The Interaction Specificity of Peptides with DNA. Evidence for Peptide β-Sheet–DNA Binding†

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ABSTRACT: Proton magnetic resonance studies (^{1}H NMR) of the interaction of oligopeptide amides of defined sequence (and containing the amino acid, phenylalanine) with salmon sperm DNA are reported. The extent of upfield chemical shifts, $\Delta\delta$, and signal line broadening of the aromatic protons (in the presence of excess DNA) are found to depend on the primary sequence and stereochemistry of α carbons of the amino acids in the oligopeptide amides. The results obtained with 21 different di-, tri-, tetra-, penta-, and hexapeptide amides are found to be consistent with a model whereby the peptide assumes a slightly 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).

In the preceding paper (Gabbay et al., 1976), evidence was presented which indicates that L-lysyl-L-phenylalaninamide (**1**) and the diastereomer L-lysyl-D-phenylalaninamide (**2**) interact in a stereospecific manner with salmon sperm DNA. In particular, the ^{1}H NMR, viscometric and flow dichroism data suggest that the aromatic ring of **1** is "partially inserted" between base pairs of DNA whereas the aromatic ring of **2** points outward toward the solvent. In order to account for this specificity it is necessary to conclude that the ϵ- and α-amino groups of the N-terminal L-lysyl residue interact in a stereospecific manner with DNA which dictates the positioning of the aromatic ring of the C-terminal phenylalanine residue of **1** and **2**. Such effect is schematically illustrated in Figure 1. It should be noted that no significant differences in the binding of L- and D-phenylalaninamide to DNA have been found by ^{1}H NMR studies (Gabbay et al., 1973), and, therefore, the specificity observed with **1** and **2** cannot be attributed to the chirality of the phenylalanine residue by itself.

In this paper, the syntheses of oligopeptide amides containing an N-terminal L-lysine and L-phenylalanine were undertaken in order to determine the effect of the primary sequence and the stereochemistry of the α carbons of the peptides on the binding of the aromatic ring (used in this case as a probe) to DNA.

Materials and Methods

Synthesis. The peptide amides were synthesized using the classical mixed anhydride procedure (Anderson et al., 1967), from N-Cbz[1] protected amino acids and amino acid esters (Sigma Chemicals). The steps involved (i) the coupling reaction, (ii) hydrolysis of the ester to the acid (or aminolysis of the ester to the amide) with steps (i) and (ii) being repeated as required. The intermediates were isolated, purified, and checked for optical purity at each step. The N-Cbz group was removed by hydrogenation in a Paar shaker in methanol–acetic acid mixture (99.9:0.1) using 10% Pd on carbon catalyst. The acetate salts of the peptide amides were converted to the chlorides via anion exchange chromatography using Amberlite CG-400 (100–200 mesh) resin. All products were checked for purity and authenticity by ^{1}H NMR, circular dichroism, infrared, paper chromatography, and elemental analysis. It should be noted that the circular dichroism spectra of the peptide amides, **3–16,** were taken; however, due to the large positive CD contribution (below 240 nm) of the L-phenylalanine residue (which is present in all the peptide amides), the data are not useful for the estimation of the optical purity of the products. Since several of the peptide amides, **1–16,** are diastereomeric (and therefore differ in physical properties) optical purity was demonstrated via thin-layer chromatography as well as by differences in the ^{1}H NMR spectra. In addition, the peptide coupling reactions employed in the syntheses were carried out under conditions which have been shown to proceed with no detectable racemization (Anderson et al., 1967).

Analytical Methods. Proton magnetic resonance spectra were recorded on a Varian XL-100-15 spectrometer at 34° employing a Nicolet Technology Corporation Fourier transform accessory. At the low concentration of peptide amides (≈2–10 m*M*) several accumulations are required. Sonicated low molecular weight salmon sperm DNA was used at 60–70 m*M* phosphate/liter in the presence of 1 m*M* EDTA in D_2O (pD 7.0). The concentration of the peptide amides was varied from 2 to 70 m*M*. Chemical shifts (in Hz) from the internal standard sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$ (TSP) are reported.

Viscometric, circular dichroism, and ultraviolet titration studies were conducted according to Gabbay et al. (1973). Estimations of the extent of binding of the peptide amides, **1–16,** to DNA under the conditions of the ^{1}H NMR experiment were determined by equilibrium dialysis technique. Solutions of the DNA–peptide complexes used for the ^{1}H NMR studies were equilibrated against a pH 7.0 buffer. Quantitative assay for peptide concentrations was made with fluorescamine (kindly supplied by Dr. Weigele, Hoff-

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

mann-La Roche) according to previously published procedure (Gabbay et al., 1973).

Results

The interactions of the peptide amides with DNA were studied by the pulsed Fourier transform ^{1}H NMR technique. Figure 2 shows the effect of binding to salmon sperm DNA (at various base pair to peptide ratio) on the chemical shifts, δ, and ^{1}H NMR signal line broadening of the aromatic protons of the diastereomeric oligopeptide amides, **1–16.** The results are summarized in Table I together with the ^{1}H NMR signal line broadening, $\Delta\nu_{1/2}$ (in Hz) = $\Delta\nu_{1/2}{}^{\text{bound}} - \Delta\nu_{1/2}{}^{\text{free}}$, where $\Delta\nu_{1/2}{}^{\text{bound}}$ and $\Delta\nu_{1/2}{}^{\text{free}}$ are the measured line broadenings for the aromatic protons of the peptide in the presence and absence of DNA, respectively. In should be noted, that under the conditions of the ^{1}H NMR experiments, i.e., at a base pair/peptide ratio of 7.5 and 15, the peptides **1–16** are almost fully bound to DNA (>95%). This was determined quantitatively by equilibrium dialysis on the ^{1}H NMR sample itself utilizing the fluorescamine assay technique described previously (Gabbay et al., 1973).

The results which are shown in Figure 1 and Table I lead to the following observations. (1) The upfield chemical shift, $\Delta\delta$, experienced by the aromatic protons of the peptides upon binding to DNA depends on the primary structure and stereochemistry of the α carbons of the amino acids contained therein. For example, the following order of decreasing upfield chemical shift is observed: L-Lys-L-PheA (**1**) > L-Lys-L-Phe-(*N*-dimethyl)A (**5**) > L-Lys-L-Phe-(Gly-L-Leu)$_2$A (**10**) ≃ L-Lys-L-Phe-GlyA (**3**) ≃ L-Lys-L-Phe-Gly-GlyA (**6**) ≃ L-Lys-L-Phe-D-Ala-L-LeuA (**9**) ≃ L-Lys-L-Phe-Gly-L-LeuA (**12**) ≃ L-Lys-L-Phe-D-AlaA (**14**) ≃ L-Lys-L-Phe-D-Ala-L-ValA (**16**) > L-Lys-L-Phe-L-AlaA (**13**) ≃ L-Lys-L-Phe-D-Leu-L-Leu-D-LeuA (**11**) ≃ L-Lys-Gly-L-PheA (**4**) ≃ L-Lys-L-Phe-L-Ala-L-ValA (**15**) ≃ L-Lys-L-Phe-D-Leu-GlyA (**8**) > L-Lys-L-Phe-L-Leu-GlyA (**7**) > L-Lys-D-PheA (**2**).

(2) The extent of the ^{1}H NMR signal line broadening of the aromatic protons of DNA-bound peptides is also found to depend on the primary sequence and chirality of the amino acids. For example, the following order of decreasing ^{1}H NMR signal line broadening ($\Delta\nu_{1/2}$) is observed: L-Lys-L-PheA (**1**) > L-Lys-L-Phe-Gly-GlyA (**6**) ≃ L-Lys-L-Phe-GlyA (**3**) ≃ L-Lys-L-Phe-Gly-L-LeuA (**12**) ≃ L-Lys-L-Phe-D-Ala-L-ValA (**16**) > L-Lys-L-Phe-D-AlaA (**14**) ≃ L-Lys-L-Phe-D-Leu-L-Leu-D-LeuA (**11**) ≃ L-Lys-L-Phe-D-Ala-L-LeuA (**9**) ≃ L-Lys-L-Phe-diMeA (**5**) ≃ L-Lys-L-Phe-(Gly-L-Leu)$_2$A (**10**) ≃ L-Lys-L-Phe-D-Leu-GlyA (**8**) > L-Lys-L-Phe-L-Leu-GlyA (**7**) ≃ L-Lys-L-Phe-L-AlaA (**13**) ≃ L-Lys-L-Phe-L-Ala-L-ValA (**15**) ≃ L-Lys-D-PheA (**2**) ≃ L-Lys-Gly-L-PheA (**4**). (It should be noted that the values of the line broadening, $\Delta\nu_{1/2}$ (in Hz), reported in Table I represent the broadening due to binding to DNA, i.e., $\Delta\nu_{1/2} = \Delta\nu_{1/2}{}^{\text{bound}} - \Delta\nu_{1/2}{}^{\text{free}}$, where $\Delta\nu_{1/2}{}^{\text{bound}}$ and $\Delta\nu_{1/2}{}^{\text{free}}$ are the line broadenings at half signal height in the presence and absence of DNA. The effect of viscosity of the DNA solution on line broadening is found to be small, e.g., the signal line broadening of the internal standard, TSP, is found to be 1.5 ± 0.2 and 2.5 ± 0.3 Hz in the absence and presence of 70 m*M* of DNA P/l..)

The effect of increasing concentrations of the oligopeptide amides, **1–16,** on the relative specific viscosity, η_{sp}/η_{sp_0} (where η_{sp} and η_{sp_0} are the specific viscosities of DNA solution in the presence and absence of peptides, respectively) at near infinite dilution of DNA (0.2 m*M* P/l.; 0.01%) was studied at 37° using the low shear Zimm viscometer. Since the study was carried out at low concentrations of DNA, the relative values of η_{sp}/η_{sp_0} are close approximations of the relative values of the intrinsic viscosity of DNA–peptide complex to free DNA ($[\eta]^{\text{DNA-peptide}}/[\eta]^{\text{DNA}}$) (Tanford, 1961). The results of the viscometric titration study indicate that the oligopeptide amides, **3–16,** lower the specific viscosity of DNA. The magnitude of this effect is found to be intermediate between L-Lys-L-PheA (**1**) and L-Lys-D-PheA (**2**) (see Figure 2 of accompanying manuscript, Gabbay et al., 1976).

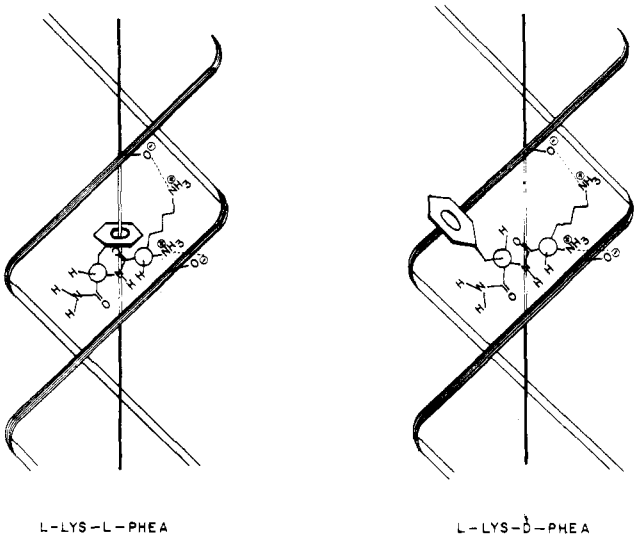


FIGURE 1: Schematic illustration of a DNA segment showing a possible mechanism by which the α- and ε-amino groups of the N-terminal L-lysine residue are anchored stereospecifically and thus dictating the positioning of the C-terminal aromatic ring of phenylalanine in the DNA–**1** and –**2** complexes.

No significant differences in the absorption spectrum of DNA are observed in the presence of the oligopeptide amides, **1–16.** Similarly, little or no change is observed in the trough at 245 nm, however, a slight decrease (2–5%) in the peak at 275 nm of the CD spectrum of DNA in the presence of **1–16** is observed. The results suggest that the oligopeptide amides do not grossly affect the structure of DNA.

The effect of **1–12** on the T_m of the helix–coil transition of salmon sperm DNA is shown in Table II. The results indicate that the peptide amides stabilize the helix with respect to the random coil and exhibit monophasic melting behavior. In addition, the data indicate that the least stabilization of the helix is obtained with peptides containing L-leucine.

It should be noted that the viscometric, absorption, circular dichroism, and T_m studies described above were carried out in 10 m*M* 2-(*N*-morpholino)ethanesulfonate (Mes) buffer (pH 6.2) (5 m*M* in Na^+).

Discussion

A great deal of interest in the past decade has centered on the recognition process between nucleic acids and protein systems (Bekhor et al., 1969; Yarus, 1973; Bartley and Chalkley, 1973; Clark and Felsenfeld, 1971; Olins and Olins, 1971; Simpson and Sober, 1970; Shih and Fasman, 1972; Johnson et al., 1972; Zimmerman, 1972; Riggs et al., 1972). It is recognized that the interaction specificity between the two macromolecules is a problem of immense complexity and probably involves numerous types of forces

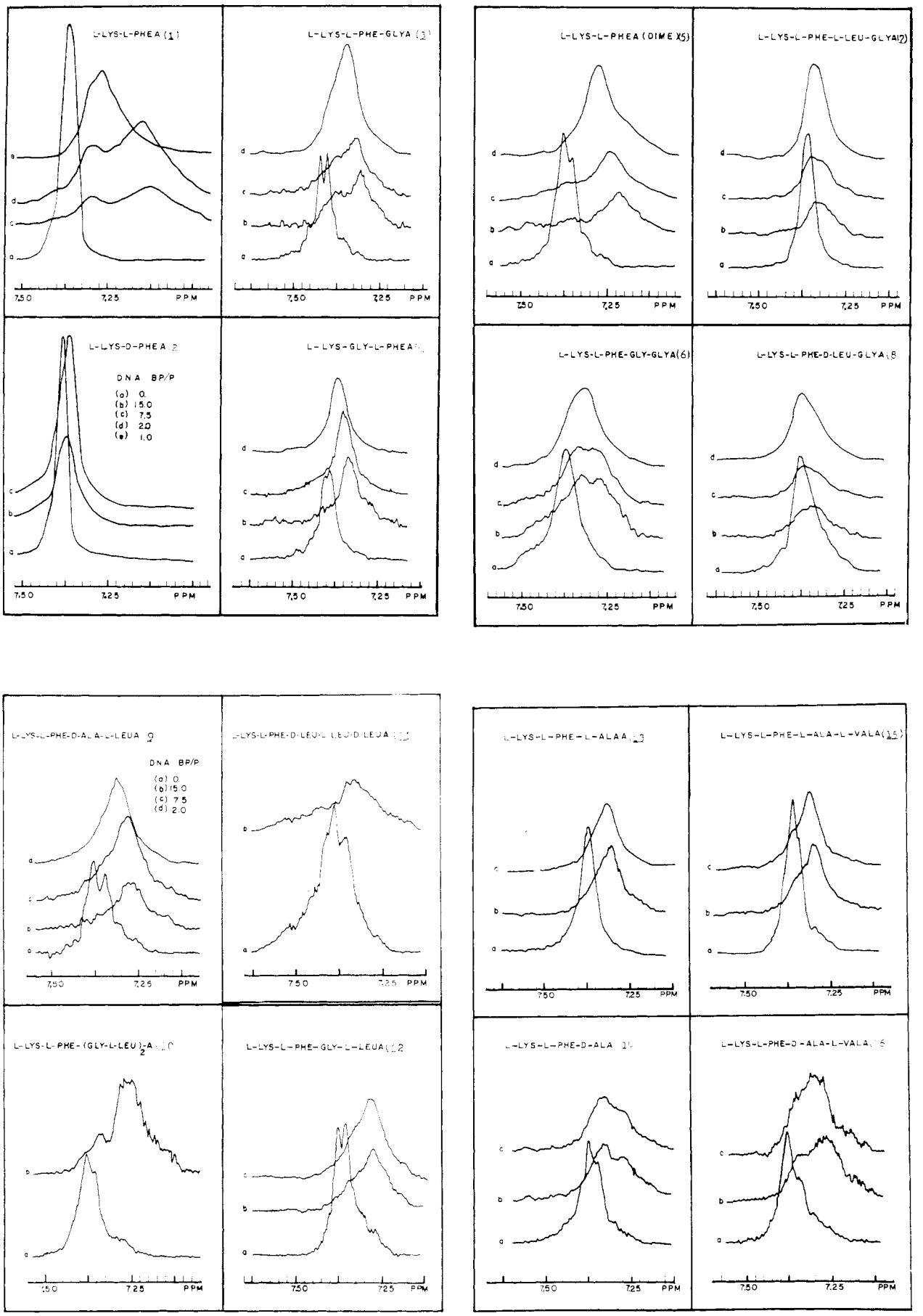


FIGURE 2: The ^{1}H NMR signal of the aromatic protons of oligopeptide amides, **1–16,** in the presence and absence of salmon sperm DNA at various base pair to peptide ratios. All of the peptide amides are almost fully bound to DNA (>95%) under the conditions of the ^{1}H NMR experiment.

Table I: The Effect of DNA on the Upfield Chemical Shift ($\Delta\delta$, in Hz) and Signal Line Broadening ($\Delta\nu_{1/2}$ in Hz) of the Aromatic Protons of Model Peptides.[a]

Peptide System	$\Delta\delta$ ($\Delta\nu_{1/2}$) in Hz[b]	
	DNA BP/Peptide	
	15.0	7.5
L-Lys-L-PheA (1)		23.5 (25)
L-Lys-D-PheA (2)	2.3 (2)	2.2 (2)
L-Lys-L-Phe-GlyA (3)	11.0 (10)	11.0 (8)
L-Lys-Gly-L-PheA (4)	6.5 (1)	6.0 (1)
L-Lys-L-Phe-diMeA (5)	15.5 (6)	13.0 (6)
L-Lys-L-Phe-Gly-GlyA (6)	10.0 (11)	8.0 (7)
L-Lys-L-Phe-L-Leu-GlyA (7)	2.5 (3)	2.5 (3)
L-Lys-L-Phe-D-Leu-GlyA (8)	3.5 (5)	3.0 (4)
L-Lys-L-Phe-D-Ala-L-LeuA (9)	11.0 (6)	10.0 (5)
L-Lys-L-Phe-(Gly-L-Leu)$_2$A (10)	13.0 (5)	
L-Lys-L-Phe-D-Leu-L-Leu-D-LeuA (11)	6.5 (7)	
L-Lys-L-Phe-Gly-L-LeuA (12)	11.0 (9)	9.0 (6)
L-Lys-L-Phe-L-AlaA (13)	7.0 (2)	6.0 (3)
L-Lys-L-Phe-D-AlaA (14)	10.0 (7)	7.5 (5)
L-Lys-L-Phe-L-Ala-L-ValA (15)	5.5 (2)	5.0 (1)
L-Lys-L-Phe-D-Ala-L-ValA (16)	11.0 (9)	8.5 (6)

[a] Sonicated low molecular weight salmon sperm DNA was used at 75 m*M* P/l. ^{1}H NMR spectra were recorded on an XL-100 Varian spectrometer using the Nicolet FT-accessory. [b] The upfield chemical shift, $\Delta\delta$ ($\delta_{free} - \delta_{bound}$, where δ_{free} and δ_{bound} are the chemical shifts in the absence and presence of DNA), and the line broadening at half signal height, $\Delta\nu_{1/2} = (\Delta\nu_{1/2}^{bound} - \Delta\nu_{1/2}^{free})$ are measured at 34°. It should be noted that the chemical shifts, δ, are measured with respect to the internal standard, TSP, and are reproducible to within ±0.2 Hz. The values of $\Delta\nu_{1/2}$ reported are not corrected for the viscosity effect of DNA solutions. The effect is small, e.g., TSP signal is found to have a $\Delta\nu_{1/2}$ of 2.5 ± 0.3 and 1.5 ± 0.2 Hz in the presence and absence of DNA.

Table II: The Effect of Peptides on the ΔT_m of the Helix–Coil Transition of Salmon Sperm DNA ($\Delta T_m = T_m - T_{m_0}$, where T_m and T_{m_0} are the melting temperatures in the presence and absence of peptide).[a]

Peptide System	ΔT_m	
	100 μ*M*	200 μ*M*
L-Lys-L-PheA (1)	7.4	9.9
L-Lys-D-PheA (2)	6.1	8.9
L-Lys-L-Phe-GlyA (3)	6.6	9.8
L-Lys-L-PheA(diMe) (5)	5.9	7.9
L-Lys-L-Phe-Gly-GlyA (6)	7.4	9.9
L-Lys-L-Phe-L-Leu-GlyA (7)	3.2	4.7
L-Lys-L-Phe-D-Ala-L-LeuA (9)	3.2	4.9
L-Lys-L-Phe(Gly-L-Leu)$_2$A (10)	4.1	5.9
L-Lys-L-Phe-D-Leu-L-Leu-D-LeuA (11)	3.0	3.9
L-Lys-L-Phe-Gly-L-LeuA (12)	5.9	8.5

[a] T_m studies were carried out in 0.01 *M* Mes buffer (pH 6.2) using 54 μ*M* P/l. of DNA and peptide concentrations of 100 and 200 μ*M*.

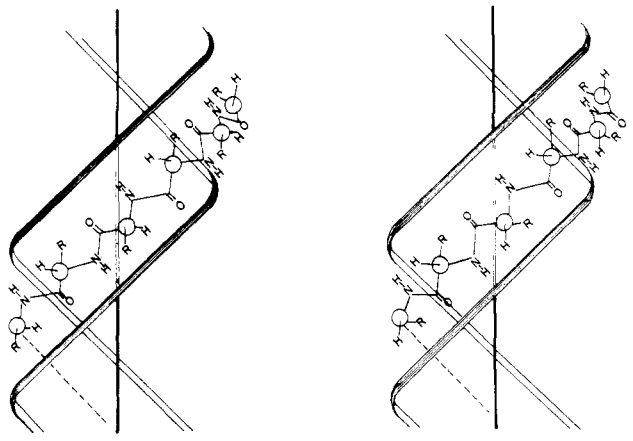
operating at several sites along the nucleic acid and protein chains. In addition, recent studies on chromatin suggest that protein–DNA binding specificity is not only a dynamic process which is continually modified during the cell cycle (Kleinsmith, et al., 1970; Gilmour and Paul, 1970; Spelsberg and Hnilica, 1970; Stein and Farber, 1972; Stein et al., 1974), but may also involve specific protein aggregates–DNA recognition (van Holde et al., 1974; D'Anna and Isenberg, 1974; Kornberg and Thomas, 1974; Kornberg, 1974; Olins and Olins, 1974).

Our efforts have taken a simplified approach to the above problem by studying the interaction specificities of small molecules with DNA (Gabbay et al., 1970, 1972, 1973; Gabbay and Glaser, 1971). These studies have revealed that the nucleic acid helix may bind to small molecules via electrostatic, H-bonding, and hydrophobic forces. Hydrophobic-type interactions are of particular interest since at least three kinds have been noted, i.e. (i) intercalation between base pairs of DNA as exemplified by aromatic cations (Lerman, 1961; Gabbay et al., 1973; Kapicak and Gabbay, 1975), (ii) "partial" insertion (or intercalation) between base pairs exemplified by sterically restricted aromatic ring containing compounds (Gabbay et al., 1973, 1976; Kapicak and Gabbay, 1975), and (iii) external hydrophobic-type binding which is noted in the nucleic acid–steroidal amine complexes (Mahler et al., 1966; Gabbay and Glaser, 1971).

In this paper, the possibility that proteins may bind to DNA via a β-chain structure is evaluated. Wilkins (1956) proposed a model based on x-ray studies whereby basic proteins are wound in a helical fashion along the grooves of DNA. The polypeptide chain appears to assume a slightly modified β-chain conformation with an approximate helical increment angle of 20° per residue. The Wilkins model has recently received added support from the observation that β chains are not linear but rather helical in nature (with dimensions similar to the DNA helix) as evidenced by the x-ray data of many proteins (Chothia, 1973). Carter and Kraut (1974) argue on the basis of the available protein x-ray data for a model of RNA–protein complex whereby the protein assumes a double-stranded helical β-chain configuration wrapped around the RNA helix. Their model, however, is specific for RNA and involves H-bonding between the 2′-OH of ribose and the polypeptide double helix.

In an attempt to provide experimental evidence for and/or against β-chain–DNA binding, the synthesis and interaction specificities of the oligopeptide amides, **1–16**, were undertaken. In the accompanying paper (Gabbay et al., 1976) evidence was presented which strongly suggests that L-lysine in the dipeptide amides, **1** and **2**, binds stereospecifically to DNA and dictates the positioning of the aromatic ring of the C-terminal residue (Figure 1). It was shown via the use of ^{1}H NMR that large upfield chemical shifts, $\Delta\delta$, and signal line broadening, $\Delta\nu_{1/2}$, are observed for the aromatic protons of L-Lys-L-PheA (**1**) in the DNA complex (Figure 2). The results are found to be consistent with the conclusion that the aromatic ring protons of **1** are in close proximity to the DNA base pairs and would therefore experience a ring current anisotropy which would lead to upfield chemical shifts (Jardetsky and Jardetsky, 1962). Since the magnitude of the ring current anisotropy experienced by the ortho, meta, and para protons of the aromatic ring of **1** depends on the geometry of the latter with respect to the base pairs in the DNA complex, large ^{1}H NMR signal line broadenings are observed (Gabbay et al., 1976). On the other hand, the ^{1}H NMR data for L-Lys-D-PheA (**2**) indicate small upfield chemical shift and signal line broadening for the aromatic ring protons which is consistent with the interpretation that the aromatic ring of **2** points outward toward the solvent. Thus, the ^{1}H NMR technique may be used to differentiate between and/or evaluate the extent of the "in" or "out" geometry of the aromatic ring of phenylalanine residues in the DNA–peptide complexes.

Figure 3 schematically illustrates the Wilkins model for peptide–DNA binding whereby the polypeptide chain assumes a helical β-sheet structure which is wrapped around the nucleic acid helix. It is noted that for polypeptides composed of L-amino acids, the side chains of the amino acid



(a) L-AMINO ACIDS (b) ALTERNATING L,D A ACIDS

FIGURE 3: Schematic illustrations of a model for peptide-DNA binding whereby the polypeptide chain assumes a helical β-sheet structure which is wrapped around the nucleic acid helix. It is noted that for polypeptides composed of L-amino acids, the side chains alternately point into and out of the helix (a). Polypeptides composed of alternating L- and D-amino acids can form two complexes with DNA, i.e., all the side chains pointing into the helix (b) or out of the helix (not shown).

residues would alternately point "into" and "out" of the helix (Figure 3a). Polypeptides composed of alternating L- and D-amino acids can form two types of complexes with DNA, i.e., all the side chains pointing into (Figure 3b) or out of the helix (not shown). The rationale for the synthesis of the oligopeptide amides, **1–16,** now becomes a little clearer, i.e., the effect of peptide chain elongation on the ^{1}H NMR signal of the aromatic ring protons of the phenylalanine residue may be used to provide experimental evidence for (or against) the Wilkins model. The initial starting point, namely, the N-terminal L-lysine residue of **1–16** is assumed to be anchored to the DNA (as evidenced from the extensive studies on the dipeptide amides, **1**, and **2**) in a stereospecific manner (see Figure 1) which would allow the side chain of the neighboring amino acid (as well as amino acids at the even-numbered positions, i.e., 4th, 6th, etc.) of the L configuration to point into the helix. In addition, it is assumed that substitution of hydrophobic amino acids of the L and D configuration at the even- and odd-numbered positions of the peptide, respectively, would allow close contact of the aromatic ring probe of L-phenylalanine with DNA base pairs if the former is present at an even-numbered position. On the other hand, it is reasoned that hydrophobic amino acids which are not "in register" with aromatic ring "probe" would compete for the internal DNA site and thus weaken the interaction of the "probe" with the DNA base pairs.

If the above model is valid it should predict that the aromatic ring in L-Lys-L-TyrA, L-Lys-L-TrpA (studied previously by Gabbay et al., 1973), L-Lys-L-PheA (**1**), L-Lys-L-Phe-GlyA (**3**), L-Lys-L-Phe-Gly-GlyA (**6**), L-Lys-L-Phe-D-Leu-GlyA (**8**), L-Lys-L-Phe-D-Ala-L-LeuA (**9**), L-Lys-L-Phe-(Gly-L-Leu)$_2$A (**10**), L-Lys-L-Phe-D-Leu-L-Leu-D-LeuA (**11**), L-Lys-L-Phe-D-AlaA (**14**), and L-Lys-L-Phe-D-Ala-L-ValA (**15**) is in close contact to the bases of DNA. Similarly, the model should predict a lesser contact between the aromatic ring "probe" and the DNA bases for the following peptides, L-Lys-D-PheA (**2**), L-Lys-Gly-L-PheA (**4**), L-Lys-L-Phe-L-Leu-GlyA (**7**), L-Lys-L-Phe-L-AlaA (**13**), and L-Lys-L-Phe-L-Ala-L-ValA (**15**). The ^{1}H NMR evidence (Table I) is completely consistent with the above, except for two peptides. For example, large upfield chemical shifts, $\Delta\delta$ (indicative of close contact to the nucleic acid bases), and large signal line broadening, $\Delta\nu_{1/2}$ (indicative of the "in" geometry), are observed for the following peptide amides: **1, 3, 6, 9, 10, 12, 14, 16,** L-Lys-L-TyrA, and L-Lys-L-TrpA (Gabbay et al., 1973); but not for **8** and **11.** The small upfield chemical shift and ^{1}H NMR signal line broadening observed for the peptide amides, **2, 4, 7, 13,** and **15,** are accurately predicted. Moreover, the previously reported ^{1}H NMR studies (Gabbay et al., 1973) which show small upfield chemical shifts and line broadening for the aromatic protons signal of L-Lys-L-Phe-L-PheA, L-Lys-L-Phe-L-LysA, and L-Lys-L-Lys-L-PheA are also predicted correctly.

The inaccurately predicted binding of the oligopeptide amides (i.e., L-Lys-L-Phe-D-Leu-GlyA (**8**) and L-Lys-L-Phe-D-Leu-L-Leu-D-LeuA (**11**)) to DNA by the model may be due to steric factors which prohibit the simultaneous "internal" binding of the L-Phe and D-Leu side chains at the 2 and 3 position of the peptides, respectively. In line with the above is the observation that substitution for the D-leucine at the 3 position by an amino acid containing a small side chain (glycine and/or D-alanine) results in a peptide in which the aromatic ring probe is in closer contact with the DNA base pairs, e.g., the peptide amides, **6, 9,** and **10.**

In summary, the ^{1}H NMR studies on the interaction specificities of 16 different oligopeptide amides (this work) and five others (from previous studies, Gabbay et al., 1973) are found to be consistent with the Wilkins (1956) model whereby the peptide chain assumes a single-stranded helical β-sheet structure which is wrapped around the nucleic acid helix. It is tempting to suggest that protein-DNA interactions are mediated via a single chain helical peptide β-sheet structure especiallly since the primary structure of histones contains a statistically significant number of sequences whereby basic (B) and/or hydrophobic (H) amino acids alternate with small hydrophylic (X) amino acids, e.g., glycine, serine, etc. (Delange and Smith, 1972). Further work in this area is in progress.

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Equilibrium Binding of Magnesium(II) by *Escherichia coli* $tRNA^{fMet}$ †

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ABSTRACT: Equilibrium dialysis measurements show that $tRNA^{fMet}_1$ in 0.17 *M* Na^+ has one strong Mg^{2+} binding site, $K = 3 \times 10^4\ M^{-1}$, and approximately 26 weak binding sites with $K = 4 \times 10^2\ M^{-1}$, with RNA concentration measured in moles of tRNA per liter and $T = 4°C$. The data fit significantly less well to a model with two strong sites and a large class of weak sites. Binding is noncooperative. Our results differ from previous experiments showing cooperative binding because the binding equilibrium is not coupled to a cooperative conformational change of the macromolecule. Measurements at relatively high Na^+ concentrations and low temperature ensure that the tRNA is in the "native" region of the conformational phase diagram for all Mg^{2+} concentrations.

Even though the three-dimensional structure of yeast phenylalanine tRNA has been determined (Kim et al., 1973; Robertus et al., 1974) and good evidence exists that all tRNAs may have a similar structure (Kim et al., 1974; Klug et al., 1974), many details of tRNA function remain to be understood. Two obvious unanswered questions are the role of the tightly bound Mg^{2+} ions, and the possible importance of tRNA conformational changes when bound to the ribosome. In this and the following paper we explore these interrelated questions. For two reasons our attention will be restricted to the behavior of tRNA separated from other macromolecular components. First, thorough characterization of the binding and conformational properties of purified tRNA seems a necessary prelude to understanding more complex systems. Second, and more persuasively, we have to admit that we do not yet possess techniques for definitive characterization of tRNA conformational properties in more complex mixtures.

The purpose of this first paper will be to establish some simple facts about the equilibrium binding of Mg^{2+} by tRNA. It has been known for some time that divalent metal ions markedly stabilize the "native" conformation of tRNA (Fresco et al., 1966). Equilibrium studies of the binding of divalent (or trivalent) ions to tRNA have been reported from several laboratories (Cohn et al., 1969; Danchin and Gueron, 1970; Sander and Ts'o, 1971; Danchin, 1972; Rialdi et al., 1972; Schreier and Schimmel, 1974; Wolfson and Kearns, 1974; Kayne and Cohn, 1974; Jones and Kearns, 1974). In the investigations in which measurements extended to low ratios of cations/tRNA it has usually been found that ion binding is cooperative: the first ions added are not bound as tightly as are subsequent increments. An exception is the report by Jones and Kearns (1974) of noncooperative Eu^{3+} binding.

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