Specificity for the Interaction of Nucleotides with Basic Polypeptides*

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ABSTRACT: Optical rotatory dispersion was used to study the interaction of the nucleotides guanosine monophosphate (GMP), inosine monophosphate (IMP), adenosine monophosphate (AMP), cytosine monophosphate (CMP), and uridine monophosphate (UMP) with poly-L-Lys, poly-L-Orn, and poly-L-Arg in water and 60% dioxane. It was shown that the nature of the nucleotide as well as the polypeptide govern the manner in which the interaction occurs, thus producing a variety of specific interactions. The *nucleotide specificity* is manifested in two ways: (1) The stabilization by the nucleotides of the helical conformation of all of the polypeptides, as shown in the far-ultraviolet region, increases with the base-stacking ability of the nucleotides. This phenomenon can be explained by steric interference of the nucleotides bound to a helical polypeptide necessitating at least partial orientation of the bases. (2) The nucleotides differ among themselves in undergoing conformational changes when oriented around the polypeptides, giving rise to changes in the near-ultraviolet optical rotatory dispersion. Since the geometry for base stacking around a helical polypeptide is quite poor, GMP appears to be the only nucleotide that undergoes appreciable base stacking. The near-ultraviolet spectra of CMP and AMP also undergo significant changes that are quite different from those displayed by GMP. The *polypeptide specificity* hinges on the nature of the side chain. The helical conformation of poly-L-Arg is stabilized more readily than that of poly-L-Lys. Nevertheless, the stacking of GMP occurs much less readily around poly-L-Arg than around poly-L-Lys. The peculiar behavior of poly-L-Arg can be explained by the ability of the guanidinium group to interact simultaneously with two oxygens of the phosphate. This interaction presumably produces a relatively rigid ring that alleviates the steric interference of the nucleotides and limits the ability of GMP to stack.

It is generally believed that histones are at least partially responsible for the regulation of genetic activity (Bradbury *et al.*, 1967). This phenomenon could occur through the recognition of specific regions of DNA by various histones which in turn would require specificity in the interaction of amino acids with nucleotides.

The first evidence of this sort of specificity came from studies involving the complexing of basic polypeptides with nucleic acids (Leng and Felsenfeld, 1966) which indicated a specificity of poly-L-Lys for (A-T)-rich nucleic acids under certain conditions.

In a recent paper, Wagner and Arav (1968) used equilibrium dialysis to study the binding of the various nucleotides to poly-L-Lys and poly-L-Arg at pH 7. They found a relatively minor variation of binding constants with poly-L-Arg, with parallels the tendency for the nucleotide bases to stack, and essentially no variation with poly-L-Lys. The limited specificity of the amino acid–nucleotide interaction in their work as well as the work suggesting a specificity of the polynucleotide–polypeptide interaction is a specificity of binding, *i.e.*, a difference in binding constant. The binding is, of course, mainly electrostatic in that the positive amino acid residue is bound to the negative nucleotide, and would not be expected to show any large variation.

It occurred to us that a more sensitive measure of the specificity of the interaction involved may be obtained by looking for changes in conformation. If a change in conformation can be produced by the interaction between nucleic acids and proteins, this change would be expected to be dependent on the geometry and structure of the particular nucleotides and amino acids involved.

In this paper our results, using optical rotatory dispersion, on the interaction of the ribonucleotides with various basic polypeptides under conditions where there exists a dramatic conformational specificity are reported.

We consider these results relevant to the problem of specific interactions of polynucleotides with polypeptides, since it is reasonable that the reactivity of the monomers is reflected in the polymeric structures. However, we wish to point out that in the polynucleotides the monomeric units are constrained by the ribose phosphate backbone, which certainly should affect these interactions.

At neutral and acidic pH values the basic polypeptides are in the random coil conformation, and their side chains are all positive. The nucleotides neutralize some of the positive charge and stabilize the helical conformation, the stable conformation which exists when the positive charges are neutralized by raising the pH (Applequist and Doty, 1962; Chaudhuri and Yang, 1968; Rifkind, 1969).

These changes involve the orientation of the nucleotides around a helical polypeptide as well as distortions in the configuration of the nucleotides. We find that the observed effects depend both on the specific polypeptide and nucleotide. In fact, by looking at the effect of the interaction of the nucleotides with poly-L-Lys, poly-L-Arg, and poly-L-Orn it is

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possible to qualitatively differentiate all the nucleotides studied.

Experimental Section

Polypeptides. Poly-L-Lys·HBr and poly-L-Orn·HBr were purchased from Pilot Chemicals, Watertown, Mass. Two lots of poly-L-Lys were used with stated molecular weights of 92,000 and 52,000 (lot no. L-64 and L-47). The poly-L-Orn had a stated molecular weight of 93,000 (lot no. O-5). Poly-L-Arg·$0.5H_2SO_4$ was purchased from Yeda Research and Development Co., Israel, and poly-L-Arg·HCl was purchased from Pilot Chemicals, Watertown, Mass. The stated molecular weights were 28,000 (lot no. AR-12 and AR-14) for poly-L-Arg·$0.5H_2SO_4$ and 50,000 (lot no. AG-4) for poly-L-Arg·HCl. Solutions (0.01 M) of the polypeptides were prepared.

In all cases an optical rotatory dispersion spectrum of the polypeptide was obtained at neutral pH in water where the polypeptide exists as a random coil, and the mean residue rotation at 205 mμ for poly-L-Lys was used to determine the actual concentration.

Other Materials. The mononucleotides were obtained from Sigma. Solutions (0.01 M) were prepared by weighing the desired amount of material and dissolving in H_2O; the actual concentrations were determined from ultraviolet spectra. The dioxane used in this work was reagent grade.

Methods. Optical rotatory dispersion spectra were measured with a Cary 60 spectropolarimeter at an ambient temperature of 24 ± 1° using 1-mm cells. Spectra were obtained from 320 mμ to the far-ultraviolet cut-off, which varied between 220 and 190 mμ. The solutions used for the optical rotatory dispersion were prepared by diluting the 0.01 M nucleic acid components and/or polypeptides with the required volumes of water, or water and dioxane, to prepare solutions 1×10^{-3} or 5×10^{-4} M in peptide and/or nucleotide. The lower final concentrations were used when solubility problems arose, and to clarify ambiguities resulting from the necessity of using the lower concentration in certain cases. The concentrations are indicated in the appropriate figures and tables. The pH was adjusted by using concentrated acid or base.

The nucleotide was generally added to the diluted polypeptide. In several instances this procedure was reversed, with the same results.

Optical rotatory dispersion spectra were measured using freshly mixed solutions of the polypeptides and the nucleotides. The solutions were then stored for 1 day and measured again in order to determine whether the differences obtained could have resulted from a failure to reach equilibrium. Several significant time variations were discovered in this manner.

The interaction of the nucleotides with the polypeptides was studied in water and a water–dioxane mixture of 60% dioxane (by volume) at apparent pH values of 6 and 4. In certain cases spectra were also obtained at pH 5.

Ultraviolet spectra were measured on a Cary 14 spectrophotometer. pH measurements were made with a Radiometer Model 25 pH meter.

Results

The nucleotides alone have an optical rotatory dispersion pattern with one Cotton effect in the region from 240 to 300 mμ with the crossover corresponding quite closely with the maximum of their absorption band (Yang *et al.*, 1966). The sign and magnitude of the Cotton effect depends on the particular nucleotide. The pyrimidine nucleotides usually have relatively large positive Cotton effects while the purine nucleotides have smaller negative Cotton effects.

The basic polypeptides which we have studied do not absorb in this region and contribute a gradually increasing negative rotation with decreasing wavelength, irrespective of the particular conformation they are in. Therefore, changes in the optical rotatory dispersion pattern of the nucleotide as a result of the interaction with the polypeptide can be readily observed, and it is possible to monitor changes being produced on the nucleotides in the presence of the polypeptides.

The contribution to the optical rotatory dispersion curves of a polypeptide arising from the peptide group are large and very well documented in both the random coil and α-helical conformations (Holzwarth and Doty, 1965). This contribution is at least qualitatively the same for all polypeptides that have been investigated, including the basic polypeptides which we have used in our study. For the polypeptides complexed with nucleotides a qualitatively similar contribution to the optical rotatory dispersion curve should be obtained. This is particularly true since the nucleotide is thought to be attached to the positively charged side chain (Wagner and Arav, 1968) and, therefore, quite far removed from the peptide group.

The random coil optical rotatory dispersion has a minimum at 205 mμ with a reduced residue rotation reported for poly-L-Lys (Davidson and Fasman, 1967) of −21,930°. The right-handed α helix has a minimum at 233 mμ with a shoulder at 210–215 mμ and a maximum at 198.6 mμ. For poly-L-Lys (Davidson and Fasman, 1967) the minimum for the α helix has a reduced residue rotation of −14,720° and the maximum a reduced residue rotation of +70,860°.

The nucleotides also contribute to the optical rotatory dispersion in the region below 240 mμ (Yang *et al.*, 1966). Nevertheless, it is frequently possible to monitor changes in the conformation of the polypeptide even in the presence of nucleotides. If there is no significant change in the optical rotatory dispersion above 240 mμ and, therefore, no drastic change is taking place at the nucleotide, it can be assumed that the optical rotatory dispersion contribution of the nucleotide below 240 mμ is approximately the same as it would be in the absence of the polypeptide. Under these conditions, a negative change in rotation at 233 mμ followed by a larger positive change in rotation further in the ultraviolet is clear evidence for an ordering of the polypeptide. When there are significant changes in the optical rotatory dispersion above 240 mμ, the possibility of suggesting a change in the conformation of the polypeptide depends on the relative magnitude of the changes in the near- and far-ultraviolet region, and the shape of the entire optical rotatory dispersion curve.

From our optical rotatory dispersion results we cannot unambiguously differentiate between a helical conformation and a β conformation (Davidson and Fasman, 1967). We have interpreted out results in terms of a helical conformation because of the role of dioxane in stabilizing the ordered conformation in the presence of the nucleotides. Dioxane affects the conformation of polypeptides in the same way as methanol (Chaudhuri and Yang, 1968). Methanol has been

TABLE I: Change in the Mean Residue Rotation at 233 mμ, as a Result of the Interaction of Nucleotides with Polypeptides, Both 1×10^{-3} M, in Water.[a,b]

	$[m]_{233} \times 10^{-3}$					
	Poly-L-Lys		Poly-L-Orn		Poly-L-Arg	
Nucleotide	pH 6	pH 4	pH 6	pH 4	pH 6	pH 4
GMP	−1.0	−1.0	−0.2	+0.1	**−11.0**	−1.5
IMP	+1.5	+4.0	+3.0	+2.0	**−8.5**	+3.0
AMP	+0.1	−0.4	−0.9	−0.6	−0.4	−0.2
CMP	+0.6	−0.4	−0.7	−0.8	+0.7	+0.2
UMP	+0.1	+0.4	+0.8	+0.3	+0.3	+0.5

[a] The helical conformation of the polypeptides has a large negative rotation at 233 mμ relative to that of the random coil. Therefore, a significantly negative value in the table indicates that the interaction stabilizes the helical conformation. [b] Boldface type is used when a large negative change in $[m]_{233}$ occurs and the entire optical rotatory dispersion spectrum is consistent with a significant stabilization of the helical conformation. Regular type indicates that the optical rotatory dispersion spectrum shows no significant change in the conformation of the polypeptide even though there may be an appreciable change in the molar rotation at 233 mμ.

shown to stabilize the helical conformation (Chaudhuri and Yang, 1968; Epand and Scheraga, 1968) and to destabilize the β conformation (Davidson and Fasman, 1967).

The interaction of the ribonucleotides GMP, IMP, AMP, CMP, and UMP with poly-L-Lys, poly-L-Orn, and poly-L-Arg was studied at pH 4 and 6 in the absence of any added electrolyte.

This pH range was chosen because it is below the p*K* for the secondary phosphate which is about 6.5 (Jordan, 1960) and above the p*K* for the protonation of the nucleotide bases (Jordan, 1960). The only exception is CMP for which the p*K* for the protonation of the base is 4.4 (Fox *et al.*, 1953). In this pH region the charge on the mononucleotides in the absence of the polypeptides is comparable with that of the polynucleotides at neutral pH where the phosphate has one negative charge and the bases are not protonated. This analysis suggests that the results at pH 6 and 4 should be very similar. However, in certain cases there are qualitative differences between the pH 6 and 4 results. In these cases the optical rotatory dispersion spectrum was also measured at pH 5 to help to determine whether the differences can be attributed to the partial ionization of the phosphate at pH 6 or the partial protonation of the ring at pH 4. It is necessary to consider the possibility of significant shifts in various p*K*'s as a result of the interaction.

Interaction of Polypeptides and Nucleotides in Water. The interaction of poly-L-Arg with GMP or IMP at pH 6 stabilizes the helical conformation. This behavior is illustrated in Figure 1 in which the optical rotatory dispersion curves obtained for poly-L-Arg + GMP at pH 6 and at pH 4 are compared with that calculated from the separate optical rotatory dispersion curves of GMP and poly-L-Arg. At pH 5 the effect of GMP was reduced and that of IMP had essentially disappeared. Therefore, the stabilization of the poly-L-Arg helix at pH 6 is presumably caused by the ionization of the phosphate. With GMP there is also a real change in the nucleotide region of the optical rotatory dispersion, which would seem to indicate some structural modification, either intra- or intermolecular, of the nucleotide (Figure 1). With all other combinations of nucleotides and polypeptides only very minor changes are produced in the optical rotatory dispersion (Table I and Figures 1B and 2B).

Interaction of Polypeptides and Nucleotides in 60% Dioxane. It has been demonstrated that the helical conformation is stabilized by dioxane (Chaudhuri and Yang, 1968; Rifkind, 1969; Iizuka and Yang, 1965). This phenomenon has been attributed to a difference in dielectric constant and hydrogen-bonding properties in the two solvents.

Since the interaction with the nucleotides in H_2O was generally not adequate to push the polypeptides into the helical conformation we decided to see if the use of dioxane–water mixtures would help. The apparent pH in 60% dioxane is approximately the same as the actual pH (Van Uitert and Fernelius, 1954). It was, therefore, possible to work in the

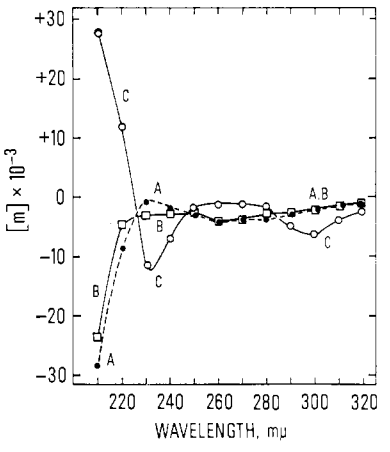


FIGURE 1: Optical rotatory dispersion spectra of GMP with poly-L-Arg in water. (A, ●) Calculated from the individual spectra of GMP and poly-L-Arg; (B, □) pH 4; (C, ○) pH 6. Concentration of GMP and poly-L-Arg 1×10^{-3} M.

TABLE II: Change in the Mean Residue Rotation at 233 mμ, As a Result of the Interaction of Nucleotides with Polypeptides in 60% Dioxane (by Volume).[a,b]

	$[m]_{233} \times 10^{-3}$					
	Poly-L-Lys		Poly-L-Orn		Poly-L-Arg	
Nucleotide	pH 6	pH 4	pH 6	pH 4	pH 6	pH 4
GMP	**−7.0**[e]	**−6.0**[c]	**−5.0**[d]	**−6.0**[c]	**−13.5**[d]	**−15.0**[e]
IMP	**−6.5**[e]	**−9.5**[c]	+1.0[d]	+0.5[d]	**−9.0**[c]	**−9.0**[c]
AMP	**−8.0**[d]	**−15.0**[c]	+1.0[c]	−4.0[c]	**−10.0**[c]	**−11.5**[c]
CMP	**−7.5**[d]	+0.1[c]	+1.5[d]	−0.1[c]	**−2.5**[e]	−2.0[c]
UMP	**−8.0**[d]	−0.2[c]	+0.6[c]	+0.1[c]	**−8.5**[c]	−0.1[c]

[a] Boldface type is used when we believe, on the basis of all of our results, that there is a significant stabilization of the helical conformation. [b] The helical conformation of the polypeptides has a large negative rotation at 233 mμ relative to that of the random coil. However, we have found that in many cases (Table III) the interaction with the polypeptide in dioxane produces large changes in the contribution of the nucleotides to the spectra. Therefore, the values listed in this table can have large positive or negative contributions from the nucleotide. The entire optical rotatory dispersion spectrum in the region of peptide absorption, and not just the value of $[m]_{233}$, is frequently necessary to indicate the conformation of the polypeptide. [c] These solutions were 1×10^{-3} M in nucleotide and polypeptide. [d] These solutions were 5×10^{-4} M in nucleotide and polypeptide. [e] These solutions were measured at concentrations both 1×10^{-3} and 5×10^{-4} M in nucleotide and polypeptide. No significant difference was found at the two concentrations.

same pH range as in water and validly compare the water and 60% dioxane results.

A comparison of Figure 2B with 2C illustrates dramatically how the substitution of 60% dioxane for water results in the conversion of the IMP–poly-L-Lys complex from a random coil configuration to a helix.

The use of a solvent with a lower dielectric constant favors noncharged species. Such a solvent can help to stabilize the nucleotide–polypeptide complex which is electrostatic in nature. A shift in the various pK's to favor the noncharged species would also be expected (Iizuka and Yang, 1965), raising the pK for the phosphate and lowering the pK for the protonation of the bases. Before proceeding with these experiments it was established that 60% dioxane did not significantly change the optical rotatory dispersion spectra of the polypeptides or nucleotides alone.

Poly-L-Lys in 60% Dioxane. The pyrimidine nucleotides CMP and UMP stabilize the helical conformation of poly-L-Lys at apparent pH of 6 and 5 but not at an apparent pH of 4 (Table II and Figure 3C). In this pH region no effect was

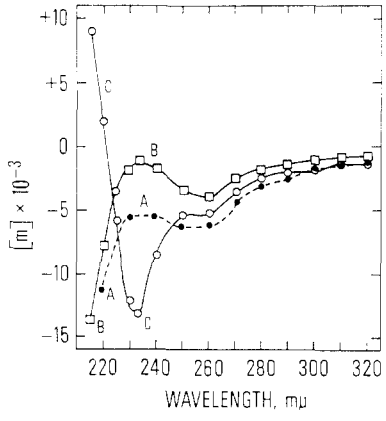


FIGURE 2: Optical rotatory dispersion spectra of IMP with poly-L-Lys at pH 4. (A,●) Calculated from the individual spectra of IMP and poly-L-Lys; (B,□) H_2O; (C,○) 60% dioxane. Concentration of IMP and poly-L-Lys, 1×10^{-3} M.

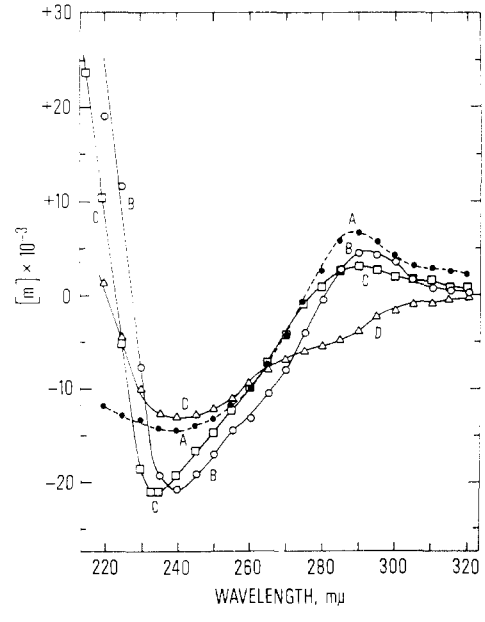


FIGURE 3: Optical rotatory dispersion spectra of CMP with the basic polypeptides in 60% dioxane at pH 6. (A,●) Calculated from the individual spectra of CMP and the polypeptides; (B,○) poly-L-Arg; (C,□) poly-L-Lys; (D,△) poly-L-Orn. Concentration of CMP and polypeptide, 5×10^{-4} M.

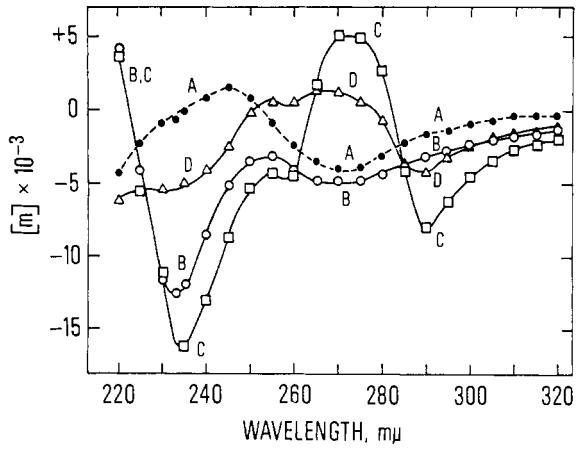


FIGURE 4: Optical rotatory dispersion spectra of AMP with the basic polypeptides in 60% dioxane at pH 4. (A,●) Calculated from the individual spectra of AMP and the polypeptides; (B,○) poly-L-Arg; (C,□) poly-L-Lys; (D,△) poly-L-Orn. Concentration of AMP and polypeptide, 1×10^{-3} M.

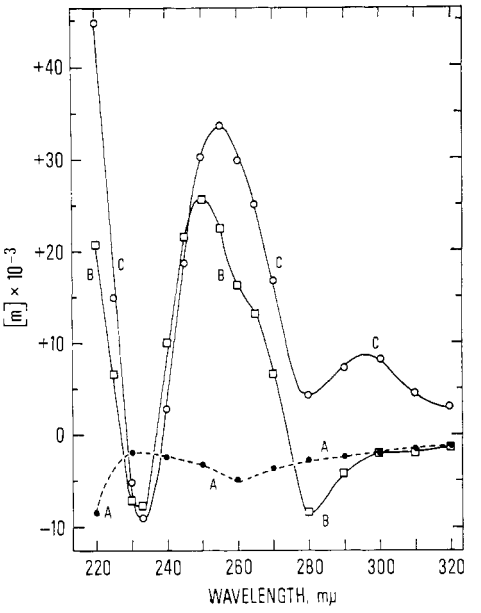


FIGURE 5: Optical rotatory dispersion spectra of GMP with poly-L-Lys in 60% dioxane. (A,●) Calculated from the individual spectra of GMP and poly-L-Lys; (B,□) pH 4; (C,○) pH 6. Concentration of GMP and poly-L-Lys, 1×10^{-3} M.

observed on the optical rotatory dispersion spectra of the pyrimidine nucleotides in the near-ultraviolet region. The stabilization of the poly-L-Lys helix by UMP at pH 6 and pH 5 must be due to the ionization of the phosphate, since the UMP base does not become protonated. The results at pH 5 imply that the polypeptide–nucleotide complex significantly lowers the pK of the secondary phosphate. The behavior of CMP cannot be unambiguously interpreted since the pK for the protonation of the CMP base occurs between pH 5 and 4.

Each of the three purine nucleotides studied, IMP, AMP, and GMP, behave very differently in their interaction with poly-L-Lys in 60% dioxane.

The IMP–poly-L-Lys reaction in the entire pH region studied (apparent pH 4–6) does not produce any affect in the near-ultraviolet region. However, there is a change in the peptide region which would clearly suggest that the poly-L-Lys assumes a helical conformation (Table II). This behavior is demonstrated by Figure 2 in which the optical rotatory dispersion curves obtained for IMP + poly-L-Lys in H_2O and in 60% dioxane are compared with the optical rotatory dispersion curve calculated from that of the nucleotide and polypeptide alone.

With AMP and GMP as shown in Figures 4C and 5, respectively, a significant change can take place in the near-ultraviolet region as well as the region of peptide absorption (Table III). The effect of the polypeptide–nucleotide interaction on GMP and AMP seems to be qualitatively different. This conclusion is suggested by a careful analysis of the respective optical rotatory dispersion curves as well as differences in the time required to reach equilibrium, and the affect of changing the pH.

The near-ultraviolet optical rotatory dispersion pattern for GMP at an apparent pH of 4 (Figure 5B) can be analyzed as a splitting of the 252 π–π^* band (Tinoco, 1964) producing one negative and one positive Cotton effect of large rotatory strengths. This spectrum would suggest that the GMP is stacking around the poly-L-Lys (Tinoco, 1964). A similar double Cotton effect, slightly displaced, was obtained by Sarkar and Yang (1965) with GMP gels, and was also attributed to stacking.

The interaction produces a near-ultraviolet optical rotatory dispersion pattern for AMP (Figure 4C) at an apparent pH of 4 that is much smaller in magnitude than that of GMP. Furthermore, the main feature is a Cotton effect at 282 mμ and nothing that would suggest a splitting of the 259 π–π^* band. The ultraviolet spectrum does not show any shift of the 259-mμ band but there is an indication of a shoulder on the near-ultraviolet side of the peak (Miles *et al.*, 1969) that can be responsible for the observed Cotton effect.

At an apparent pH of 4 the AMP change requires several hours to fully develop while the GMP effect has reached equilibrium in the 10–15 min required to prepare the solution. Raising the apparent pH to 5 and 6 has a very different effect on the interaction with AMP and GMP (Table III). For AMP the changes in the near-ultraviolet spectrum are obliterated at pH 5 and 6. AMP then behaves like the other nucleotides and only stabilizes the helical conformation of poly-L-Lys. The pK for the base protonation of AMP is 3.7 (Alberty *et al.*, 1951). Therefore, the disappearance of the near-ultraviolet effect between apparent pH 4 and 5 can be attributed to either the ionization of the phosphate or deprotonation of the base.

With GMP identical results are obtained at an apparent pH of 5 and 4. At an apparent pH of 6 the optical rotatory dispersion spectrum is similar to that obtained at pH 4. However, there is an additional Cotton effect with a maximum at 295 mμ and significant changes in the rest of the optical rotatory dispersion spectrum (Figures 5C). These changes take several hours to fully develop and are thought to represent a subtle change in the stacking of GMP produced by the secondary ionization of the phosphate.

With such large changes occurring in the near-ultraviolet

TABLE III: Specific Interactions between Nucleotides and Polypeptides That Produce Changes in the Near-Ultraviolet Optical Rotatary Dispersion in 60% Dioxane (by Volume).[a]

Nucleotide	Poly-L-Lys		Poly-L-Orn		Poly-L-Arg	
	pH 6	pH 4	pH 6	pH 4	pH 6	pH 4
GMP	+++	+++	+++	+++	−	+++
IMP	−	−	−	−	−	−
AMP	−	++	−	+	−	−
CMP	−	−	++	−	+	−
UMP	−	−	−	−	−	−

[a] The plus and minus signs are used to indicate the presence of a significant change in the near-ultraviolet region. The number of pluses indicates qualitatively the magnitude of the optical rotatory dispersion change. The detailed features of the various changes are discussed in the text and illustrated in Figures 3, 4, 5, and 6. Concentrations are as in Table II.

region for GMP it is essentially impossible to say with any certainty what causes the far-ultraviolet changes. However, stacking of the GMP would require that the polypeptide exists in an ordered conformation. Furthermore, the evidence that the other purine nucleotides stabilize the helical conformation strongly suggests that the poly-L-Lys is helical. Therefore, in curves B and C of Figure 5, the 233-mμ minimum, and the large positive rotation at shorter wavelengths is at least partially attributed to the helical polypeptide.

Poly-L-Orn in 60% Dioxane. When poly-L-Orn is used instead of poly-L-Lys the interaction with GMP produces very similar results (Table II and Table III).

While none of the other nucleotides stabilize the helical conformation of poly-L-Orn (Table II), AMP and CMP are affected in the near-ultraviolet region by the interaction (Table III). AMP at an apparent pH of 4 in the presence of poly-L-Orn has a spectrum similar to, but smaller in magnitude than, the one obtained with poly-L-Lys under the same conditions (Figure 4, curves C and D). The CMP maximum in the 290–295-mμ region (Figure 3A–C) is eliminated at an apparent pH of 6 in the presence of poly-L-Orn (Figure 3D). In both of these cases the spectra in the region of peptide absorption show no indication of stabilization of the helical conformation (Figures 3D and 4D). This behavior is unlike the changes in the near-ultraviolet region found with poly-L-Lys and poly-L-Arg which seemed to coincide with stabilization of the helical conformation.

In all other cases the interaction with poly-L-Orn in 60% dioxane produces no effect.

Poly-L-Arg in 60% Dioxane. The stabilization of the helical conformation of poly-L-Arg-HCl in 60% dioxane by the various nucleotides is qualitatively the same as for poly-L-Lys (Table II).

As shown in Figure 3B and Table II, the change in mean residue rotation at 233 mμ that results from the interaction of CMP and poly-L-Arg at pH 6 in 60% dioxane is very small. This result would seem to indicate that the helical conformation of poly-L-Arg is not stabilized by CMP. However, the optical rotatory dispersion curve shown in Figure 3B has a large positive rotation at 220 mμ as expected for a helix. We, therefore, believe that CMP does stabilize the helical conformation of poly-L-Arg and that the shift of the 233-mμ minimum to 240–245 mμ is the result of a change in the contribution of CMP to the optical rotatory dispersion curve.

The change in the near-ultraviolet region with AMP at an apparent pH of 4 which was observed with poly-L-Lys and to a limited extent with poly-L-Orn was not observed with poly-L-Arg (Figure 4B and Table III).

At an apparent pH of 4 in 60% dioxane the optical rotatory dispersion pattern which has been explained as due to GMP stacking around a helical polypeptide is still present with poly-L-Arg. However at an apparent pH of 6 the base stacking which is observed with poly-L-Lys and poly-L-Orn has completely disappeared (Figure 6 and Table III).

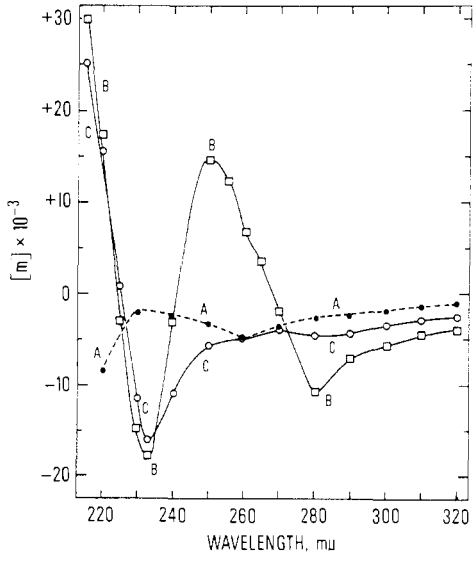


FIGURE 6: Optical rotatory dispersion spectra of GMP with poly-L-Arg in 60% dioxane. (A,●) Calculated from the individual spectra of GMP and poly-L-Arg; (B,□) pH 4; (C,○) pH 6. Concentration of GMP and poly-L-Arg, 5×10^{-4} M.

Discussion

Nucleotide Specificity. There are two different types of nucleotide specificity which we have observed. (1) The

variation in the ability of the nucleotides to stabilize the helical conformation of the polypeptides. (2) Specific changes of certain nucleotides as a result of the interaction with the polypeptides.

From the results for the stabilization of the helical conformation it is possible to differentiate qualitatively among most of the nucleotides studied (Tables I and II). GMP is the only nucleotide to stabilize the helical conformation of poly-L-Orn. IMP together with GMP, but unlike all the other nucleotides, stabilize the poly-L-Arg helix at pH 6 in H_2O. AMP together with the other purine nucleotides, but unlike the pyrimidine nucleotides, stabilize the helical conformation of poly-L-Arg and poly-L-Lys at pH 4 in 60% dioxane. All of the nucleotides, including UMP and CMP, stabilize the helical conformation of poly-L-Arg and poly-L-Lys at pH 6 in 60% dioxane.

The order in which the nucleotides stabilize the helical conformation of the polypeptides is thus GMP > IMP > AMP > CMP $\approx$ UMP. This order parallels that usually given for the tendency of the bases to stack (Ts'o *et al.*, 1963). For this reason we feel that this base specificity can be at least partially explained by the extent to which the nucleotides are willing to restrain their freedom of rotation and the mobility around the nucleotide–polypeptide linkage. This restriction permits partial parallel orientation of adjacent bases and is actually a direct measure of the base stacking tendency.

Many properties of nucleotides are very well correlated with base stacking. Even the minor variation in binding observed by Wagner and Arav (1968) follows the same order. Therefore, it is possible that variations of binding constants can contribute to the stabilization of the helical conformation. The coil → helix transition is favored by the neutralization of positive charge. Because the helix–coil transitions are very cooperative a small change in the charge neutralization can produce a large change in the helical content (Applequist and Doty, 1962).

The second type of nucleotide specificity is also quite interesting. We observe that under the proper conditions the interaction between the polypeptides and the nucleotides produces specific effects on certain nucleotides (Table III).

The difference in the behavior of GMP from that of the other nucleotides (Figures 5 and 6) has already been interpreted as base stacking around the helical polypeptide. It is possible to rationalize these results if nucleotides are bound to the polypeptides in such a way that the average position of the nucleotides is quite unfavorable for parallel base stacking. Counteracting this unfavorable geometry for nucleotide stacking there is, however, the consideration that the lysine residues are suspended in space some distance away from the helix. These residues have considerable freedom of movement, both lateral and vertical. Nucleotides with relatively low-stacking tendency would conceivably leave the side chains in positions as far apart from each other as possible. Nucleotides with higher stacking tendency could reorganize the side chains to permit greater nucleotide stacking to occur. The unique behavior of GMP is perhaps the result of GMP being the only nucleotide with a strong enough base-stacking tendency to energetically outweigh the entropic freedom of the side chains.

The changes in the optical rotatory dispersion spectra found with AMP and CMP (Figures 3 and 4) do not seem to be the result of parallel base stacking. In fact it would be very difficult to explain why AMP stacks when IMP does not and why CMP stacks when IMP and AMP do not. These effects perhaps require certain specific substituents at particular positions on the purine or pyrimidine and are the result of the nucleotides being forced into more rigid configurations when crowded along the polypeptide chain.

The effects noted in Table III, while they all probably involve the interaction of the nucleotides with each other, are really somewhat diverse phenomena, as is indeed indicated by the different conditions under which they are obtained. For this reason, there is no correlation between the results shown in Table III and the usual stacking tendency of the nucleotides. On the other hand, the results of Table II clearly indicate that the ability to stabilize the polypeptide is correlated with the stacking tendency of the nucleotides.

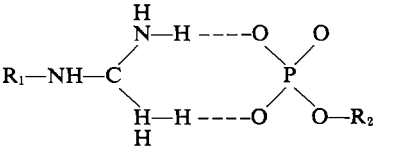
Polypeptide Specificity. The differences obtained among the three basic polypeptides studied are equally dramatic. In order to discuss these differences it is necessary to first compare the stability of the helical conformations of these three polypeptides in the absence of nucleotides. Such a comparison has been made (Rifkind, 1969) and it has been demonstrated that the helical conformations of poly-L-Arg and poly-L-Lys are more stable than that of poly-L-Orn. The poly-L-Arg and poly-L-Lys helices are of comparable stability; however, it is possible to preferentially stabilize the helical conformation of poly-L-Arg or poly-L-Lys because of differences in the binding properties of the amino and guanidinium groups.

It is now possible to explain why the helical conformations of the three polypeptides studied are stabilized to a different extent by interacting with nucleotides.

The results with poly-L-Orn can be explained in terms of the relative instability of its helical conformation. GMP with the strongest base-stacking tendency presumably stacks around the helical polypeptide and is the only nucleotide to stabilize the poly-L-Orn helix.

The only difference between the polypeptide helicity of poly-L-Arg and poly-L-Lys is that IMP and GMP stabilize the poly-L-Arg helix at pH 6 even in water (Table I). Nevertheless the large base-stacking effect on GMP in 60% dioxane has completely disappeared at pH 6 (Figure 6C).

An explanation of these results necessitates a qualitative difference between the interaction of nucleotides with lysine residues and arginine residues at pH 6. We have previously reported (Rifkind, 1969) that perchlorate preferentially stabilizes the helical conformation of poly-L-Arg at pH 6. We have also found that trace amounts of sulfate which remain after exhaustive dialysis of poly-L-Arg·$0.5H_2SO_4$ are able to stabilize the helical conformation of poly-L-Arg. The structural similarity among perchlorate, sulfate and the phosphate of the nucleotide suggest the possibility in all three cases for the formation of a hydrogen-bonded ringed structure similar to the one shown for a nucleotide and a guanidinium group



A comparison of the rigidity of this structure relative to the mobility around the electrostatic bond between an amino

group and a nucleotide strongly suggests an explanation for the unique behavior of poly-L-Arg at pH 6. We have already proposed that the stabilization of the helical conformation by the bulky nucleotides requires a restraint on the mobility of the nucleotides. With poly-L-Arg the rigid ring serves as this restraint and therefore the poly-L-Arg helix is stabilized by GMP and IMP even in H_2O. As we have noted earlier, the poor geometry for the stacking of the bases around the helical polypeptide requires that, in order for the GMP base–base interaction to occur, groups of several nucleotides must be able to rearrange themselves into a geometry more favorable for stacking. The same ring structure which can help the nucleotides to stabilize the helical conformation of poly-L-Arg, therefore, can prevent the GMP from assuming a favorable geometry for base stacking and there is no base-stacking observed with poly-L-Arg at pH 6.

Why do the differences between poly-L-Lys and poly-L-Arg show up at pH 6 and not at pH 4? This can be explained by the additional ionization of the phosphate when the pH is raised from pH 4 to 6.

Perchlorate with a -1 charge stabilizes the poly-L-Arg helix (Rifkind, 1969). We have also observed that, when poly-L-Arg·$0.5H_2SO_4$ is dissolved in acid and exhaustively dialyzed *vs.* water, the trace amounts of sulfate remaining are as effective as 0.01 M ClO_4^- in stabilizing the poly-L-Arg helix. This difference suggests that the ringed structure which we have proposed can occur when there is one negative charge on the nucleotide as with perchlorate, since the negative charge can be partially carried by two oxygens. However, when there is a -2 charge on the nucleotide, as with sulfate this interaction becomes very much favored, since it makes possible the partial neutralization of both negative charges.

Therefore, at pH 4, far removed from the second p*K* for the phosphate, it is possible that there is an equilibrium between the ringed structure preferred at pH 6 and an electrostatic bond similar to that obtained with poly-L-Lys. The change in the near-ultraviolet region when AMP is added to poly-L-Lys and poly-L-Orn at pH 4 in 60% dioxane is not observed with poly-L-Arg (Table III and Figure 4). This indicates that even at pH 4 there is perhaps a difference between the interaction of the nucleotides with a guanidinium group and an amino group.

Possible Biological Implications. Nucleotide components play a role in numerous enzymatic systems as substrates, activators, or inhibitors. We have shown that the conformation of a simple homopolymer can be controlled by subtle differences in the tendency of bases to stack or to engage in other intermolecular interactions. Therefore, more complicated enzymatic systems can be expected to be able to differentiate various bases by their ability to change the conformation of another molecule. This sort of conformational specificity may be as important to consider as the more frequently discussed binding specificity.

The difference which we find between the interaction of an arginine side chain and a lysine side chain may perhaps help to explain the different role played by these two basic amino acids in histones as well as other proteins. While the lysine residue appears to interact essentially electrostatically, the arginine residue may be capable of the formation of a ringed structure. The formation of this structure would fix the position of the nucleotide relative to the arginine residue and place much greater restraints on the conformation of the histone and nucleic acid in the region where arginine is bound. Furthermore, the very strong affinity of poly-L-Arg for certain types of anions suggests the possibility of specifically displacing arginine residues from the residues of a nucleic acid with a single minus charge, by multivalent phosphates, *i.e.*, mononucleotides, trinucleotides, or P_i.

On the basis of the results of Gratzer and McPhie (1966) and some of our preliminary experiments with polynucleotides, it does not look as if the interaction of basic polypeptides with polynucleotides can induce a coil → helix transition in the polypeptide as the mononucleotides do. This is not surprising since the polynucleotides with the ribose phosphate backbones would be expected to be incompatible with a helical polypeptide. Nevertheless, the polynucleotides would neutralize the positive charge on the polypeptide perhaps even more effectively. For the neutral polypeptide the helical conformation is the most stable conformation. The polynucleotide must thermodynamically compensate for preventing the polypeptide from assuming a helical conformation. This compensation requires a distortion of the nucleic acid structure and is very likely responsible for the changes that occur when polypeptides or histones bind to nucleic acids (Lees and von Hippel, 1968).

The specific changes in the near-ultraviolet which we have found to take place with AMP and CMP imply that the nucleic acid–polypeptide interaction can possibly recognize a particular nucleotide. The only demonstrated specificity for polypeptides and nucleic acids is the preference of poly-L-Lys, relative to poly-L-Arg, for (A-T)-rich regions (Leng and Felsenfeld, 1966). It is tempting to speculate that perhaps the ability of poly-L-Lys and not poly-L-Arg to affect the configuration of AMP is related to the ability of poly-L-Lys to recognize (A-T)-rich regions of nucleic acids.

The observations made in this section are suggestive of the way in which our findings can be of biological significance, but they must be considered speculative at this time.

Acknowledgments

The authors thank Mrs. Joan Green for valuable technical assistance.

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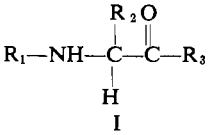
On the Specificity of Papain*

John de Jersey†

ABSTRACT: Good substrates for papain such as *p*-nitrophenyl hippurate contain an α-acylamino group. To investigate the interaction between this group and the active site of the enzyme, a number of *p*-nitrophenyl esters in which the group has been modified or removed have been examined as substrates. A quantitative estimate of the importance of the acylamino group in the acylation reaction has thus been obtained. The oxazolin-5-one and *p*-nitrophenyl ester of Bz-DL-Ala have been used to show that the acylamino group is the major factor in establishing the stereospecificity of both acylation and deacylation reactions. The effects of indole, benzene, and benzyl alcohol on the acylation of papain by 4,4-dimethyl-2-phenyloxazolin-5-one and on the corresponding deacylation reaction have been determined.

Papain (EC 3.4.4.10), one of several proteolytic enzymes present in papaya latex (Kunimitsu and Yasunobu, 1967), has been reported to have a broad specificity (Smith and Kimmel, 1960; Hill, 1965). However, the main emphasis has been on the determination of the specificity of the enzyme for the side chain of the L-α-amino acid contributing the carboxyl group to the bond being hydrolyzed (R_2 in I). The nature of this side chain is a major specificity determinant



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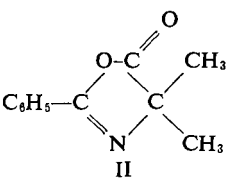
in catalysis by trypsin and α-chymotrypsin. By contrast, kinetic constants (k_{cat} and K_m) for papain-catalyzed hydrolyses are relatively insensitive to variation in R_2. For example, k_{cat} for the best substrate known, α-*N*-Z-L-LyspNp[1] (46 sec^{-1}) (Bender and Brubacher, 1966) is only a factor of 8 greater than k_{cat} for *N*-Z-GlypNp (5.9 sec^{-1}). Because of this, an attempt has been made to determine the importance of some of the other possible interactions between the enzyme and the substrate. In particular, the importance of the α-acylamino group (R_1—NH—) has been investigated by testing as substrates for papain a series of compounds in which the acylamino group has been progressively removed or modified.

Several experiments have indicated that the active site of papain may contain a hydrophobic region which interacts with the leaving group of the substrate. For example, Brubacher and Bender (1966) found that L-tryptophanamide was about 100 times as efficient as glycinamide in catalyzing the deacylation of *trans*-cinnamoyl-papain. D-Tryptophanamide was only about half as efficient as glycinamide. On this basis, it was proposed that hydrophobic compounds might affect the rates of the acylation and deacylation reactions of papain in much the same way as indole increases the rate of deacylation of acetyl-chymotrypsin (Foster, 1961). 4,4-Dimethyl-2-phenyloxazolin-5-one (II) was chosen as a substrate to test the effect of indole, benzene, and benzyl alcohol on these reactions. This oxazolinone has been considered previously as a substrate for papain (de Jersey and



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[1] Abbreviations used are: Z, benzyloxycarbonyl; Bz, benzoyl; But, butyryl; pNp, *p*-nitrophenyl ester; Et, ethyl ester; Sar, sarcosine.