysis at pH 7.0 in the presence of 0.02 *M* acetate. Under these conditions, the declining order of association constants for nucleotide interactions with both poly-L-lysine and poly-L-arginine was guanosine 5′-monophosphate > adenosine 5′-monophosphate > cytidine 5′-monophosphate > uridine 5′-monophosphate. The same hierarchy was found for turbidity development (phase separation).

Wagner (14) was the first to point out that, at low nucleotide concentrations, binding of mononucleotides by polybasic amino acids was essentially noncooperative, followed Langmuir binding curves, and did not produce phase separation. However, as nucleotide concentration was increased at constant polyamino acid concentration, the binding became cooperative and phase separation occurred. Wagner also found that the binding affinities of nucleotides for poly-L-arginine was in the order guanosine 5′-monophosphate > adenosine 5′-monophosphate > cytidine 5′-monophosphate.

Wulff *et al.* (15) reported a continuation of this work; they studied the binding of 5′-ribonucleotides to poly-L-lysine using circular dichroism. These experiments were carried out at 1°, pH 7, in the presence of 0.02 *M* KF. Poly-L-lysine and nucleotide concentrations were 1.4 and 0.5 m*M*, respectively. Under these conditions, only guanosine 5′-monophosphate showed circular dichroic spectra that differed significantly from the sum of the spectra of the individual components. The investigators concluded that the binding of guanosine 5′-monophosphate resulted in a cooperative ordering of guanosine 5′-monophosphate molecules, probably mediated by increased base stacking. There was some indication from the data, based on circular dichroism effects at ~220 nm, that the poly-L-lysine was assuming a more helical conformation.

Rifkind and Eichorn (16) carried out an extensive study of the effect of the 5′-ribonucleotides on the helicity of poly-L-lysine, poly-L-arginine, and poly-L-ornithine. They employed optical rotatory dispersion and used equimolar amounts of monomer and polymer at either 1×10^{-3} or 5×10^{-4} *M*. Phase separation did not occur. They studied the interaction at pH 4 and 6 in both water and 60% dioxane. In water, the only nucleotides causing significant increases in helicity of any of the polyamino acids were guanosine 5′-monophosphate and inosine 5′-monophosphate (with poly-L-arginine at pH 6).

In 60% dioxane, all 5′-nucleotides induced helicity in poly-L-lysine at pH 6. At pH 4, guanosine 5′-monophosphate, inosine 5′-monophosphate, and adenosine 5′-monophosphate induced helicity in poly-L-lysine. Poly-L-ornithine became more helical only with guanosine 5′-monophosphate at both pH 6 and 4. Poly-L-arginine became more helical with all nucleotides at pH 6 (guanosine 5′-monophosphate > adenosine 5′-monophosphate > inosine 5′-monophosphate > uridine 5′-monophosphate > cytidine 5′-monophosphate) and with guanosine 5′-monophosphate > adenosine 5′-monophosphate > inosine 5′-monophosphate at pH 4. Changes in the optical rotatory dispersion spectra at ~233 nm were used to measure helicity in the polyamino acid. Greater helical ordering of the nucleotide was observed at longer wavelengths where it was found that guanosine 5′-monophosphate became more ordered than the rest of the nucleotides, probably due to its greater ability to self-associate. Rifkind and Eichorn (16) concluded that the overall ability of the 5′-ribonucleotides to induce helicity in polyamino acids was guanosine 5′-monophosphate > inosine 5′-monophosphate > adenosine 5′-monophosphate > cytidine 5′-monophosphate ≈ uridine 5′-monophosphate, which is in agreement with base-stacking tendency (17).

These results, together with those already mentioned, make it clear that the association of the nucleotides with the basic polyamino acids consists of a primary electrostatic attraction, without which the interaction does not take place, and a secondary one, the

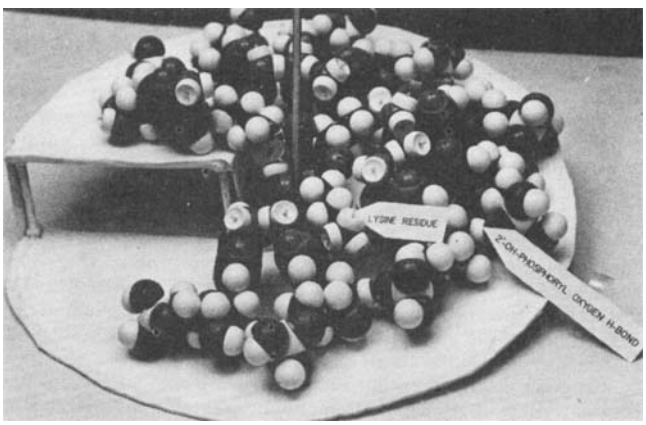


Figure 2—*Photograph of a molecular model of an α-helix of poly-L-lysine inside and complementary helix of associated adenosine 5′-monophosphate monomers outside. Note lysine side chains intercalated between adenine rings. Adenine rings are base stacked, and the nucleotides are in an approximate* anti-*conformation. The outside of the complex is a continuous sequence of ribose phosphates stabilized by hydrogen bonding of 2′-OH on one nucleotide to the phosphoryl oxygen of the next. Although the model shows hydrogen bonding of the —NH_2 of one adenine to the —NH_2 of the next, this is not a known property of adenine–adenine interactions and may not be operative in actuality.*

association of the nucleotides with themselves. The extent of cooperation of these two attractions then determines the final strength of binding.

The cooperative nature of these interactions at high nucleotide concentrations has been treated in detail (14, 18).

In model-building experiments (12), the 2′-OH groups of the nucleotides may have participated in nucleotide–nucleotide interactions through hydrogen bonding of the ribose on one nucleotide to the phosphoryl oxygen on a neighboring nucleotide. Several studies (18, 19) examined the importance of the 2′-OH group. Circular dichroic spectra showed that guanosine 5′-monophosphate and guanosine 3′-monophosphate (with 2′-OH groups) both caused changes in the spectra of poly-L-lysine at short wavelengths, indicating polyamino acid conformational change, and at high wavelengths, indicating association of the bases. However, deoxyguanosine 5′-monophosphate and deoxyguanosine 2′-monophosphate, lacking 2′-OH groups, caused only minor spectral changes.

In equilibrium dialysis experiments with the same set of nucleotides, it was found that nucleotides having the 2′-OH group possessed considerably greater nearest neighbor interaction energies than those lacking the 2′-OH group. The stacking coefficients decreased in the order guanosine 3′-monophosphate > guanosine 5′-monophosphate > deoxyguanosine 5′-monophosphate > guanosine 2′-monophosphate. The 2′-OH group is obviously an important factor in stabilizing these interactions.

Wagner (20) also explored the effect of variations in amino acid side-chain length on the interaction of polybasic amino acids with guanosine 5′-monophosphate. Polyarginine, polyhomoarginine, polyornithine, and polylysine were studied at 0°, pH 7, and in the presence of 0.02 *M* sodium acetate. The binding constants under these conditions decreased in the order polyarginine > polyhomoarginine > polyornithine > polylysine. Thus, the side chains with stronger basicity and shorter chain length had greater affinity for the nucleotide. The cooperativity of guanosine 5′-monophosphate interaction was about the same with all polymers. Circular dichroism results showed that poly-L-lysine fit best into the ordered guanosine 5′-monophosphate structure even though the other polymers had greater binding constants.

NMR Studies of Adenosine 5′-Monophosphate with Poly-L-lysine—In an NMR study of monoamino acid–mononucleotide interactions at high concentrations (21), the authors observed that adenosine 5′-monophosphate, but not the other three common mononucleotides, caused changes in the chemical shifts of amino acids. Furthermore, all three amino acids studied (lysine, arginine, and proline) showed these changes. The changes in chemical shift were downfield from the reference signal. This kind of change indicates that the amino acid side chain is oriented in the same plane as the nucleotide base ring. Since none of the other nucleotides caused such changes, it was decided that there was a generalized amino acid–adenosine 5′-monophosphate association.

The same interaction with poly-L-lysine and adenosine 5′-monophosphate was then studied. First a polymer having a molecular weight of about 100,000 was tried. After the addition of adenosine 5′-monophosphate, the lysine protons were not easily resolved. A polymer with a molecular weight of 3000 was then used. At a poly-L-lysine concentration of 0.46 *M* and pH 8.0, additions of adenosine 5′-monophosphate did not cause phase separation but did cause downfield shifts of the lysine side-chain protons (Fig. 3) as the adenosine 5′-monophosphate concentration increased. In the presence of the poly-L-lysine, the ring protons of the adenine ring showed an upfield shift with the H_8 proton shifting more than the H_2. Such upfield shifts result when base stacking occurs.

Schweizer *et al.* (9) earlier reported such a shift of the adenine proton signals of adenosine 5′-monophosphate in the presence of poly-L-lysine, but these investigators did not mention changes in the position of the lysine proton signals. These data may indicate that the lysine side chain is approaching the edge of the adenine ring in the same plane as the ring and that polylysine induces tighter stacking of the adenosine 5′-monophosphate rings. The greater shift of the H_8 proton signals would occur if the imidazole rings of the purines overlapped more than the six-membered rings. Such an offset of the rings suggests helical ordering. These data also suggest why poly-L-lysine binds preferentially to adenine–thymine-rich regions of DNA. The basis for this specificity of binding is probably the affinity of the lysine side chains for the adenine rings exposed in the grooves of the adenine–thymine regions of DNA.

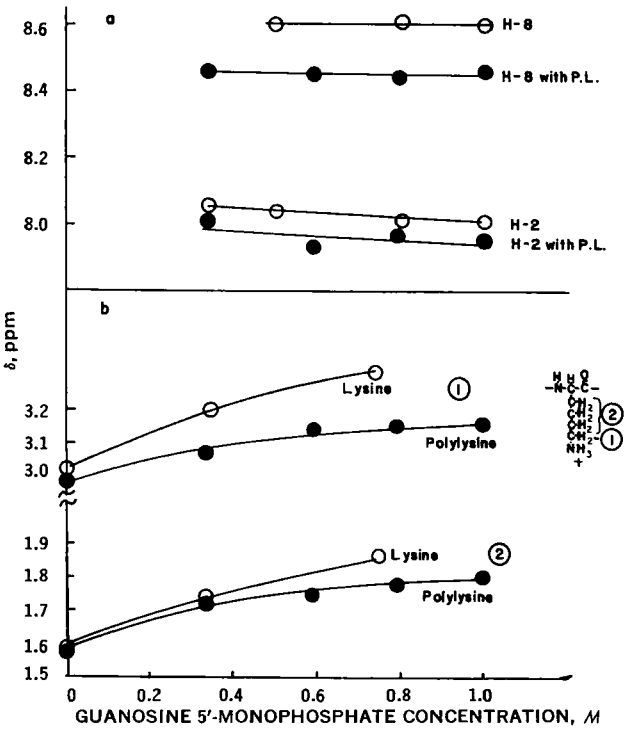


Figure 3—(*a*) *Positions of PMR signals of the protons of the adenine ring of adenosine 5′-monophosphate in aqueous (D_2O) solution at pH 8.0 and various adenosine 5′-monophosphate concentrations without* (○) *and with* (●) *poly-L-lysine (0.46* M *residue basis). An internal standard, 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionic acid sodium salt, was used.* (*b*) *Positions of the amino acid side-chain protons of lysine* (○) *and poly-L-lysine* (●), *as identified in the formula, at various adenosine 5′-monophosphate concentrations at pH 8.0. Positions are relative to the internal standard. All data were collected at 25°.*

The important role played by base stacking in polyamino acid–nucleotide interactions suggests the possibility of ring–ring interactions between aromatic amino acids and DNA. There have been some reports of such interactions (22, 23). These studies have generally shown ring stacking of the aromatic amino acids with the rings of the nucleic acid bases.

SUMMARY

The results reviewed here show that the interactions of nucleotides with polybasic amino acids are strongly influenced by the nucleotide–nucleotide interactions that occur once the monomers are bound to the polyamino acid. The charge–charge interactions between negative phosphate groups and positively charged amino acid side chains are common features of all pairs of reactants. The specificity of the interaction is determined to a large extent by the variations in self-associating tendencies of the bound nucleotides.

When the monomer binding sites on the polymer are close enough to each other to allow bound monomers to interact, a cooperative binding occurs which is dependent on the specific nucleotide–nucleotide interactions. Polymer structure influences the binding process because the structure of the polymer determines the spatial relationships of the monomer binding sites. Thus, the monomer binding is influenced by such factors as the length of the amino acid side chain on the polyamino acid and helix–coil transitions in the polymer.

Both specific and nonspecific interactions can be influenced by changes in experimental conditions affecting the forces responsible for the binding. Specificity is enhanced by conditions favorable to base stacking, and binding can be prevented entirely by conditions that interfere with charge–charge interactions.

On the basis of experience with mononucleotide–polybasic amino acid interactions, it is possible to make important inferen-

ces regarding the interactions of polypeptides and nucleic acids. The coplanar interaction of lysine side chains and adenine rings suggests that exposed adenine rings in adenine–thymine-rich regions of nucleic acids may serve as important binding sites for regions of polypeptide chains having amino acid residues of appropriate structure.

Finally, these studies have important implications for the synthesis of drugs intended to interact with specific regions of nucleic acids. Such drugs should be designed to take advantage of the specificity that can be achieved by cooperative self-association once initial attachment is made to the nucleic acid.

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Drug–Biomolecule Interactions: Mechanisms and Kinetics of Interactions of Biomolecules at Interfaces

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Abstract □ At the air–water interface, the area occupied by the molecules of each component of mixed monomolecular layers of cholesterol with hexadecyl alcohol, hexadecylic acid, or hexadecylamine was independent of the presence of the other component. The values of the free energy of mixing of these lipids were within the range of the entropic factor of the free energy even at high surface pressures. Mixed monolayers of cholesterol and bovine serum albumin showed a similar independence of the area per molecule and of the free energy of mixing values when the concentration of the protein was expressed in terms of amino acid residues per molecule of protein forming the mixed monolayer. Higher values of the free energy of mixing were obtained for mixed monolayers of cholesterol with dipalmitoyl lecithin and dipalmitoyl phosphatidylethanolamine than were expected from an entropic factor. The interaction between monomolecular layers of lipidic biomolecules with bulk subphase components, the energy of activation, interaction kinetics, and effects of added electrolytes were also studied. The implications of these data to a mechanism of action are discussed.

Keyphrases □ Phospholipid monolayer interactions—mechanisms and kinetics at interfaces □ Interfaces—mechanisms and kinetics of interactions, surface pressure and surface potential □ Monolayers, phospholipid—mechanisms and kinetics of interactions of biomolecules of interfaces □ Drug–biomolecule interactions—mechanisms and kinetics of interactions of biomolecules at interfaces □ Interactions—drugs with biomolecules, symposium

Drug molecules incorporated into body fluids are distributed and directed to receptors located in tissue cells. If the receptors form part of the cell membrane, as seems to be the case for some local anesthetics (1) and bactericides (2), the interaction with the receptor alters the permeability of the cell membrane and thus completely defines the pharmacological activity of the drug. If the receptors are located in subcellular