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Stereospecific Binding of Diastereomeric Peptides to Salmon Sperm Deoxyribonucleic Acid. Further Evidence for Partial Intercalation†

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ABSTRACT: A series of diastereomeric dipeptide amides, containing an N-terminal L-lysyl residue and a C-terminal L- or D-amino acid with a derivatized aromatic ring on the side chain, was synthesized to determine the dependence of (1) the chirality of the N-terminal amino acid α-carbon and (2) the length of the N-terminal amino acid side chain for intercalation of the aromatic ring. The nature of the complex between the peptide and DNA (i.e., electrostatic, intercalative, or a combination of these) was determined by UV and CD studies, viscometric titrations, and ^{1}H NMR studies. The results of these studies reveal distinct differences in the binding site of the aromatic rings of the various peptides. In particular, the results suggest that the α- and ϵ-amino groups of the lysyl residue bind electrostatically to adjacent phosphates on the DNA backbone in a *stereospecific* manner. As a result of this stereospecificity, the aromatic rings of the peptides with the L-L designation point toward the DNA helix, while those of the peptides of the L-D designation point away from the helix. This is completely consistent with previously reported work [Gabbay, E. J., Adawadkar, P. D., & Wilson, W. D. (1976) *Biochemistry 15*, 146; Gabbay, E. J., Adawadkar, P. D., Kapicak, L., Pearce, S., & Wilson, W. D. (1976) *Biochemistry 15*, 152]. The results also indicate a great dependence on the length of the side chain for intercalation of the aromatic ring. Specifically, if the side chain is long enough, and flexible enough, the aromatic ring can fully or partially intercalate, regardless of the chirality of the N-terminal amino acid α-carbon. However, if the side chain is too short, only partial intercalation is observed for peptides of the L-L designation, and no intercalation is observed for peptides of the L-D designation.

The recognition processes of proteins to specific sequences of nucleic acids is of considerable interest in several laboratories due to the number of different types of protein/DNA complexes found in the living cell. Because of the complexity of studying the interactions of two macromolecules, our approach has been to synthesize and study model systems using small oligopeptides and DNA of various AT/GC content (Gabbay et al., 1972, 1973). The model oligopeptides we have chosen contain aromatic amino acids whereby the aromatic moiety of the amino acid may partially intercalate into the base pairs of DNA. In particular, the results from previously presented studies (Adawadkar et al., 1975; Gabbay et al., 1976a,b) utilizing diastereomeric dipeptides, L-lysyl-L-phenylalaninamide (**1**) and L-lysyl-D-phenylalainamide (**2**), indicated that there is a stereospecific binding of the α- and ϵ-amino groups of the N-terminal L-lysyl residue to the nucleic acid backbone which then dictates the positioning of the aromatic ring either toward the interior of the helix for **1** or away from the helix for **2**. Due to steric constraints for **1**, the aromatic ring may only partially intercalate into the base pairs of the nucleic acid, resulting in a bend at the point of insertion. We wish to present further evidence in support of this mode of interaction of

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‡ Professor Edmond J. Gabbay died unexpectedly during the completion of this work. He was a bright scientist and a good friend, who will be sorely missed. It is for these reasons that this report is dedicated to his memory.

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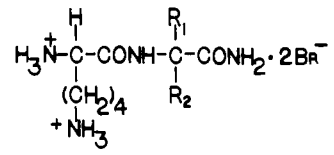

COMPOUND	R_1	R_2
L-LYS-L-PHEA (1)	-H	$-CH_2$-Ph
L-LYS-D-PHEA (2)	$-CH_2$-Ph	-H
L-LYS-L-pNO_2-PHEA (3)	-H	$-CH_2$-Ph-NO_2
L-LYS-D-pNO_2-PHEA (4)	$-CH_2$-Ph-NO_2	-H
L-LYS-L-DNP-DABA (5)	-H	$-(CH_2)_2-NH$-Ph(NO_2)-NO_2
L-LYS-D,L-DNP-DABA (6)	$-(CH_2)_2-NH$-Ph(NO_2)-NO_2, -H	-H, $-(CH_2)_2-NH$-Ph(NO_2)-NO_2
L-LYS-L-DNP-ORNA (7)	-H	$-(CH_2)_3-NH$-Ph(NO_2)-NO_2
L-LYS-D-DNP-ORNA (8)	$-(CH_2)_3-NH$-Ph(NO_2)-NO_2	-H

FIGURE 1: Dipeptide amides **1–8** discussed in this report. Note that all are pure diastereomeric isomers with the exception of **6**, which is a mixture of both L-L and L-D diastereomers. The synthesis of optically pure D-N^{γ}-Dnp-DABA was not possible due to the unavailability of D-DABA and the difficulty of resolving DL-DABA; thus, L-Lys-D-Dnp-DABA was not synthesized.

peptides containing aromatic amino acids with nucleic acids using dipeptides **3–8** which have a derivatized aromatic amino acid at the C-terminus (see Figure 1). Dipeptides **3** and **4** were synthesized for a direct comparison to the unsubstituted analogues **1** and **2** because of the distinct differences in the NMR chemical shifts of the ortho and para protons of the aromatic ring for **3** and **4**. The Dnp[1] containing dipeptides **5–8** were synthesized not only because of the different chemical shifts of the various aromatic protons in their NMR spectra but also because of their well-defined λ_{max} and large extinction coefficients (see Table I). These physical properties allows UV/vis and CD studies to be performed. The results of these studies are consistent with the stereospecific binding of the N-terminal L-lysyl residue and subsequent partial intercalation of a sterically constrained aromatic moiety or full intercalation of an unconstrained aromatic moiety.

Materials and Methods

Synthesis. The N^{γ}-Dnp derivatives of L- and DL-diaminobutyric acid and the N^{δ}-Dnp derivatives of L- and D-ornithine were prepared according to Sanger (1945). L- and D-pNO_2-phenylalanine and all the Dnp derivatives were then converted to their ethyl esters and then their amides by standard procedures (Greenstein & Winitz, 1961). Coupling of the amino acid amides to Cbz_2-L-lysine (Sigma Chemical Co.) was accomplished by using the mixed anhydride procedure (Anderson et al., 1967). Removal of the Cbz group was performed with HBr/HOAc (Greenstein & Winitz, 1961). The peptide amides were fully converted to bromide salts via anion-exchange chromatography by using Amberlite CG400 (100–200 mesh) resin (Baker Chemicals). All products were checked for purity and authenticity by ^{1}H NMR, ultraviolet spectroscopy, thin-layer chromatography, and elemental analysis. It should be noted that the peptide coupling reaction used in the above syntheses has been shown to proceed with no detectable racemization (Anderson et al., 1967).

Table I: Spectrophotometric Results for the Dipeptide Amides **3–8** of Figure 1

compound	λ_{free}[a] (nm)	ϵ_{free}[b]	λ_{DNA}[a] (nm)	ϵ_{DNA}[b]	% H[c]
L-Lys-L-pNO_2-PHEA (3)	275	8.75×10^3	ND[d]	ND	ND
L-Lys-D-pNO_2-PHEA (4)	275	8.75×10^3	ND	ND	ND
L-Lys-L-Dnp-DABA (5)	360	1.55×10^4	365	1.0×10^4	55
L-Lys-DL-Dnp-DABA (6)	360	1.55×10^4	365	1.0×10^4	55
L-Lys-L-Dnp-ORNA (7)	360	1.55×10^4	365	1.0×10^4	55
L-Lys-D-Dnp-ORNA (8)	360	1.55×10^4	365	1.0×10^4	55

[a] λ_{free} and λ_{DNA} are the wavelength maxima for the dipeptides in the absence and presence of excess DNA, respectively. [b] ϵ_{free} and ϵ_{DNA} are the extinction coefficients of the dipeptides at λ_{free} and λ_{DNA}, respectively. [c] % H is the percent hypochromicity for the DNP chromophores in the presence of excess DNA. [d] ND is not determinable due to the absorption of DNA at λ_{DNA} for dipeptides **3** and **4**. These measurements were carried out in 10 mM Mes (5 mM Na^+, pH 6.2) at 25 °C with salmon sperm DNA.

Analytical Methods. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. ^{1}H NMR spectra were recorded on a JEOL-FX 100 Fourier transform NMR spectrometer at various temperatures. Sonicated low molecular weight DNA was used at 72 mM phosphate/L in the presence of 1 mM EDTA in D_2O (pD 7.0). The concentration of peptide varied from 5 to 23 mM. Chemical shifts were determined relative to internal standard sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ (TSP). Ultraviolet and visible absorption spectra were recorded with a Cary-17D UV/vis spectrophotometer, and circular dichroism (CD) measurements were recorded with a JASCO J-20 spectropolarimeter at ambient temperature (24 ± 1 °C). Viscosity measurements were performed with a low-shear Zimm viscometer (Beckman Instrument Co.).

Binding studies were performed to determine the apparent binding constants of the various peptides. Photometric titrations, where small aliquots of a peptide/DNA solution were added to a peptide solution and the resultant absorbances at 360 nm were recorded, were performed for all the Dnp dipeptides. For the pNO_2-phenylalanyl-containing peptides, **3** and **4**, whose λ_{max} overlaps with that of DNA, the binding data were determined via equilibrium dialysis at various peptide/DNA ratios. Analysis of the binding data for both techniques was performed by using the site exclusion model of the McGhee–von Hippel (1974) equation:

$$r/C_f = K_a(1 - nr)[(1 - nr)/[1 - (n - 1)r]]^{n-1} \quad (1)$$

where r is the ratio of bound peptide to DNA base pair, C_f is the concentration of free peptide, K_a is the apparent binding constant, and n is the exclusion parameter.

All solutions containing DNA and peptides were prepared in Mes buffer with deionized water. Salmon sperm DNA (8 BA, ϵ_p = 6500; Worthington Biochemicals) was found to be free of any detectable protein contaminants.

[1] Abbreviations: Lys, lysyl; PHEA, phenylalaninamide; DABA, diaminobutyric acid amide; ORNA, ornithine amide; Dnp, dinitrophenyl; Cbz, carbobenzoxy; NMR, nuclear magnetic resonance; TSP, sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$; CD, circular dichroism; Mes, 2-(*N*-morpholino)ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

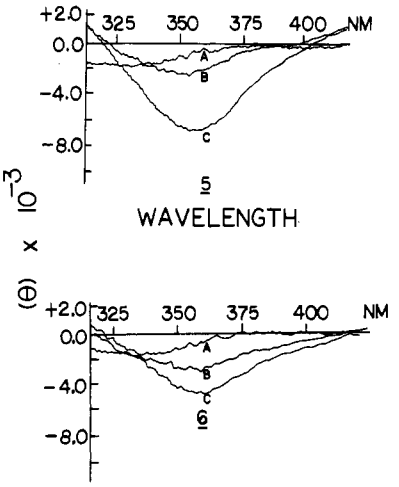

FIGURE 2: Intrinsic and DNA-induced CD spectra for peptides **5** and **6** are shown with molar ellipticity plotted as a function of wavelength. The DNA base pair to peptide ratio is (A) 0, (B) 1.5, and (C) 12. The peptide and peptide/DNA solutions were prepared in 10 mM Mes (5 mM Na^+, pH 6.2).

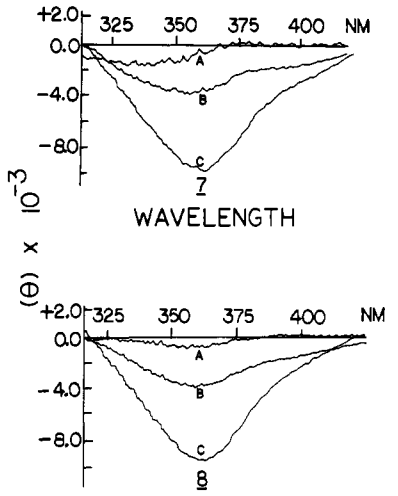

FIGURE 3: Intrinsic and DNA-induced CD spectra for peptides **7** and **8** are shown with molar ellipticity plotted as a function of wavelength. Experimental conditions are as in Figure 2.

Results

Ultraviolet and Visible Absorption Studies. Spectrophotometric data for the peptides are shown in Table I. All peptides have a well-defined λ_{max} in the region 260–400 nm with extinction coefficients on the order of 8.75×10^3 for **3** and **4** and 1.55×10^4 for the Dnp-containing peptides **5–8**. For the Dnp-containing peptides, two effects can be seen upon binding to DNA: (1) there is a bathochromic shift in λ_{max} from 360 to 365 nm, and (2) there is a large accompanying hypochromic shift on ϵ_{max}. The percent hypochromicity was calculated according to the equation: $\% H = 100(1 - A_b/A_f)$, where A_b and A_f are the areas under the absorption curves for the bound and free peptide, respectively. Due to the large absorption of DNA at λ_{DNA} for the peptide/DNA complexes of **3** and **4**, the % H could not be determined.

Circular Dichroism Studies. The CD spectra of the Dnp-containing peptides in the absence and presence of DNA are shown in Figures 2 and 3. There is little or no CD for the free dipeptides. However, in the presence of DNA, there is an induced CD for all the Dnp peptides. The extent of the induced CD, in the presence of excess DNA, is the same for the diastereomeric peptides **7** and **8**, while **5** experiences a greater induced CD than that of **6**, which is a racemic mixture of both the L-L and L-D dipeptides. Furthermore, **7** and **8** have a much larger induced CD than **5**.

Table II: Effect of the Dipeptide Amides **3–8** on the Helix–Coil Transition Temperature for Salmon Sperm DNA, Poly(I)-Poly(C), and Poly[d(A-T)][a]

peptide system	ΔT_m[b] salmon sperm DNA	poly(I)-poly(C)	poly-[d(A-T)]
L-Lys-L-pNO$_2$-PHEA (**3**)	1.8	3.8	4.0
L-Lys-D-pNO$_2$-PHEA (**4**)	0.9	0.4	1.2
L-Lys-L-Dnp-DABA (**5**)	7.5	7.0	10.6
L-Lys-DL-Dnp-DABA (**6**)	4.3	4.3	7.7
L-Lys-L-Dnp-ORNA (**7**)	>30	6.7	14.3
L-Lys-D-Dnp-ORNA (**8**)	14.9	1.1	7.0

[a] The T_m measurements consisted of 1.26×10^{-4} M nucleic acid in 10 mM Mes (5 mM Na^+, pH 6.2) in the absence or presence of 25 μM dipeptide. [b] $\Delta T_m = T_m - T_{mo}$, where T_m and T_{mo} are the melting temperatures of the nucleic acids in the presence and absence of the dipeptide, respectively.

Table III: Apparent Binding Constants for Dipeptides **3–8**

compound	method[a]	K_{app}[b]
L-Lys-L-pNO$_2$-PHEA (**3**)	1	5.4×10^2
L-Lys-D-pNO$_2$-PHEA (**4**)	1	4.9×10^2
L-Lys-L-Dnp-DABA (**5**)	2	2.5×10^5
L-Lys-DL-Dnp-DABA (**6**)	2	2.1×10^5
L-Lys-L-Dnp-ORNA (**7**)	2	1.9×10^5
L-Lys-D-Dnp-ORNA (**8**)	2	2.3×10^5

[a] Determinations were made via equilibrium dialysis by using 3.0×10^{-4} M DNA in 10 mM Mes (pH 6.2) at 5 mM Na^+ with various peptide concentrations (method 1) or via photometric titrations using a Cary 17-D spectrophotometer with 1.125×10^{-5} M peptide in 6.6 mM phosphate buffer (pH 7.2) at 5 mM Na^+ (method 2). [b] Calculated with $n = 4.5$ in the site exclusion form of the McGhee–von Hippel (1974) equation.

Melting Temperature Studies. The effect of the peptides on the T_m (melting temperature) of the helix to coil transition for three different nucleic acids was determined. Several observations can be made from the T_m data presented in Table II. First, all peptides stabilize the helix relative to the random coil, and the transition exhibits a monophasic behavior. Furthermore, Dnp compounds **5–8** stabilize the helix more than pNO_2-phenylalanyl-containing compounds **3** and **4**. Second, all Dnp peptides, in general, stabilize poly[d[(A-T)] to a greater extent than poly(I)–poly(C), which is a general phenomenon observed in the binding of drugs to poly[d(A-T)]. Finally, for a given set of diastereomers, the compound having the L-L designation stabilizes the helix to a greater extent than the compound with the L-D designation.

The interpretation of these data is complicated by the fact that the helix–coil transition involves the interaction of the peptide (P) not only with the helix (H), but also with the coil (C) as shown in the equation

$$H + P \overset{K_H}{\rightleftharpoons} H\text{–}P \rightleftarrows C\text{–}P \overset{K_C}{\rightleftharpoons} C + P$$

It can be assumed that K_H is greater than K_C since the T_m is increased in the presence of the dipeptide. However, Gabbay & Kleinman (1970) have pointed out that this conclusion is valid only when K_H and K_C are determined directly.

Binding Studies. Apparent binding constants were determined for the peptides via equilibrium dialysis or photometric titrations and subsequent treatment of the binding data by using eq 1. The results are presented in Table III. These binding constants are estimated values and were determined by using $n = 4.5$. Although these values are approximate and

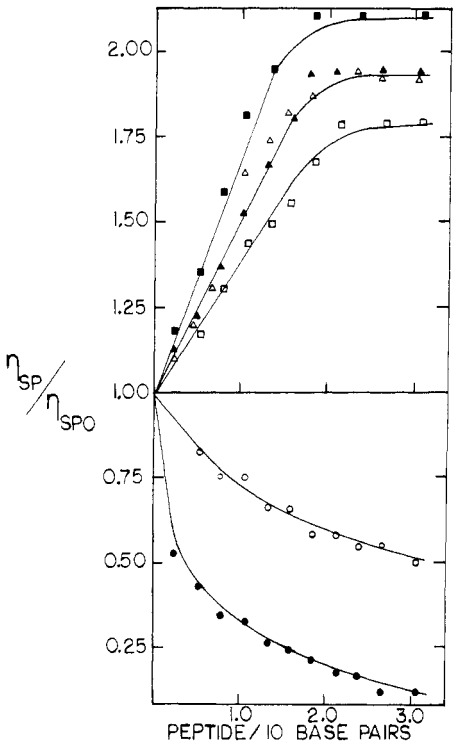

FIGURE 4: Viscometric titrations of salmon sperm DNA with peptides **3** (●), **4** (○), **5** (■), **6** (□), **7** (▲), and **8** (△) are shown with the ratio of the reduced specific viscosity of the DNA/peptide complex, η_{sp}, to the reduced specific viscosity of free DNA, η_{spo}, as a function of the peptide to 10 base pair ratio. The DNA concentration for these titrations was 3.0×10^{-4} in 10 mM Mes (5 mM Na^+, pH 6.2). All titrations were performed at 37.5 °C.

are not reflective of the true binding constants, they can be used to compare the relative binding affinities of the peptides within the series. The data indicate that Dnp peptides **5–8** bind with similar affinities, as do pNO_2-phenylalanyl peptides **3** and **4**. Furthermore, the Dnp peptides apparently form a tighter complex with DNA than do the pNO_2-phenylalanyl peptides.

Viscosity Studies. The effect of increasing the concentration of peptide on the relative reduced specific viscosity, η_{sp}/η_{spo} (where η_{sp} and η_{spo} are the reduced specific viscosities of the DNA solution in the presence and absence of peptide, respectively), was studied at 37 °C by using the low-shear Zimm viscometer. Since the study was carried out at low DNA concentration, the relative values of η_{sp}/η_{spo} are close approximations of the relative values of the intrinsic viscosity of a DNA–peptide complex to free DNA ($[\eta]/[\eta]_0$).

The results are shown in Figure 4 and indicate that **3** and **4** decrease the reduced specific viscosity of a DNA solution, with **3** inducing a much larger decrease than its diastereomer **4**. On the other hand, peptides **5–8** increase the reduced specific viscosity of the DNA solution. These data suggest that **3** and **4** have a different mode of binding than peptides **5–8**. Furthermore, Dnp peptides **5–8** saturate the binding sites of the DNA at 2.2 peptides per 10 base pairs, which is equivalent to $n = 4.5$.

^1H Nuclear Magnetic Resonance Studies. The ^1H NMR studies conducted in this work were performed to determine the effect of binding of the peptides to DNA on (1) the line broadening of the aromatic proton NMR signals of the peptide and (2) the upfield shifting of these resonances. In these experiments, the peptides are fully bound as determined by equilibrium dialysis. The results, shown in Figures 5–7 and summarized in Table IV, lead to the following observations.

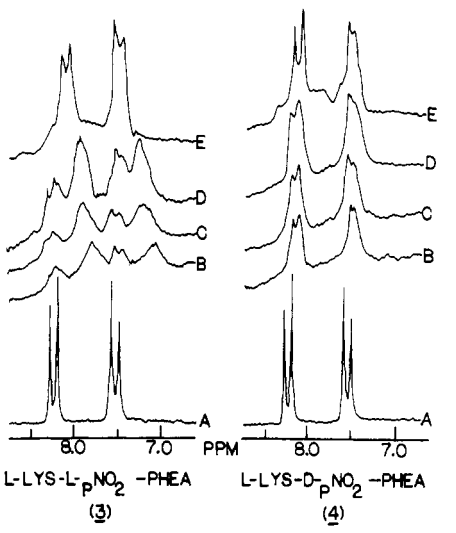

FIGURE 5: 100-MHz NMR spectra of peptides **3** and **4** in the absence and presence of sonicated DNA are shown with the DNA base pair to peptide ratio at (A) 0 (37 °C), (B) 10 (37 °C), (C) 7 (37 °C), (D) 5.5 (37 °C), and (E) 5.5 (90 °C). The spectrometer was operating in the pulse mode, and 100 scans were obtained before applying the Fourier transformation. Temperatures were verified with ethylene glycol.

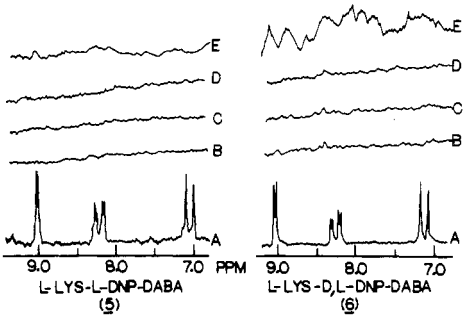

FIGURE 6: 100-MHz NMR spectra of peptides **5** and **6** in the absence and presence of sonicated DNA are shown with the DNA base pair to peptide ratio at (A) 0 (37 °C), (B) 15 (37 °C), (C) 10 (37 °C), (D) 7 (37 °C), and (E) 7 (90 °C). The experimental conditions were as in Figure 5.

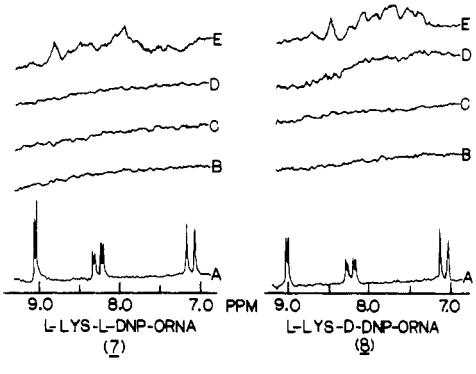

FIGURE 7: 100-MHz NMR spectra for peptides **7** and **8** in the presence and absence of sonicated DNA are shown with the DNA base pair to peptide ratio at (A) 0 (37 °C), (B) 15 (37 °C), (C) 10 (37 °C), (D) 7 (37 °C), and (E) 7 (90 °C). Experimental conditions were as in Figure 5.

First, the NMR spectra for **3** in the presence of DNA is more complex than those for diastereomer **4** in the presence of DNA. In particular, the spectra for **3** in the presence of DNA (as compared to free peptide) show one set of peaks that are only slightly upfield shifted ($\Delta\delta \simeq 0.044$) and only slightly broadened ($\Delta\nu_{1/2} \simeq 6$ Hz) and another set of peaks that are both considerably upfield shifted ($\Delta\delta \simeq 0.45$) and broadened

Table IV: Aromatic Ring Proton Chemical Shifts and Line Widths in the Absence and Presence of Salmon Sperm DNA[a]

compound	position[b]	δ_{free}[c]	δ_{DNA}[c]	$\Delta\delta$	$\Delta\nu_{1/2}$[d] (Hz)
L-Lys-L-pNO$_2$-PHEA (3)	H_1	7.533 ($J_{1,2}$ = 8.54 Hz)	7.088	0.445	10.95
			7.490	0.043	5.47
	H_2	8.233	7.778	0.455	9.86
			8.189	0.044	6.55
L-Lys-D-pNO$_2$-PHEA (4)	H_1	7.559 ($J_{1,2}$ = 8.79 Hz)	7.514	0.045	6.57
	H_2	8.231	8.189	0.044	5.48
L-Lys-L-Dnp-DABA (5)	H_3	7.146 ($J_{3,4}$ = 9.52 Hz)	ND[e]	ND	ND
	H_4	8.310	ND	ND	ND
	H_5	9.087 ($J_{4,5}$ = 2.69 Hz)	ND	ND	ND
L-Lys-DL-Dnp-DABA (6)	H_3	7.155 ($J_{3,4}$ = 9.73 Hz)	ND	ND	ND
	H_4	8.354	ND	ND	ND
	H_5	9.091 ($J_{4,5}$ = 2.69 Hz)	ND	ND	ND
L-Lys-L-Dnp-ORNA (7)	H_3	7.147 ($J_{3,4}$ = 9.76 Hz)	ND	ND	ND
	H_4	8.295	ND	ND	ND
	H_5	9.078 ($J_{4,5}$ = 2.69 Hz)	ND	ND	ND
L-Lys-D-Dnp-ORNA (8)	H_3	7.141 ($J_{3,4}$ = 9.77 Hz)	ND	ND	ND
	H_4	8.272	ND	ND	ND
	H_5	9.082 ($J_{4,5}$ = 2.68 Hz)	ND	ND	ND

[a] Measurements were carried out with low molecular weight DNA at 72 mM P/L in the presence of 1.0 mM EDTA in D_2O (pD 7.0) at 37 °C. [b] H_1 is meta to the nitro group while H_2 is ortho; H_3 is ortho to the carbon attached to the side chain, H_4 is meta to this carbon, and H_5 is the meta position between the two nitro groups. [c] δ_{free} and δ_{DNA} are the chemical shifts, relative to internal TSP, in the absence and presence of DNA at peptide/DNA = 10, respectively. [d] $\Delta\nu_{1/2} = \nu_{1/2,DNA} - \nu_{1/2,free}$, where $\nu_{1/2,DNA}$ and $\nu_{1/2,free}$ are the line widths of the NMR resonances at half-height in the presence and absence of DNA, respectively. [e] ND is not determinable due to the complete line broadening of these resonances in the presence of DNA (see the text).

($\Delta\nu_{1/2} \simeq 10$ Hz). On the other hand, the NMR spectra for **4** in the presence of DNA (as compared to free dipeptide) show only one set of peaks. These peaks are upfield shifted and broadened to an extent which is approximately equal to the first set of peaks for **3** in the presence of DNA. These results indicate that the aromatic ring of **3** is in a different environment than that of **4**, which can be attributed solely to the chirality of the C-terminal amino acid α-carbon (i.e., L vs. D). It should be noted that the line broadening decreases at higher temperatures as the DNA begins to "melt out", freeing bound peptide. Furthermore, the effect of the viscosity of the DNA solution on the line broadening is found to be small (e.g., the signal line width of internal TSP is found to be 1.2 ± 0.2 and 2.1 ± 0.3 Hz in the absence and presence of DNA, respectively).

Second, the ^{1}H NMR signals for Dnp-containing dipeptides **5**–**8** are completely broadened into the base line and are indistinguishable from the base-line noise at all DNA base pair to dipeptide ratios. These results indicate a close proximity of the Dnp ring for each dipeptide to the base pairs of the DNA (Jardetsky & Jardetsky, 1962; Pople et al., 1959).

Discussion

Much research has been devoted to the study of the recognition specificity of proteins for nucleic acids. Due to several types of forces operating at several sites along the nucleic acid backbone, this type of study is of immense complexity. The problem can be simplified by studying the interaction specificities of small oligopeptides with DNA as undertaken in this and previously reported work (Gabbay, 1977). The focal point of this laboratory has been to observe full, partial, or no intercalation of the aromatic rings of specifically designed and synthesized molecules and then to establish criteria which could then be used to predict the extent of intercalation of aromatic rings of other molecules. From the work with reporter molecule I (Figure 8), it was shown that there is a dependence on the length between the aromatic ring and the quaternary ammonium group for full or partial intercalation. Specifically, if the length was long enough ($N \geq 2$), the aromatic ring could fully intercalate; however, if the length was too short ($N = 1$), then the aromatic ring could only partially

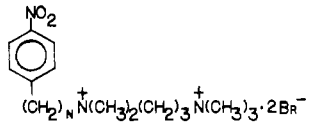

FIGURE 8: Reporter molecule I is shown with N = 1, 2, 3, or 4.

intercalate (Kapicak & Gabbay, 1975). That there is a dependence on the chirality of the C-terminal amino acid α-carbon was shown by the work with diastereomeric dipeptides **1** and **2**, as previously discussed.

In an attempt to provide additional experimental evidence for (or against) these two criteria, the synthesis of the peptides discussed in this paper was undertaken. The series consisted of pairs of diastereomeric dipeptides containing an N-terminal L-lysyl residue and a C-terminal amino acid of either the L or D designation and having a side chain with an aromatic ring at various distances from the α-carbon. On the basis of the two criteria establixhed above, it was predicted that those peptides of the L-D designation would not show intercalation since the aromatic ring would be pointed away from the helix. For the dipeptides of the L-L designation, the aromatic ring would point toward the helix, but the extent of intercalation would depend upon the distance from the aromatic ring to the α-carbon.

The experimental results at least partially support the predictions. In particular, the viscosity and the ^{1}H NMR studies for the diastereomeric pNO$_2$-phenylalanyl containing peptides, **3** and **4**, lead to the conclusion that the aromatic ring of **3** is partially intercalated into the base pairs of DNA, while the aromatic ring of **4** is not. There is a much larger increase in the viscosity of the DNA/peptide complex for **3** as opposed to **4**, which can be attributed to bending the DNA at the point of insertion of the aromatic ring of **3**, resulting in a shortening of the helix length. The small decrease in viscosity of the complex with **4** can be attributed solely to electrostatic binding (Cohen & Eisenberg, 1969, and references therein). The partial intercalation of the aromatic ring of **3** is further verified by examination of the ^{1}H NMR spectra of the peptide in the presence of DNA. The appearance of the set of peaks that are considerably upfield shifted and broadened indicates that the aromatic protons responsible for these resonances are in

close proximity to the ring current of the DNA base pairs (Jardetsky & Jardetsky, 1962; Pople et al., 1959). However, the aromatic resonances of **4** are only slightly upfield shifted and broadened in the presence of DNA, which indicates that the aromatic ring of **4** is pointing out into solution, with no intercalation. The appearance of the set of peaks in the spectra of **3** (in the presence of DNA) which are also only slightly upfield shifted and broadened can be accounted for by considering three possible explanations.

First, the differential shifting and broadening of the aromatic protons of **3** in the NMR spectra in the presence of DNA could arise if the peptide was a mixture of the two possible diastereomers (i.e., both the L-L and the L-D peptides). Since optically pure starting amino acids were used in the synthesis, and since all coupling and deprotection steps have been shown to proceed with no detectable racemization, the possibility of a diastereomeric mixture of peptides can be ruled out.

A second, more probable explanation is that there is slow exchange between intercalated and nonintercalated sites. However, T_1 studies with peptides **1** and **2** in the presence of DNA gave identical relaxation times for the aromatic protons for both peptides. It was therefore concluded that the differences observed in the NMR spectra of **1** compared to **2** was due strictly to differences in the chemical shifts of the ortho, meta, and para protons, not to differences in the chemical exchange or tumbling rates (Gabbay, 1977). Due to the similarity of binding of peptides **1** and **2** to **3** and **4**, it can be assumed that the differences in the shifting and broadening of the aromatic resonances for **3** in the presence of DNA is not due to a slow exchange between binding sites.

A third explanation is based on considering two possible geometries of insertion. The two are (1) a sideways geometry or (2) a straight-in geometry (see Figure 9). Under the assumption that there would be restricted rotation around the C–C bonds connecting the aromatic ring to the peptide backbone once the aromatic ring is wedged between the base pairs of the DNA, the two ortho positions would be in very different environments, as would the two meta positions, for a sideways geometry of insertion. One would expect to observe two sets of resonances in the NMR spectrum of the DNA complex: one set for the ortho and meta positions pointing toward the interior of the helix (inside positions) and one set for the ortho and meta positions pointing away from the helix (outside positions). Furthermore, the inside positions would be upfield shifted to a much greater extent than the outside positions due to their closeness to the ring current of the base pairs. For a straight-in geometry, the two ortho positions are nearly identical, as are the meta positions. The geometry would lead to a much greater upfield shift of the ortho positions than for the meta positions, resulting in only one set of peaks in the NMR spectrum of the DNA complex. This set would include the relatively unshifted meta proton resonances and the considerably shifted ortho proton resonances. Since the spectra recorded for the DNA complex of **3** have two distinct sets of peaks, it is concluded that the aromatic ring of **3** sits in the groove with a sideways geometry (as in Figure 9A).

Further evidence for this geometry comes from comparing the magnitude of the shifting ($\Delta\delta$) of the aromatic resonances for the various positions on the ring. In particular, both inside positions should be shifted to approximately the same extent, and both outside positions should be shifted to approximately the same extent. Examination of the data in Table III indicates that two peaks are shifted by about 0.45 ppm in the DNA complex (as compared to the aromatic resonances for the free peptide), corresponding to the inside positions, and two peaks

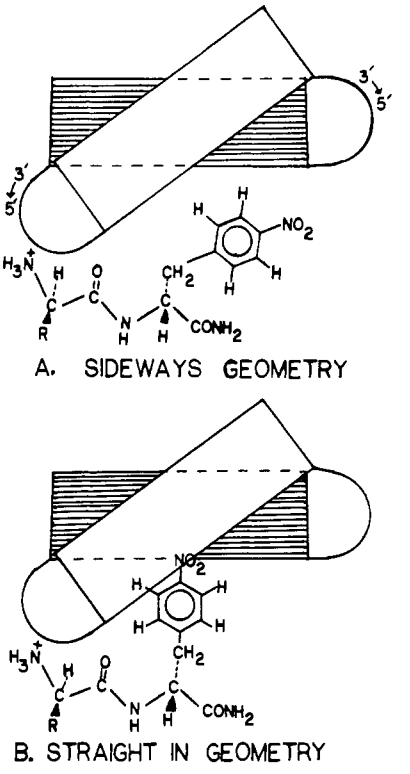

FIGURE 9: Two possible geometries for the partial insertion of the aromatic ring of peptide **3** are shown with (A) representing a sideways geometry and (B) representing a straight-in geometry (see text). These schematic diagrams show the top view of the aromatic ring partially sandwiched between two adjacent base pairs. The side chain of the L-lysyl residue is depicted as R-, and it should be noted that the quaternary ammonium group of this side chain is electrostatically bound to the next phosphate.

are shifted by only about 0.044 ppm, corresponding to the outside positions. These results are completely consistent with the prediction for the sideways geometry. Furthermore, the extent of shifting of the resonances of **4** in the presence of DNA is of the same order as the outside proton resonances for **3** in its DNA complex, indicating that these outside protons are away from the ring current of the base pairs.

For Dnp-containing peptides **5** and **7**, the data from the viscosity and NMR studies indicate that the aromatic rings of these compounds are fully intercalated, as was predicted, due to the large increase in the viscosity of the DNA/dipeptide complexes and the complete line broadening of the aromatic proton resonances of these peptides in the NMR spectra of their DNA complexes. However, the data indicate that the aromatic ring of **8** (the diastereomer of **7**) also fully intercalates, which is in opposition to the prediction. This conclusion could be explained by one of two mechanisms: (1) intercalation is favored over electrostatic binding; (2) electrostatic binding occurs, and because of the flexibility and length of the side arm, intercalation of the aromatic ring still occurs. In other words, the side arm is long enough, after electrostatic binding of the α- and ϵ-amino groups of the lysyl residue, to allow the aromatic ring, which is seeking a hydrophobic environment, to swing around toward the helix and then intercalate. Since both **7** and **8** bind with about equal affinity, mechanism 2 is reasonable.

The data from compound **6** can also support this mechanism. Whereas diastereomers **7** and **8** behave identically in the presence of DNA, compounds **5** and **6** do not behave the same. Since **6** is a 1/1 mixture of both the L-L and the L-D diastereomers, one would expect both diastereomers to behave the same (as the case for **7** and **8**) if mechanism 2 is indeed

operative. However, for the L-D diastereomer, the side arm may not be long enough to allow the ring to fully intercalate after swinging around toward the interior of the helix. Thus, **6** would be a mixture of a full intercalator (the L-L diastereomer, which would behave identically with **5**) and a partial intercalator (the L-D diastereomer). If this were the case, one would expect to see mixed results from studies with this diastereomeric mixture of peptides. This is, indeed, the case.

So that additional support for mechanism 2 could be provided, model studies were performed. For reporter molecule I with $N = 1$, the distance between the aromatic ring and the quaternary ammonium nitrogen is on the order of 4 Å, while this distance is on the order of 5 Å with $N = 2$. Furthermore, the distance between the aromatic ring and the α-carbon of dipeptide **1** or **3** is also approximately 4 Å. Therefore, the minimum critical distance between the aromatic ring and the backbone to which it is attached must be between 4 and 5 Å to allow for full intercalation. For dipeptides **5** and **7**, this distance is approximately 6 and 7.5 Å, respectively. This is certainly long enough to allow full intercalation of their aromatic rings. For compound **8**, after the aromatic ring is swung around and pointed toward the helix, the distance between the ring and the α-carbon is about 4.5 Å. This distance is apparently long enough to allow full intercalation. However, for L-Lys-D-Dnp-DABA, this distance is only on the order of 3 Å, which is insufficient to allow full intercalation. It should be noted that the model studies were performed by assuming a certain rigidity in the peptides after binding. More experimental evidence is needed to support this mechanism, e.g., doing the experiments with optically pure L-Lys-D-Dnp-DABA.

In conclusion, this work has provided evidence for the stereospecific binding of the dipeptides studied followed by intercalation of the aromatic ring if the side arm, connecting the aromatic ring to the peptide backbone, is sufficiently long enough, regardless of the chirality of the N-terminal amino acid. The results support the hypothesis that the side chain of the N-terminal amino acid of the D designation points out toward the solvent but adds the condition that intercalation may still occur if the side arm is flexible and long enough.

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Registry No. **3**, 84849-52-5; **4**, 84849-53-6; **5**, 84849-54-7; **6**, 84849-55-8; **7**, 84849-56-9; **8**, 84849-57-0; poly[d(A-T)], 26966-61-0; poly(I)–poly(C), 24939-03-5.

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