Kornberg, A. (1948), *J. Biol. Chem. 176,* 1475.
Kornberg, A. (1962), in Horizons in Biochemistry, Kasha, M., and Pullman, B., Ed., New York, N Y., Academic Press, p 251.
Kornberg, A. (1974), DNA Synthesis, San Francisco, Calif., W. H. Freeman, p 41.
Lehman, I. R. (1974), *Enzymes, 3rd Ed., 10,* Chapter 8.
Lehninger, A. L. (1965), Bioenergetics, New York, N.Y., W. A. Benjamin, p 184.
Miyake, A., and Stockmayer, W. H. (1965), *Makromol. Chem. 88,* 90.
Modrich, P., and Lehman, I. (1973), *J. Biol. Chem. 248,* 7502.
Peller, L. (1961), *Biochim. Biophys. Acta 47,* 61.
Peller, L. (1966), *Proc. Natl. Acad. Sci. U.S.A. 55,* 1025.
Peller, L. (1975), *Biochem. Biophys. Res. Commun. 63,* 912.
Peller, L., and Barnett, L. (1962), *J. Phys. Chem. 66,* 680.
Pisetsky, D., Berkower, I., Wickner, R., and Hurwitz, J. (1972), *J. Mol. Biol. 71,* 557.
Podolsky, R. J., and Morales, M. F. (1956), *J. Biol. Chem. 218,* 945.
Podolsky, R. J., and Sturtevant, J. M. (1955), *J. Biol. Chem. 217,* 603.
Roberts, J. D., and Caserio, M. C. (1965), Basic Principles of Organic Chemistry, New York, N.Y., W. A. Benjamin, p 714.
Schekman, R., Weiner, A., and Kornberg, A. (1974), *Science 186,* 987.
Trevelyan, W. E., Mann, P. E., and Harrison, J. S. (1952), *Arch. Biochem. Biophys. 39,* 419.
Watson, J. D. (1970), Molecular Biology of the Gene, 2nd ed, New York, N.Y., W. A. Benjamin pp 150–159.
Weiss, B., and Richardson, C. C. (1967), *Proc. Natl. Acad. Sci. U.S.A. 57,* 1021.
Wells, R. D., and Inman, R. B. (1973), DNA Synthesis in Vitro, Baltimore, Md., University Park Press.
Zimm, B. H. (1960), *J. Chem. Phys. 33,* 1349.

Stereospecific Binding of Diastereomeric Peptides to Salmon Sperm DNA†

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ABSTRACT: Studies of the interaction specificities of L-lysyl-L-phenylalaninamide (**1**) and the diastereomeric dipeptide amide, L-lysyl-D-phenylalaninamide (**2**), with salmon sperm DNA reveal distinct differences in the binding site of the aromatic ring of the phenylalanine residue. The results of ^{1}H nuclear magnetic resonance (NMR), spin-lattice relaxation rates, viscometric, and flow dichroism studies indicate the aromatic ring of **1** is "partially" inserted between base pairs of DNA whereas the aromatic ring of **2** points outward toward the solution. The terminal L-lysyl residue presumably interacts stereospecifically with DNA helix thus dictating the positioning of the aromatic ring of the C-terminal phenylalanine residue. In the accompanying paper (E. J. Gabbay et al. (1976), *Biochemistry,* following paper in this issue), the interaction of several oligopeptide amides (containing the N-terminal L-Lys-L-Phe residue) with DNA is examined. The results are found to be consistent with stereospecific binding of the terminal L-lysyl residue, and in addition, the evidence suggests that oligopeptides may bind to DNA via a modified single-stranded β-sheet structure which is wrapped around the nucleic acid helix in a manner similar to that described by M. H. F. Wilkins ((1956), *Cold Spring Harbor Symp. Quant. Biol. 21,* 75).

The mechanism(s) by which proteins of defined amino acid sequence may recognize specific sequences of nucleic acid has been the subject of considerable interest in many laboratories. Our approach to this problem has been centered on model systems composed of small oligopeptide amides interacting with DNA of various AT/GC compositions (Gabbay et al., 1972, 1973). Although the relationship of these model studies to the overall problem of the recognition process between macromolecules (DNA and proteins) is not immediately obvious, nonetheless, the results obtained are valuable in elucidating the interaction specificities of nucleic acids with small oligopeptide systems. Preliminary study on the interaction specificities of L-lysyl-L-phenylalaninamide (**1**) and the diastereomeric peptide L-lysyl-D-phenylalaninamide (**2**) to DNA has been presented (Adawadkar et al., 1975). The results indicate that stereospecific peptide–DNA complexes are obtained whereby the aromatic rings of **1** and **2** point into and out of the helix, respectively. In this paper, further evidence is presented in support of the above mode of interaction of DNA with **1** and **2**. The results suggest that the ϵ- and α-amino groups of the N-terminal L-lysyl residue interact stereospecifically with the DNA helix thus dictating the positioning of the aromatic ring of the C-terminal phenylalanine residue. In the accompanying paper (Gabbay et al., 1976), the interaction of several oligopeptides, containing the N-terminal L-Lys-L-Phe residue, with DNA is examined. The results are found to be consistent with stereospecific binding of the ter-

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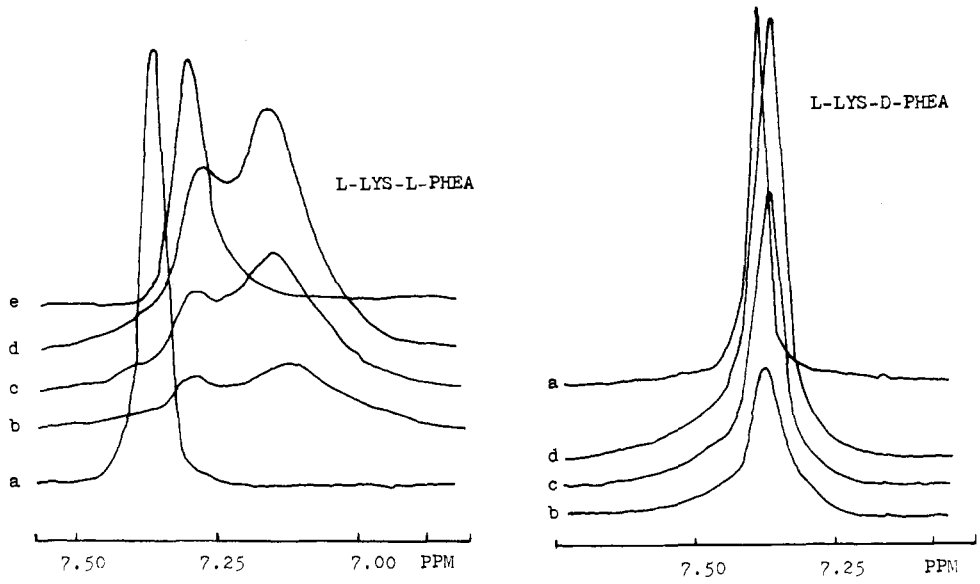


FIGURE 1: The ^{1}H NMR signal of the aromatic protons of L-Lys-L-PheA (**1**) and L-Lys-D-PheA (**2**) (a and b, respectively); in the absence (a) and presence of DNA at a base pair to peptide ratio of 7.5 (b), 3.6 (c), 2.4 (d), and 0.5 (e). See Table I for details.

minal L-lysyl residue and, in addition, the evidence suggests that oligopeptides may bind to DNA via a modified single-stranded β-sheet structure which is wrapped around the nucleic acid helix in a manner similar to that described by Wilkins (1956).

Materials and Methods

Synthesis. The peptide amides were synthesized using the classical mixed-anhydride procedure (Anderson et al., 1967) from carbobenzoxy-N-protected amino acids (Sigma Chemicals) and amino acid esters (Sigma Chemicals). The N-Cbz[1] dipeptide esters were converted to the amides according to standard procedure (Greenstein and Winitz, 1961) and the N-Cbz group removed by hydrogenation in a Paar shaker in methanol–acetic acid mixture (99.9:0.1). The acetate salts of the peptide amides were converted to the chlorides via anion exchange chromatography using Amberlite CG-400 (100–200 mesh) resin (Baker Chemicals). All products were checked for purity and authenticity by ^{1}H nuclear magnetic resonance (NMR), circular dichroism, ultraviolet, infrared, paper chromatography, and elemental analysis. It should be noted that the peptide coupling reaction employed in the above syntheses has been shown to proceed with no detectable racemization (Anderson et al., 1967). Circular dichroism spectra and chromatographic purity of the intermediates and the diastereomeric peptide amides are consistent with optically pure products.

Analytical Methods. Analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. Infrared spectra were recorded on a Perkin-Elmer Model 337. ^{1}H NMR spectra were performed on a Varian XL-100-15 spectrometer at 34°. The spin–lattice relaxation times, T_1, were measured by the inversion recovery method with a Nicolet Technology Corporation FT accessory. Pulse widths of 49 (180°) and 24.5 μsec (90°) were used, and 50 scans were accumulated for each delay time. Twenty seconds was taken as the infinite delay time and 4K transform was used. Sonicated low molecular weight salmon sperm DNA was used at 60–70 mM phosphate/liter in the presence of 1 mM EDTA in D_2O (pD 7.0). The concentration of the peptide amides was varied from 2 to 70 mM. Chemical shifts (in Hz) from the internal standard sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$ (TSP) are reported. It is found that the chemical shifts, δ, and T_1 values are reproducible to ±0.2 Hz and ±10%, respectively.

Circular dichroism measurements were recorded on a Jasco J-20 spectropolarimeter at ambient temperature (24 ± 1°). Viscosity studies were performed with a low-shear Zimm viscometer (Beckman Instrument Co.) for native DNA and Ubbelohde viscometer for low molecular weight DNA. Ultraviolet and visible absorption measurements were recorded on a Cary 15 spectrophotometer. Quantitative assay for peptide concentrations (equilibrium dialysis studies) was made with fluorescamine (kindly supplied by Dr. Weigele, Hoffmann-La Roche) according to previously published procedure (Gabbay et al., 1973). Flow dichroism measurements were carried out at 25 ± 1° at 260 nm using the Cary 15 spectrometer with a Glan-Taylor calcite polarizing prism. DNA (0.5–3.0 mM P/l.) solution was flowed through a quartz capillary (0.415 mm radius) by means of a Sage syringe pump. The shear rate in all experiments was maintained constant at 2600 sec^{-1}.

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

Results

^{1}H Nuclear Magnetic Resonance Studies. Figure 1 shows the effect of binding to salmon sperm DNA (at various base pair to peptide ratio) on the chemical shift, δ, and signal broadening of the aromatic protons of L-Lys-L-PheA (**1**) and L-Lys-D-PheA (**2**). The results are summarized in Table I together with the measured values of the spin–lattice relaxation times, T_1, in the presence and absence of DNA. Several interesting observations can be made.

(1) In the presence of DNA (i.e., base pair to peptide ratio of 7.5, 3.6, 2.4, and 0.5) large differences between the chemical shifts and line broadening of the aromatic protons

[1] Abbreviations used are: Cbz, carbobenzoxy; TSP, sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$; Mes, 2-(*N*-morpholino)ethanesulfonate.

Table I: The Chemical Shift, δ, the Spin–Lattice Relaxation Time, T_1, and Signal Line Broadening ($\Delta\nu_{1/2}$), of the Aromatic Protons of L-Lys-L-PheA (**1**) and L-Lys-D-PheA (**2**) in the Presence and Absence of Salmon Sperm DNA.[a]

	Chemical Shift, δ (Hz), T_1 (sec), and $\Delta\nu_{1/2}$ (Hz)				
		Base Pair/Peptide			
System	Free	7.5	3.6	2.4	0.5
L-Lys-L-PheA	736.5 (2.02) (5.7)	713.0 (0.57) 730.5 (0.77) (30.7)	714.5 (0.62) 729.5 (0.80) (28.0)	715.5 (0.61) 728.0 (0.79) (23.2)	730.0 (1.4) (7.1)
L-Lys-D-PheA	738.1 (2.02) (4.2)	735.9 (0.66) (6.5)	736.0 (0.71) (6.5)	736.0 (0.72) (6.5)	

[a] Sonicated low molecular weight salmon sperm DNA was used at 60–70 m*M* phosphate/l. in the presence of 1 m*M* EDTA in D_2O (pD 7.0). The concentration of **1** and **2** was varied from 4 to 15 m*M*. Spectra were recorded at 34°C using a Varian XL-100-15 spectrometer equipped with a Nicolet Technology FT accessory. Chemical shifts (Hz) from the internal standard sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TSP) are reported. It should be noted that the chemical shifts, δ, are accurate to ±0.2 Hz and T_1 values are accurate to ±10%. The T_1 value of the internal standard, TSP, is not affected by the presence of DNA (T_1 = 3.4 ± 0.2). The line broadening ($\Delta\nu_{1/2}$) is measured at half signal height.

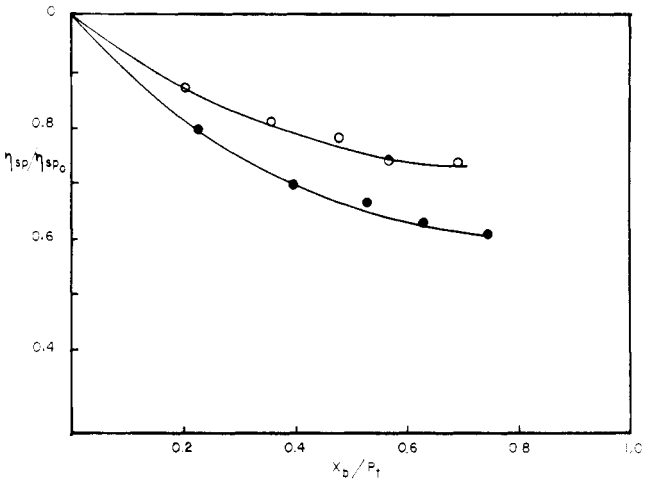


FIGURE 2: The effect of bound L-Lys-L-PheA (●) and L-Lys-D-PheA (○) on the relative specific viscosity of DNA (X_b represents the concentration of bound peptide and P_t the total DNA concentration in P/l.). Viscosity measurements were carried out at near infinite dilution of native salmon sperm DNA (0.26 m*M* in P/l.) in 10 m*M* 2-(*N*-morpholino)ethanesulfonate (Mes) buffer (pH 6.2) (5 m*M* in Na^+) using the low shear Zimm viscometer at 37.5°.

of L-Lys-L-PheA and L-Lys-D-PheA are observed (Figure 1). For example, the L-Lys-D-PheA (**2**) exhibits slight broadening and upfield chemical shift under conditions where the peptide is 100% bound to DNA. [The binding constants of **1** and **2** to DNA were determined by equilibrium dialysis and found to be 8000 and 6000, respectively. Under the conditions of the ^{1}H NMR experiments shown in Figure 1, the dipeptide amides are totally bound to DNA at base pair to peptide ratio greater than 2.0.] On the other hand, the ^{1}H NMR signal of L-Lys-L-PheA in the presence of DNA (base pair to peptide ratio of 7.5) exhibits two broad resonance lines which are shifted upfield with respect to the free peptide by 6.0 and 23.5 Hz, respectively. At low base pair to peptide ratio (i.e., less than one, see Figure 1a), L-Lys-L-PheA exhibits a single ^{1}H NMR resonance line for the aromatic protons which is indicative of fast exchange between the free and the various possible DNA binding sites of **1**.

(2) The apparent spin–lattice relaxation times, T_1, for the aromatic protons of **1** and **2** are found to be identical for the free state, i.e., T_1 = 2.02 sec. In the presence of DNA (base pair/peptide = 7.5), two apparent values are measured for **1**, e.g., T_1 = 0.57 and 0.77 sec for the high and low field signals, respectively (Figure 1a, Table I). The apparent T_1 value for the diastereomeric dipeptide amide, L-Lys-D-PheA, in the presence of DNA is also observed to be similar in magnitude, i.e., 0.66 sec. It should be noted that the measured T_1 values are found not to be sensitive to dissolved oxygen in solution; however, considerable variations in T_1 values are obtained if paramagnetic impurities are present in the DNA. The work reported in this paper was carried out with sonicated DNA which has been extensively dialyzed against EDTA and with ^{1}H NMR samples which have been placed under nitrogen atmosphere. The ^{1}H NMR relaxation data suggest that the phenyl rings of **1** and **2** in the DNA complex experience restriction in tumbling of equal magnitude. However, the differences in chemical shifts and line broadening suggest that the aromatic rings of **1** and **2** are in different chemical environments in the DNA complexes (see Discussion).

Viscometric Studies. The effect of bound L-Lys-L-PheA (**1**) and L-Lys-D-PheA (**2**) on the relative specific viscosity of native salmon sperm DNA (mol wt $\simeq 6 \times 10^6$) solution at 37° is shown in Figure 2. Viscometric measurements were carried out at near infinite dilution of the nucleic acid (0.26 m*M* P/l., i.e., 0.01% in DNA) in 10 m*M* Mes buffer (pH 6.2) (5 m*M* Na^+) using the low shear Zimm viscometer. It is observed that the dipeptide amide, L-Lys-L-PheA (**1**), exhibits a larger decrease in the relative specific viscosity (η_{sp}/η_{sp_0}) where η_{sp} and η_{sp_0} are the specific viscosities in the presence and absence of peptide, respectively, of the DNA solution than the corresponding diastereomer, L-Lys-D-PheA (**2**). The decrease in viscosity of the DNA solution by the peptide amides **1** and **2** may be explained in terms of (i) shortening of the helix length and/or (ii) via peptide induced intramolecular aggregation effect (Muller and Crothers, 1968). In order to rule out the intramolecular aggregation possibility, the effect of bound L-Lys-L-PheA (**1**) and L-Lys-D-PheA (**2**) on the relative specific viscosity of rod-like sonicated salmon sperm DNA (mol wt $\leq 5 \times 10^5$) at 17 and 35° was studied and the results are shown in Figure 3. The viscometric measurements were also carried out at near infinite dilution of the nucleic acid (0.2 m*M* in P/l., i.e., 0.01% in DNA) in 10 m*M* Mes buffer (pH 6.2) (5 m*M* Na^+) using the Ubbelohde viscometer. Results identical with those obtained with the high molecular weight native DNA are observed, i.e., greater decrease in the η_{sp}/η_{sp_0} value in the presence of **1** as compared to **2**. In addition, titration of the DNA–**2** complex (at a bound peptide to phosphate ratio of 0.64) with L-Lys-L-PheA (**1**) results in an additional lowering of η_{sp}/η_{sp_0} to a value approaching that of DNA–**1** complex (Figure 3b). It is therefore reasonable to

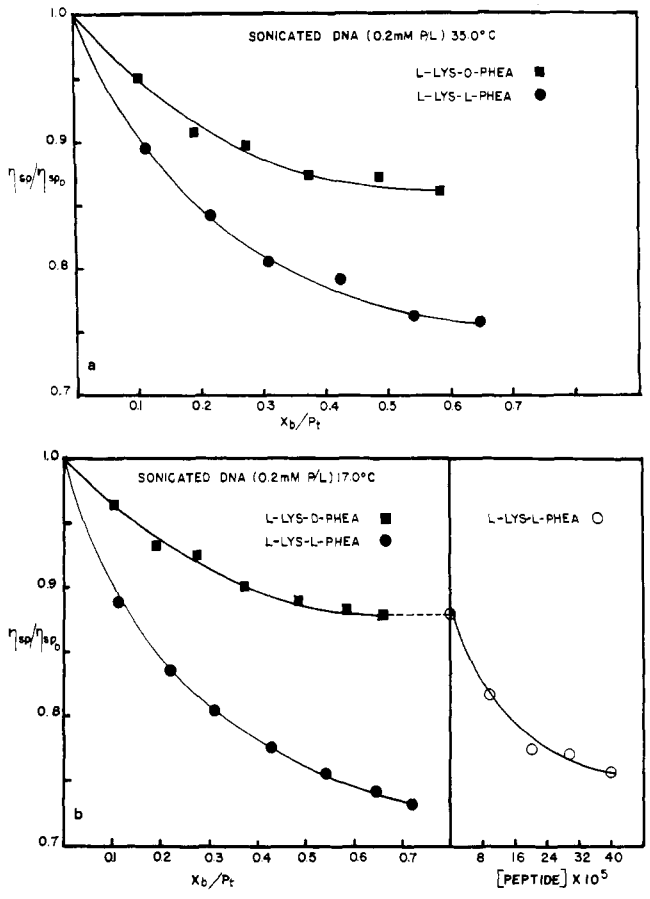


FIGURE 3: The effect of bound L-Lys-L-PheA (●) and L-Lys-D-PheA (■) on the relative specific viscosity of sonicated low molecular weight DNA (mol wt $\leq 5 \times 10^5$) at 35° (a) and 17° (b).

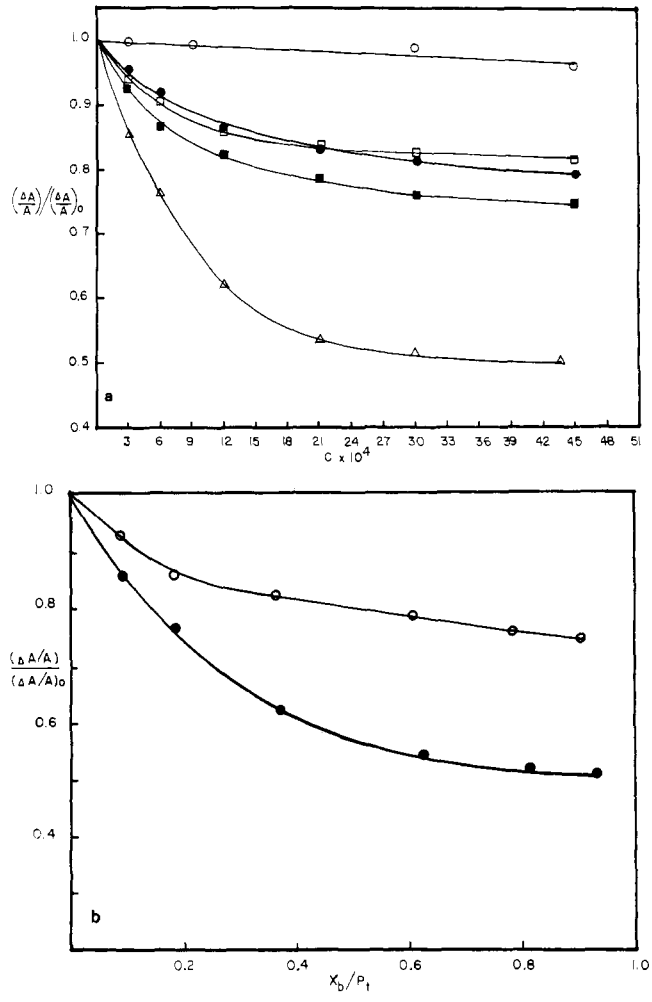


FIGURE 4: (a) The effect of increasing concentration of NaCl (○), 1,5-diaminopentane·2HCl (□), L-Lys-L-LeuA (●), L-Lys-D-PheA (■), and L-Lys-L-PheA (△) on the relative reduced dichroism of DNA. (b) The effect of bound L-Lys-L-PheA (●) and L-Lys-D-PheA (○) on the relative reduced dichroism of DNA. It should be noted that at the highest peptide concentration used in these studies, the peptide contribution to the absorbance at 260 nm is found to be less than 1%.

conclude that the lower viscosity of the DNA solutions induced by the peptides is due to the shortening of the helix length rather than an intramolecular aggregation phenomenon.

Flow Dichroism. The effect of increasing concentrations of NaCl, 1,5-diaminopentane·2HCl, L-lysyl-L-leucinamide, and the dipeptide amides, L-Lys-L-PheA (**1**) and L-Lys-D-PheA (**2**), on the relative reduced dichroism of native salmon sperm DNA (at 3 m*M* P/l.) is shown in Figure 4a. Figure 4b shows the effect of *bound* L-Lys-L-PheA (**1**) and L-Lys-D-PheA (**2**) on the relative reduced dichroism. It is noted that the value of the reduced dichroism ratio, $(\Delta A/A)/(\Delta A/A)_0$ (where $\Delta A = A_{\parallel} - A_{\perp}$ and A is the absorbance of a stationary DNA solution at 260 nm; $(\Delta A/A)$ and $(\Delta A/A)_0$ refer to the reduced dichroism of DNA–peptide complex and free DNA, respectively), is significantly diminished in the presence of 1,5-diaminopentane·2HCl and the L-lysine containing dipeptide amides. The observed decrease in the relative reduced dichroism of DNA solution is more pronounced in the presence of L-Lys-L-PheA (**1**) than with the diastereomer L-Lys-D-PheA (**2**). The latter shows effects similar in magnitude to L-Lys-L-LeuA and 1,5-diaminopentane·2HCl (Figure 4a). Identical results are also obtained at lower DNA concentration (0.5 m*M* P/l.) which indicates that the effects are caused by a molecular conformational change in the DNA rather than via intermolecular aggregation effect induced by the peptide binding.

Uv and CD Spectral Studies. Interactions of the peptide amides, **1** and **2**, with salmon sperm DNA were studied by uv absorption and circular dichroism techniques. Little or no difference is detected in the absorption spectrum of DNA in the presence of the dipeptide amides. Similarly, little or no change is observed in the trough at 245 nm; however, a slight decrease in the peak at 275 nm of the CD spectrum of DNA in the presence of **1** and **2** is observed (Figure 5).

Discussion

The interactions of oligopeptides of defined sequence with DNA have been studied extensively by many investigators. In particular, it has been found that the aromatic amino acids in peptides may bind to DNA via an *intercalation* mechanism (Brown, 1970; Helene et al., 1971a,b; Grabbay et al., 1972, 1973; Dimicoli and Helene, 1974; Raszka and Mandel, 1971; Friedman and Ts'o, 1971). Brown (1970) was first to suggest the "bookmark" hypothesis whereby the aromatic rings of amino acids may behave as bookmarks and thus anchor the proteins to specific sequences of nucleic acids. Recent studies (Dimicoli and Helene, 1974; Gabbay et al., 1973) on tryptophanyl, tyrosyl, and phenylalanyl-containing peptides utilizing ^{1}H NMR, fluorescence, equilibrium dialysis, viscometric, and circular dichroism techniques have presented evidence which is con-

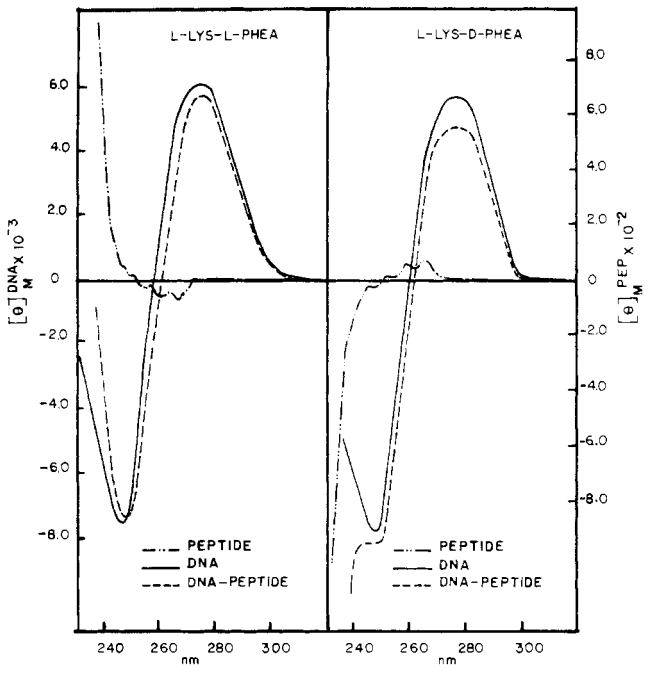
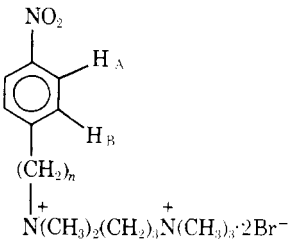


FIGURE 5: The circular dichroism spectra of salmon sperm DNA in the presence and absence of the dipeptide amides, **1** and **2**. The CD experiments were carried out utilizing 45.8 μM P/l. of DNA and large excess of the peptide amides (800 μM) in 10 mM Mes buffer (pH 6.2) (5 mM Na^+) at 24 ± 1°C.

sistent with the intercalation model. In addition, work from this laboratory (Kapicak and Gabbay, 1975) on model systems, I, has revealed that the extent of insertion of the aromatic ring between base pairs of DNA has profound effect



I, n = 1-4

on the tertiary structure of the latter, i.e., either shortening or lengthening of the helix. For example, it is found that at an n = 1, the nitrophenyl ring of I is "partially" inserted between base pairs of DNA as evidenced by the lower viscosity of the DNA-I complex and by the lack of ^{1}H NMR signal broadening of the aromatic protons. Moreover, it is found that the A protons of the aromatic ring of I (n = 1) experience a greater upfield chemical shift than the B protons in the DNA-I complex which is consistent with the "wedge type partial insertion" model (Kapicak and Gabbay, 1975). On the other hand, with n = 3 and 4, the aromatic cations, I, cause enhanced viscosity of DNA solutions and the ^{1}H NMR signals of the aromatic protons are totally broadened and indistinguishable from baseline noise. Peptides containing the aromatic amino acids, tryptophan, phenylalanine, and tyrosine, exhibit effects identical with the aromatic cation, I, where n = 1, i.e., "partial" insertion between base pairs of DNA leading to shortening of the DNA length (Gabbay et al., 1973). Presumably, the single methylene group (CH_2), between the peptide backbone and the aromatic ring of tyrosine, tryptophan, and phenylalanine is not sufficient to allow for "full" insertion and lengthening of the helix. In this work, additional evidence is presented which is consistent with the above interpretation.

Stereospecific Binding of L-*Lys*-L-*PheA* (**1**) *to DNA.* The proton magnetic resonance data (Figure 1a and b) indicate that the protons of the aromatic ring of L-Lys-L-PheA (**1**) experience a large upfield chemical shift (23.5 Hz) and line broadening, whereas the aromatic protons of the diastereomeric peptide L-Lys-D-PheA is relatively unaffected upon binding to DNA. A model which assumes that the aromatic ring of **1** points into the helix (i.e., partial insertion between base pairs of DNA) and the aromatic ring of **2** points outward toward the solvent can best explain the data. The larger upfield chemical shift experienced by the aromatic protons of **1** as compared to **2** is indicative of closer contact to the DNA base pairs and is due to ring current anisotropy (Jardetsky and Jardetsky 1962; Pople et al., 1959). On the other hand, the large ^{1}H NMR signal broadening of the aromatic protons of **1** ($\Delta\nu_{1/2}$ = 31 Hz) as compared to **2** ($\Delta\nu_{1/2}$ = 6.5 Hz) could be explained by several mechanisms: (i) slower tumbling rates of the aromatic ring of **1** in the DNA complex as compared to **2**, (ii) slow exchange between various DNA binding sites for DNA-**1** as compared to DNA-**2** complex, (iii) larger differences in the chemical shifts are experienced by the ortho, meta, and para protons of the aromatic protons of **1** in the DNA complex as compared to **2**, and/or (iv) combination of all three mechanisms. In order to discriminate between the above alternatives, the spin-lattice relaxation time (T_1) measurements were performed on the DNA-**1** and -**2** complexes under conditions of total binding. Since (a) the value of T_1 is determined (among other things) by the correlation time (τ_c) and the mean residence time (τ_m) (Pople et al., 1959; Mildvan and Cohn, 1970; Dwek, 1973) and (b) the observation that the T_1 values of the aromatic protons of **1** and **2** in the DNA complex are nearly identical ($T_1 \simeq 0.65$ sec, Table I) it is concluded that the tumbling rate ($1/\tau_c$) and the chemical exchange rate ($1/\tau_m$) of the aromatic protons of **1** and **2** in the DNA complex are very similar in magnitude. Therefore, the large ^{1}H NMR signal line broadening observed for the aromatic protons of **1** in the DNA complex ($\Delta\nu_{1/2}$ = 31 Hz) can only be due to large differences in the chemical shifts experienced by the ortho, meta, and para protons. The observation of two ^{1}H NMR signals for the aromatic protons of **1** in the presence of excess DNA (Figure 1a) would result from the greater upfield chemical shift experienced by the meta and para protons than by the ortho protons as a consequence of ring current anisotropy of neighboring base pairs (Figure 6). The observed relative areas of the two aromatic signals of DNA-**1** are consistent with this interpretation. The results of the flow dichroism (Figure 4) and viscometric (Figures 2 and 3) studies provide added support in favor of the "partial insertion (or intercalation)" model. The selective lowering of the relative specific viscosity and reduced dichroism of DNA solution upon binding the dipeptide amide, **1**, suggests that the effective length of the DNA helix is smaller in the DNA-**1** than in the DNA-**2** complex. Tilting (or bending) of the helix at the point of insertion of the aromatic ring of the dipeptide amide, **1**, between base pairs (schematically shown in Figure 6) would adequately account for all the observed data. The aromatic ring of L-Lys-D-PheA (**2**), on the other hand, points outward toward the solvent and experiences (i) small upfield chemical shifts and (ii) similar chemical shifts, δ, for the ortho, meta, and para protons ($\Delta\nu_{1/2}$ = 6.5 Hz, Table I) in the DNA complex. The results of the rela-

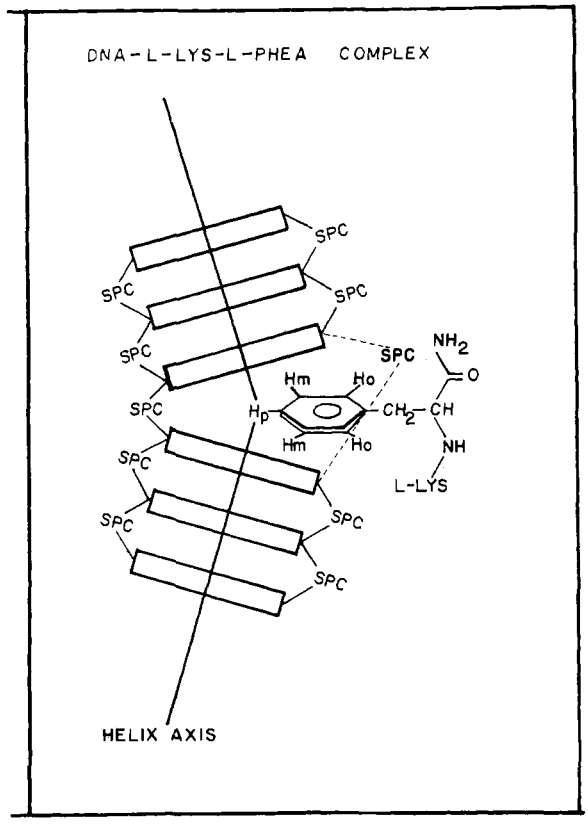


FIGURE 6: Schematic illustration of a segment of DNA duplex showing partial insertion of the aromatic ring of L-Lys-L-PheA (**1**). Under these conditions, the para and meta protons (H_p and H_m) are expected to undergo greater upfield chemical shifts than the ortho protons (H_o) due to ring current anisotropy of the neighboring bases of DNA (see Discussion).

tive specific viscosity and reduced dichroism of DNA solution upon binding the dipeptide amide, **2**, are consistent with the above interpretation. For example, the effects on the viscosity and dichroism of DNA exhibited by L-Lys-D-PheA are similar to those observed for L-Lys-L-LeuA and 1,5-diaminopentane·2HCl (Figure 4, and also Gabbay et al., 1973). Such effects are presumably due to shielding of neighboring negatively charged phosphate groups by the positively charged counterions which would lead to electrostatic constriction of the DNA polymer (see Cohen and Eisenberg, 1969, and references therein).

In summary, it is found that the diastereomeric dipeptide amides, L-Lys-L-PheA (**1**) and L-Lys-D-PheA (**2**), interact stereospecifically with DNA. Since it has been previously shown by Gabbay et al. (1973) that L- and D-phenylalaninamide interact similarly to DNA, it is concluded that the N-terminal L-lysyl residue of **1** and **2** binds stereospecifically to DNA and dictates the positioning of the aromatic ring of the C-terminal phenylalanine residue.

References

Adawadkar, P., Wilson, W. D., Brey, W., and Gabbay, E. J. (1975), *J. Am. Chem. Soc. 97,* 1959.

Anderson, G. W., Zimmerman, J. E., and Callahan, F. M. (1967), *J. Am. Chem. Soc. 89,* 5012.

Brown, P. E. (1970), *Biochim. Biophys. Acta 213,* 282.

Cohen, G., and Eisenberg, H. (1969), *Biopolymers 35,* 251.

Dimicoli, J. L., and Helene, C. (1974), *Biochemistry 13,* 714, 724.

Dwek, R. A. (1973), Nuclear Magnetic Resonance in Biochemistry, Oxford, Clarendon Press.

Friedman, S., and Ts'o, P. O. P. (1971), *Biochemistry 10,* 3099.

Gabbay, E. J., Adawadkar, P. D., Kapicak, L., Pearce, S., and Wilson, W. D. (1976), *Biochemistry,* following paper in this issue.

Gabbay, E. J., Sanford, K., and Baxter, S. (1972), *Biochemistry 11,* 3429.

Gabbay, E. J., Sanford, K., Baxter, C. S., and Kapicak, L. (1973), *Biochemistry 12,* 4021.

Greenstein, J. P., and Winitz, M. (1961), Chemistry of the Amino Acids, New York, N.Y., Wiley.

Helene, C., Dimicoli, J. L., and Brun, F. (1971b), *Biochemistry 10,* 3802.

Helene, C., Montenry-Garestier, T., and Dimicoli, J. L. (1971a), *Biochim. Biophys. Acta 254,* 349.

Jardetsky, O., and Jardetsky, C. D. (1962), *Methods Biochem. Anal. 9,* 235.

Kapicak, L., and Gabbay, E. J. (1975), *J. Am. Chem. Soc. 97,* 403.

Mildvan, A. S., and Cohn, M. (1970), *Adv. Enzymol. Relat. Areas Md. Biol. 33,* 1.

Muller, W., and Crothers, D. M. (1968), *J. Mol. Biol. 35,* 251.

Pople, J. A., Schneider, W. G., and Bernstein, H. J. (1959), High-Resolution Nuclear Magnetic Resonance, New York, N.Y., McGraw-Hill.

Raszka, M., and Mandel, M. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 1190.

Wilkins, M. H. F. (1956), *Cold Spring Harbor Symp. Quant. Biol. 21,* 75.