Specific Interaction of Peptides with Nucleic Acids†

Edmond J. Gabbay,* Karl Sanford, and C. Stuart Baxter

ABSTRACT: The interactions of over 50 peptides and peptide amides with salmon testes DNA is reported. The results of the temperature-dependent proton magnetic resonance, ultraviolet, circular dichroism, and viscometric studies are presented. It is found that peptides containing aromatic amino acids interact selectively with the nucleic acid *helix* to form a "sticky complex." An intercalation model, in which the aromatic residue of the peptide is partially inserted between base pairs, accounts for the observed results.

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, 1969; Haynes *et al.*, 1970; Shapiro *et al.*, 1969a,b; Shih and Bonner, 1970; Sober *et al.*, 1966; Clark and Felsenfeld, 1971; Simpson, 1970). It is recognized that the interaction specificity between the two macromolecules, *i.e.*, nucleic acids and proteins, is a problem of immense complexity and probably involves numerous types of forces operating at several sites along the nucleic acid and protein chains. Our laboratory has taken a simplified approach to the problem which involves the delineation of the interaction specificities of small molecules with nucleic acids. For example, electrostatic, H-bonding, and hydrophobic forces have been shown to play a role in the binding process. Hydrophobic type interactions are of particular interest since at least two kinds have been noted, *i.e.*, (i) intercalation between base pairs of nucleic acids as exemplified by the aromatic residues of the reporter molecules (Gabbay, 1969; Passero *et al.*, 1970; Gabbay *et al.*, 1970; Gabbay and DePaolis, 1971), and (ii) external hydrophobic type binding which is noted in the binding of steroidal amines with nucleic acids (Gabbay *et al.*, 1969; Gabbay and Glaser, 1971). In principle, therefore, nucleic acids may utilize the above forces, singly or in combination, to bind to polypeptide chains. This paper reports preliminary studies of the interactions of several di- and tripeptides and the corresponding amides with nucleic acids. The effects of the primary sequences of the peptides on the T_m of the helix–coil transition and on the viscosity of salmon testes DNA are reported. Moreover, temperature-dependent studies of the proton magnetic resonance spectra of peptide–nucleic acids complexes are utilized, together with ultraviolet and circular dichroism studies, to elucidate the specificity of nucleic acid interactions.

Experimental Section

Material and Methods. Salmon testes deoxyribonucleic acid (DNA 8BA, Ep^{260} 6500) was purchased from Worthington Biochemical Corp. Stock solutions of the DNA were made in 0.01 M 2-(*N*-morpholine)ethanesulfonic acid (MES) buffer and 0.005 M Na^+ at pH 6.2 at 5.0–6.0 $\times 10^{-3}$ mole of P/l. T_m determinations were run in 1-ml quartz cuvets thermostated with a Haake constant-temperature water circulator equipped with a Neslab temperature programmer. A Gilford Model 240 spectrophotometer equipped with automatic recording accessories was used, and the temperature of the cell compartment was measured directly with an iron–constantan thermocouple connected to a Leeds-Northrup Model 8680 potentiometer. Ultraviolet absorption spectra were recorded on a Cary 15 spectrometer at ambient temperature between 220 and 320 nm. Circular dichroism spectra were recorded on a Jasco J-20 in the region of 220–320 nm. Viscosity measurements were carried at two different concentrations of DNA, *i.e.*, 2.6 $\times 10^{-4}$ and 6 $\times 10^{-4}$ M in P/l., and varied concentrations of peptide systems in pH 6.2, 0.01 M MES buffer at 37.0° using a low shear Zimm viscometer.

Proton magnetic resonance spectra were determined on a Varian Model XL-100 spectrometer equipped with a variable-temperature probe. Sonicated salmon testes DNA (mol wt $\leq$500,000) was used at 0.16 mole of P/l. in D_2O in 5 $\times 10^{-3}$ M sodium phosphate buffer (pD 7.0 $\pm$ 0.2). Denatured DNA was obtained by heating native DNA at 100° for 10 min followed by quenching to 0°. Sodium 2,2-dimethyl-2-silapentanesulfonate was used as the pmr internal standard in D_2O at 0.1% concentration. The concentration of the peptide systems was 0.02 M.

The amino acid amides, dipeptides, dipeptide amides, tripeptides, and tripeptide amide systems were either purchased from Cyclo Chemical Corp. or were synthesized in our laboratories *via* the mixed anhydride procedure (Anderson *et al.*, 1967). The peptides were checked for authenticity by pmr spectra and, in some cases, by paper chromatography, infrared and circular dichroism spectra. Peptides that were locally synthesized were also checked by carbon and hydrogen analysis and are indicated in Tables I and II.

Results and Discussion

General Considerations of the Proton Magnetic Resonance Studies. The line widths and chemical shifts of the proton magnetic resonance (pmr) signals of a small molecule bound to a macromolecule may reveal considerable information concerning the nature of the binding process. For example, three types of binding may be distinguished. Type I binding, which is characteristic of a rigid macromolecule–small molecule complex, leads to total line broadening of the pmr signals. This effect has been observed for molecules which intercalate

† From the Department of Chemistry, University of Florida, Gainesville, Florida 32601. *Received February 7, 1972.* This work is part of a series, XXVI, "Topography of Nucleic Acid Helices in Solution." The authors acknowledge the support of the U. S. Public Health Service Grants GM 17503 and GM 18653 and the National Science Foundation Grant GB 16044. Taken in part from K. S. thesis to be submitted in partial fulfillment for the Ph.D. degree (University of Florida), 1972.

* To whom correspondence should be addressed. Recipient of a Career Development Award (1970).

TABLE I: Peptides and Peptide Amides Which Exhibit Line Broadening and Upfield Chemical Shift of the Proton Magnetic Resonance Signals on Binding to DNA.

No.	Compound	No.	Compound
1	L-Phenylalanyl-L-arginine	19	L-Phenylalanyl-L-phenylalaninamide
2	L-Arginyl-L-phenylalanine	20	L-Phenylalanyl-L-tyrosinamide
3	L-Leucyl-L-phenylalanine	21	L-Tyrosyl-L-phenylalaninamide
4	L-Phenylalanyl-L-leucinamide	22	L-Phenylalanylglycylglycinamide
5	L-Leucyl-L-phenylalaninamide	23	Glycylglycyl-L-phenylalaninamide
6	L-Phenylalanylglycinamide	24	L-Alanyl-L-tyrosinamide
7	Glycyl-L-phenylalaninamide	25	Glycyl-L-tyrosinamide
8	L-Histidyl-L-phenylalaninamide	26	L-Histidyl-L-tyrosinamide
9	L-Alanyl-L-phenylalaninamide	27	L-Lysyl-L-tyrosine
10	L-Phenylanyl-L-alaninamide	28	L-Lysyl-L-tyrosinamide
11	L-Phenylalanyl-L-serinamide	29	L-Tyrosylglycylglycine
12	L-Phenylalaninamide	30	Glycylglycyltyrosinamide
13	D-Phenylalaninamide	31	L-Tryptophanamide
14	β-Alanyl-L-phenylalaninamide	32	D-Tryptophanamide
15	L-Phenylalanyl-L-tryptophanamide	33	Glycyl-L-tryptophanamide[a]
16	L-Lysyl-L-phenylalanine[a]	34	L-Alanyl-L-tryptophanamide[a]
17	D-Lysyl-D-phenylalanine[a]	35	L-Tryptophylglycinamide
18	L-Lysyl-L-phenylalaninamide[a]	36	L-Tryptophyl-L-leucinamide

[a] These peptides were synthesized in our laboratory. All others were purchased from Cyclo Chemical Corp.

between base pairs in DNA as well as for strongly bound large hydrophobic cations, *e.g.*, steroidal amines (see Gabbay *et al.*, 1970, and references therein). In both cases, the small molecule experiences *strongly restricted tumbling* in the DNA complex, leading to an unaveraged chemical shift of the individual protons and total line broadening (Pople *et al.*, 1959; Jardetsky and Jardetsky, 1962). None of the peptides reported in this paper exhibit type I binding with DNA (see below).

Type II binding, which is characteristic of molecules exhibiting a *line-broadened pmr signal* as well as an upfield chemical shift, is noted with certain peptide systems on *binding to DNA* (Table I). In all cases, these peptides contain an aromatic amino acid, *e.g.*, phenylalanine, tyrosine, and tryptophan. The line broadening and upfield chemical shift of the pmr signals are observed to be more pronounced for the protons of the aromatic amino acids of the DNA-bound peptides (see below). It should be noted that this effect may arise by two distinctly different mechanisms, *i.e.*, (i) weak restriction of molecular tumbling in the DNA–complex and/or (ii) slow rate of exchange between the various DNA binding sites and the unbound state (Jardetsky and Jardetsky, 1962). It should be noted that in either mechanism a "sticky" DNA complex is formed.

TABLE II: Peptides and Peptide Amides Which Exhibit Rapid Molecular Tumbling in the DNA Complex as Evidenced by a Lack of Line Broadening and Upfield Shifts of the Proton Magnetic Resonance Signals.

L-Lysyl-L-lysine
L-Lysyl-L-leucine
D-Leucyl-D-leucine
L-Leucyl-L-leucine
D-Leucyl-L-leucine
L-Alanyl-L-leucinamide
L-Leucyl-L-leucinamide
L-Alanylglycinamide
L-Histidylglycine
L-Histidyl-1-histidine
Glycyl-1-histidine
L-Histidyl-1-proline
L-Histidylglycylhistidine
Glycylglycylglycine
Glycylglycylglycinamide
Glycylglycylglycylglycinamide
L-Alanyl-L-alanyl-1-alanine

Type III binding to DNA is noted for molecules which have a high affinity to nucleic acids but exhibit *no line broadening and upfield chemical shift of the pmr signals*. Among these are the polyammonium salts, *i.e.*, $R_3N^+(CH_2)_nN^+R_3 \cdot 2Br^-$, where R = H and CH_3, and n = 2–6; spermidine derivatives, $R_2HN^+(CH_2)_3N^+HR(CH_2)_4N^+HR_2 \cdot 3Cl^-$; and spermine derivatives, $[R_2HN^+(CH_2)_3N^+HR(CH_2)_2]_2 \cdot 4Cl^-$, where R = H and CH_3. For example, Gabbay *et al.* (1970) have found that the pmr spectra of the polyammonium salts are nearly identical in the presence and absence of DNA. The absence of line broadening and upfield chemical shift of the pmr signals in type III binding is indicative that the bound molecule is undergoing rapid tumbling as well as fast exchange between the various binding sites of DNA. A number of peptides listed in Table II show this type of behavior. It is noted that they all contain the simple aliphatic and polar amino acids, *e.g.*, glycine, alanine, histidine, serine, and lysine.

Pmr Studies of the Effect of the Primary Structure of Peptides on DNA Binding. A number of interesting results have been obtained by the temperature-dependent pmr studies of the peptides and peptide amides listed in Table I in the presence and absence of DNA. A summary of the results is presented below together with some illustrative examples of the pmr spectra wherever possible.

The temperature-dependent partial pmr spectra of the

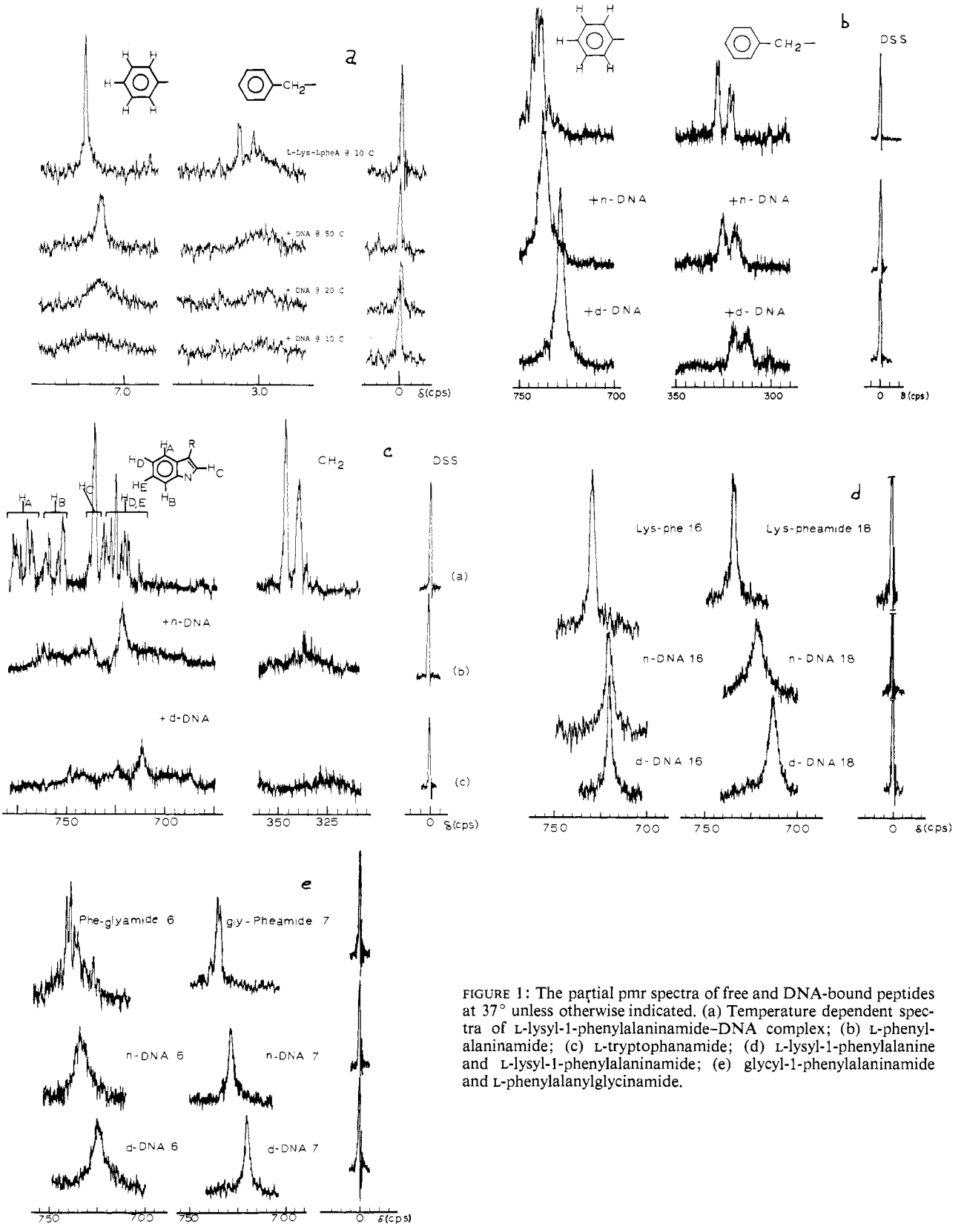

FIGURE 1: The partial pmr spectra of free and DNA-bound peptides at 37° unless otherwise indicated. (a) Temperature dependent spectra of L-lysyl-1-phenylalaninamide–DNA complex; (b) L-phenylalaninamide; (c) L-tryptophanamide; (d) L-lysyl-1-phenylalanine and L-lysyl-1-phenylalaninamide; (e) glycyl-1-phenylalaninamide and L-phenylalanylglycinamide.

DNA–L-lysyl-L-phenylalaninamide complex is shown in Figure 1a. It is noted that pmr signals of the phenyl protons and the methylene protons of the phenylalanine residue are extensively broadened and upfield shifted at low temperatures in the presence of native salmon testes DNA. Restricted tumbling and/or slow exchange between different binding sites is indicated for the interaction of this system with DNA.

Moreover, the simple aromatic amino acid amides, *e.g.*, L-phenylalaninamide (**12**) and L-tryptophanamide (**31**) also show line broadening and upfield chemical shift of the pmr signals of the aromatic protons on binding to native and

TABLE III: Change in Chemical Shift, $\Delta\delta$, of the Pmr Signals of the Aromatic Protons of the Dipeptides and Dipeptide Amides in the Presence and Absence of Native and Denatured DNA ($\Delta\delta = \delta_f - \delta_{DNA\ complex}$ in cps) at 37° in D_2O.

Peptide System	n-DNA	d-DNA
L-Lysyl-L-phenylalanine	10	10
D-Lysyl-D-phenylalanine	12	5
L-Lysyl-L-phenylalaninamide	12	21
L-Lysyl-L-tyrosine	0	10
L-Lysyl-L-tyrosinamide	17	16
L-Leucyl-L-phenylalanine	4	5
L-Leucyl-L-phenylalaninamide	11	18
L-Histidyl-L-phenylalanine	0	3
L-Histidyl-L-phenylalaninamide	6	11

TABLE IV: Effect of Primary Sequence on the Change in Chemical Shift, $\Delta\delta$, of the Pmr Signals of the *Aromatic* Protons of the Peptide Systems in the Presence and Absence of Native and Denatured DNA ($\Delta\delta = \delta_f - \delta_{DNA\ complex}$).

Peptide System	$\Delta\delta$ (cps) n-DNA	d-DNA
L-Arginyl-L-*phenylalanine*	4	7
L-*Phenylalanyl*-L-arginine	1	2
Glycyl-L-*phenylalanin*amide	8	16
L-*Phenylalanyl*glycinamide	3	12
L-Alanyl-L-*phenylalanin*amide	9	15
L-*Phenylalanyl*-L-alaninamide	5	10
β-Alanyl-L-*phenylalanin*amide	4	10
L-Leucyl*phenylalanin*amide	11	18
L-*Phenylalanyl*-L-leucinamide	5	14
L-Phenylalanyl-L-*tyrosin*amide	6	6
L-*Tyrosyl*-L-phenylalaninamide	2	13
L-Tyrosyl-L-*phenylalanin*amide	13	20
L-*Phenylalanyl*-L-tyrosinamide	2	3
L-Phenylalanyl-L-*phenylalanin*amide	11	19
L-*Phenylalanyl*-L-phenylalaninamide	0	1
L-*Tryptophan*amide	13	28
D-*Tryptophan*amide	13	26
Glycyl-L-*tryptophan*amide	12	24
L-Alanyl-L-*tryptophan*amide	13	28
L-*Tryptophyl*glycinamide	13	24
L-*Tryptophyl*leucinamide	15	28
L-Histidyl-L-*phenylalanin*amide	6	11
L-*Phenylalanyl*-L-serinamide	5	11
L-*Phenylalanyl*glycylglycinamide	2	6
Glycylglycyl-L-*phenylalanin*amide	4	9

denatured DNA (Figure 1b,c). It is noted that the aromatic protons of the indole ring of **31** are considerably more broadened and upfield shifted than the protons of the phenyl ring of **12** in the presence of native DNA. These results are not surprising since it might be expected that the rate of tumbling of the bicyclic indole ring of **31** in the DNA complex would be lower than that of the monocyclic phenyl ring of **12**. It is also observed that the pmr signals of the aromatic protons in the denatured DNA–amide complexes are more broadened and upfield shifted than are the corresponding complexes with native DNA at 37°. Identical results are also obtained with the corresponding enantiomers, **13** (D-phenylalaninamide) and **32** (D-tryptophanamide), which indicate a lack of stereospecificity in the binding process.

As might be expected, the dipeptide amides interact with the bases of DNA to a greater extent than the corresponding dipeptides. This effect is noted in the pmr spectra of the DNA complex as an enhanced upfield chemical shift, $\Delta\delta$, ($\delta_{free} - \delta_{DNA\ complex}$) of the aromatic protons of the dipeptide amides as compared to the dipeptides. The results are tabulated for several systems in Table III and shown for lysylphenylalanine and its amide derivative in Figure 1d. It should be noted that structural specificity plays a role in the binding to native DNA since neither lysyltyrosine nor histidylphenylalanine show an upfield chemical shift of the pmr signal of the aromatic protons in the presence of native DNA (n-DNA). This in contrast to lysylphenylalanine and leucylphenylalanine which show a definite upfield shift in the presence of n-DNA. Moreover, such structural specificity is absent in the interaction of the dipeptides with denatured DNA (d-DNA).

A sequence effect on the upfield chemical shift, $\Delta\delta$, of the pmr signals of the aromatic protons in the presence of DNA is noted. Table IV lists the upfield shift, $\Delta\delta$, of the aromatic proton signals of several dipeptide amides, shown for glycylphenylalaninamide and phenylalanylglycinamide in Figure 1e. It is noted that a larger upfield chemical shift, $\Delta\delta$, is observed for the aromatic protons if the aromatic residue is at the carboxyl-terminal end of the dipeptides and/or dipeptide amides in the case of phenylalanyl and tyrosyl residues. These results indicate that the aromatic ring of phenylalanine and tyrosine are in closer proximity to the base pairs of DNA when present at the carboxyl-terminal than in the N-terminal position of the dipeptide amide. If it is assumed that the positively charged ammonium group, N^+H_3, of the dipeptide amide is in close proximity to the phosphate anion on the periphery of the helix, it is not surprising that the above sequence specificity is observed. It should be noted that the tryptophan-containing dipeptide amides do not show the sequence specificity observed for the phenylalanyl and tyrosyl residues. For example, glycyl-L-tryptophanamide and L-tryptophylglycinamide show upfield chemical shifts, $\Delta\delta$, in the presence of DNA which are of the same magnitude as those obtained with L- and D-tryptophanamide and other tryptophan-containing dipeptide amides, *i.e.*, approximately 12–15 cps (Table IV). The results suggest that the indole ring of the tryptophan residue has a greater tendency to interact with the base pairs of n-DNA than do the phenyl and *p*-hydroxyphenyl ring of the phenylalanine and tyrosine residues.

Several additional observations could be made from the results shown in Tables III and IV. (i) L-Lysyl-L-phenylalanine interacts with the bases of denatured DNA to a greater extent than its enantiomer, D-lysyl-D-phenylalanine (Table III). (ii) Increasing the distance between the positively charged N-terminal amino group of the peptide and the aromatic residues beyond 6 chemical bonds lowers the interaction of the latter with the base pairs in DNA. This effect is observed by comparing the upfield chemical shifts, $\Delta\delta$, between (a) L-alanyl-L-phenylalaninamide and β-alanyl-L-phenylalaninamide, and (b) glycyl-L-phenylalaninamide and glycylglycyl-L-phenylalaninamide (Table IV). In each case $\Delta\delta$ decreases, *i.e.*, from 9 to 4 cps for a and 8 to 4 cps for b. (iii) The extent

TABLE V: Effect of Peptides and Peptide Amides 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} is the Melting Temperature in the Presence and Absence of Peptide).[a]

System	ΔT_m	System	ΔT_m
L-PheA	1.5	L-Phe-L-AlaA	5.2
D-PheA	−2.2	L-Ala-L-PheA	1.5
L-TrpA	2.1	β-Ala-L-PheA	6.8
		L-Ala-L-TyrA	8.8
D-TrpA	2.1	L-HisGlyA	13.5
Gly-L-PheA	4.7	L-His-L-PheA	10.6
L-Phe-GlyA	0.8	L-His-L-TyrA	11.6
L-Phe-L-SerA	0.9	L-Lys-L-Phe[b]	5.8
L-Ser-L-PheA	3.0	L-Lys-L-PheA[b]	17.4
L-Phe-L-PheA	0.2	L-Lys-L-LeuA[b]	18.8
L-Phe-L-TyrA	5.3	L-Lys-L-TyrA[b]	18.6
L-Tyr-L-PheA	2.5	Cadaverine[b]	22.5
GlyglyPheA	4.3		
PheGlyGlyA	1.9		
GlyGlyGlyA	5.8		

[a] T_m studies were carried out in 0.01 M MES buffer, pH 6.2, using 8.4×10^{-5} M P/l. of DNA and a peptide concentration of 2.5×10^{-3} M. [b] The peptide concentration used for the study was 5×10^{-4} M.

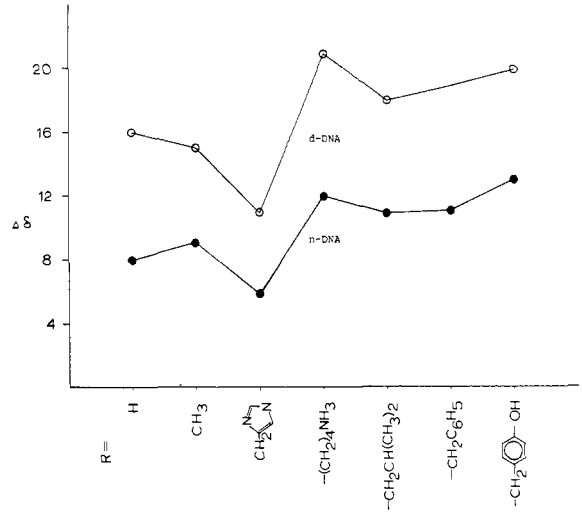

FIGURE 2: The effect of the N-terminal amino acid residue on the upfield shift ($\Delta\delta$) of the pmr signal of the aromatic protons of the C-terminal phenylalanyl residue of the dipeptide amide on binding to salmon testes native and denatured DNA.

of the upfield shift, $\Delta\delta$, of the C-terminal aromatic protons of phenylalanine in dipeptide amides is dependent on the N-terminal amino acid. The effect is illustrated in Figure 2 where it is noted that for the L,L-dipeptide amides, $N^+H_3CHR\text{-}CONHCH(CH_2C_6H_5)CONH_2$, the nature of the R group affects the $\Delta\delta$ of the aromatic protons upon binding to DNA. For example, when R is a glycyl, alanyl, or histidyl residue a lower upfield shift, $\Delta\delta$, was observed. However, if R is an apolar residue, *i.e.*, leucyl, phenylalanyl, or tyrosyl, or contains an extra positive charge, *i.e.*, R = lysyl residue, a larger upfield shift is observed for the C-terminal aromatic protons, which is indicative of an enhanced stacking interaction with the base pairs of DNA.

In summary, the pmr data indicate that the aromatic amino acids, when present in dipeptides and/or dipeptide amides, experience an upfield chemical shift and line broadening which is indicative of a ring current anistropic effect and a "sticky complex," respectively. The effect is dependent on the primary structure of the dipeptide and/or the dipeptide amide for phenylalanine- and tryosine-containing systems. Tryptophan-containing peptides show a greater upfield chemical shift of the aromatic protons on binding to native DNA than the phenylalanine and tyrosine peptides and moreover the magnitude of $\Delta\delta$ is independent of the sequence. In addition, apolar amino acid residues, when present in the dipeptide amides, enhance the upfield chemical shift, $\Delta\delta$, of the aromatic protons of phenylalanine and tyrosine. A similar effect is observed in the presence of amino acid residues containing an extra positive charge, *e.g.*, lysine.

The pmr studies are consistent with a model wherein the aromatic ring of the peptide is partially intercalated between base pairs of DNA. Both the upfield chemical shift of the pmr signals, which is indicative of ring current anistropy, and the "sticky complex," suggested by the line-broadened spectra, are in agreement with this interpretation (Gabbay *et al.*, 1970).

Ultraviolet (uv) and Circular Dichroism (CD) Spectral Studies. The interactions of the peptides and peptide amides listed in Tables I and II with native salmon sperm DNA were also studied by uv absorption and CD techniques. Little or no difference is detected in the absorption spectrum of DNA in the presence of the peptide systems, *i.e.*, intensity at 260 nm varied by ±3%. Similarly, little or no change is observed in the peak at 275 nm and the trough at 246 nm of the CD spectrum of DNA in the presence of the above systems. The results suggest that no gross alteration in native DNA structure is occurring.

Melting Temperature Studies. The effect of various peptide systems on the melting temperature, T_m, of the helix–coil transition of DNA was studied and the results are summarized in Table V. Several interesting points may be made from these data. (i) A primary sequence effect on the stabilization of the DNA helix is observed. (ii) With one exception, *i.e.*, L-Phe-L-AlaA *vs.* L-Ala-L-PheA, the dipeptide amides with aromatic residues at the carboxyl-terminal end stabilize the DNA helix to a greater extent than do the corresponding N-terminal isomers. (iii) Tyrosyl residues in the dipeptide amides stabilize the helix to a greater extent than do the corresponding phenylalanine-containing systems. (iv) Cadaverine (1,5-diaminopentane) shows a greater stabilization of the helix than does the corresponding α-substituted cadaverines, *i.e.*, the lysyl dipeptide amides. (v) The dipeptide, as one would expect, stabilizes the helix to a lesser extent than does the corresponding dipeptide amide, *i.e.*, L-Lys-L-Phe *vs.* L-Lys-L-PheA.

It is not clear how to interpret the T_m data since they are complicated by the fact that they involve relative interaction of the peptide with helix and with the random coil. However, it is significant to note that the T_m studies are consistent with the pmr data in at least one respect, *i.e.*, the dipeptide amides with C-terminal aromatic residues elicit a greater upfield chemical shift and line broadening of the pmr signals as well as a higher T_m of the helix–coil transition as compared to the corresponding dipeptide amides with N-terminal aromatic residues.

It should be noted that the pmr studies appear at first

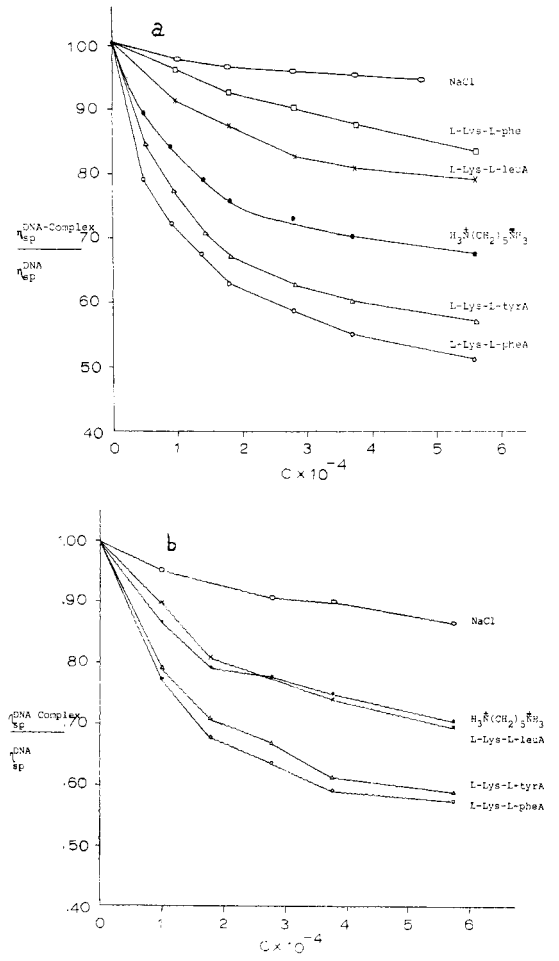

FIGURE 3: The effect of increasing concentrations of peptides, Na^+, and $N^+H_3(CH_2)_5N^+H_3 \cdot 2Br^-$ on the specific viscosity, η_{sp}, of DNA at (a) 6.0×10^{-4} mole of nucleotide/l. and (b) 2.6×10^{-4} mole of nucleotide/l.

hand to contradict the T_m results. For example, a greater upfield shift, $\Delta\delta$, and line broadening are observed for the aromatic protons of the peptides in the presence of denatured DNA than native DNA at 37°. The results are consistent with a higher affinity of the peptides to denatured than to native DNA at 37°. At higher temperatures, *i.e.*, at the T_m of the helix–coil transition ($\approx 60°$), the relative affinities of n- and d-DNA to the peptides could be reversed leading to a net stabilization of helix. This effect has already been observed for the binding of amino acid amides to nucleic acids by direct binding studies (Gabbay and Kleinman, 1970).

Viscosity Studies. A number of investigators have demonstrated that planar molecules such as Acridine Orange, ethidium bromide, and proflavine may intercalate between base pairs in DNA (Lerman, 1961; Drummond *et al.*, 1966; Cohen and Eisenberg, 1969). This phenomenon usually leads to an increase in the length of the helix with a concomitant increase in the viscosity of the solution. In order to define the nature of the binding process of the peptides listed in Tables I and II, viscosity measurements were made at two different concentrations of DNA, and the results are shown in Figure 3. It should be noted that intrinsic viscosity measurements were carried to infinite dilution for the above systems. The results are found to be uninformative since the value of the intrinsic viscosity at infinite DNA dilution in the presence of other molecules will and does approach the value of the intrinsic viscosity of free DNA at infinite dilution, *i.e.*, since the binding constant of the small molecule to DNA is finite, the complex will be disassociated at the lower concentrations. Instead, the effect of increasing concentrations of various molecules on the η_{sp} of DNA solution at low concentrations of the latter is presented in Figure 3a and 3b.

As seen from Figure 3, the aromatic amino acid containing dipeptide amides, *e.g.*, L-Lys-L-PheA, cause dramatic decreases in the specific viscosity, η_{sp}, of DNA. On the other hand, the simple diammonium salts, $N^+H_3(CH_2)_5N^+H_3$ and the nonaromatic amino acid containing dipeptide amides, *e.g.*, L-Lys-L-LeuA decreases the η_{sp} of DNA to a much lesser extent. A decrease in the viscosity of DNA solution is noted in the presence of increasing salt concentrations (Cohen and Eisenberg, 1969) presumably due to electrostatic constriction of the polymer at high ionic strength. The anomalous results obtained with aromatic amino acid containing peptide amide at both high and low concentrations of DNA cannot be explained on the basis of an ionic effect alone. We, therefore, propose an intercalation model whereby the aromatic residue is partially inserted between base pairs resulting in slight bending at the point of complexation. Such a model is totally consistent with that suggested by the proton magnetic resonance studies. It should be noted that Müller and Crothers (1968) also observed a decrease in viscosity of high molecular weight DNA solution upon intercalation of actinomycin. They propose, however, a model whereby an enhanced "cross-linking" of the DNA chains occur in the presence of actinomycin.

In summary, the peptide–nucleic acid studies reported in this paper are consistent with a model whereby the aromatic residues are partially intercalated between base pairs of DNA. The proton magnetic resonance data indicate a selective broadening and upfield chemical shifts of the aromatic protons of the peptides (containing aromatic amino acids) on binding to DNA. Moreover, the extent of line broadening and upfield chemical shift depends on the primary structure of the dipeptide, *i.e.*, increases with larger adjacent nonpolar amino acid residues (Figure 2). For example, the pmr signal of the aromatic protons of phenylalanine residues in the dipeptide amide derivatives, $N^+H_3CHRCONHCH(CH_2O)$-$CONH_2$, is upfield shifted and broadened to a greater extent as the size of the apolar side chain (R group) of the adjacent amino acid is increased (Table IV). In addition, the selective lowering of the specific viscosity of DNA by the aromatic amino acid containing peptides as opposed to the nonaromatic amino acid containing peptides is also in agreement with the partially intercalated model (Figure 3). Interactions of this type may be expected to lead to tilting of adjacent base pairs and hence to a decrease in the effective length of the helix. Denaturation of the DNA helix at the point of complex formation with the peptides is ruled out by the results of the T_m, uv, and CD studies. The peptides are found to stabilize the helix to heat denaturation and show little effect on the uv and CD spectra of DNA. The results of Helene *et al.* (1971) utilizing fluorescence and pmr techniques on the studies of tyramine and tryptamine binding to DNA and its monomer components are in agreement with our data and indicate that the planar aromatic indole ring is intercalated between base pairs of DNA.

The role of intercalation as a means of specific recognition of protein–nucleic interaction is difficult to evaluate with the present data. However, since there are ten distinctly different intercalation sites in DNA, it is felt that a "selective book mark" recognition hypothesis proposed by Gabbay *et al.*

(1972) may be involved in nucleic acid recognition. Further work is presently in progress to determine the specificity of binding of the above-mentioned systems to DNA.

References

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

Bekhor, I., Kung, G. M., and Bonner, J. (1969), *J. Mol. Biol. 39*, 351.

Clark, R. J., and Felsenfeld, G. (1971), *Nature (London), New Biol. 229*, 101.

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

Drummond, N. J., Pritchard, N. J., Simpson-Gildmeister, V. F. W., and Peacocke, A. R. (1966), *Biopolymers 4*, 971.

Gabbay, E. J. (1969), *J. Amer. Chem. Soc. 91*, 5136.

Gabbay, E. J., and DePaolis, A. (1971), *J. Amer. Chem. Soc. 93*, 562.

Gabbay, E. J., DeStefano, R., and Sanford, K. (1972), *Biochem. Biophys. Res. Commun. 46*, 155.

Gabbay, E. J., Gaffney, G., and Glaser, R. (1970), *Ann. N. Y. Acad. Sci. 171*, 810.

Gabbay, E. J., Gaffney, B., Glaser, R., and Denney, D. Z. (1969), *Chem. Commun.*, 1507.

Gabbay, E. J., and Glaser, R. (1971), *Biochemistry 10*, 1665.

Gabbay, E. J., and Kleinman, R. (1970), *Biochem. J. 117*, 247.

Haynes, M., Garret, R. A., and Gratzer, W. B. (1970), *Biochemistry 9*, 4410.

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

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

Lerman, L. S. (1961), *J. Mol. Biol. 3*, 18.

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

Passero, F., Gabbay, E. J., Gaffney, B., and Kurucsev, T. (1970), *Macromolecules 3*, 158.

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

Shapiro, J. T., Leng, M., and Felsenfeld, G. (1969a), *Biochemistry 8*, 3219.

Shapiro, J. T., Stannard, B. S., and Felsenfeld, G. (1969b), *Biochemistry 8*, 3233.

Shih, T. Y., and Bonner, J. (1970), *J. Mol. Biol. 48*, 469.

Simpson, R. J. (1970), *Biochemistry 9*, 4814.

Sober, H. A., Schlossman, S., Yaron, A., Latt, S. A., and Rushizky, G. W. (1966), *Biochemistry 5*, 3608.

Yarus, M. (1969), *Annu. Rev. Biochem. 38*, 841.

Acylation of Transfer Ribonucleic Acid with the *N*-Hydroxysuccinimide Ester of Phenoxyacetic Acid†

Stanley Friedman

ABSTRACT: Uncharged rat liver tRNA can be eluted from BD-cellulose columns with 0.8 M sodium chloride, with the exception of $tRNA^{Phe}$, which can only be eluted with 0.8 M sodium chloride containing ethanol. When the uncharged rat liver tRNA is reacted with the *N*-hydroxysuccinimide ester of phenoxyacetic acid, there is an increase in the amount of tRNA that is eluted in the presence of ethanol. Greater than 50% of the tRNAs for tyrosine, threonine, isoleucine, cysteine, and asparagine can now be eluted only in the presence of ethanol. Only a small fraction of the tRNA from *Escherichia coli* and yeast shifted into the ethanol fraction after reaction with phenoxyacetoxysuccinimide. The rate of acylation of rat liver and *E. coli* tRNA with [^{14}C]phenoxyacetoxysuccinimide was greater than that with tRNA isolated from yeast. Furthermore, all the incorporated radioactivity could be hydrolyzed from the yeast tRNA by incubation in 0.34 M ammonia, whereas only a fraction of the radioactivity incorporated into *E. coli* and rat liver tRNA could be made acid soluble by this procedure. Only one acylated nucleoside could be isolated from an enzymic digest of rat liver tRNA. A compound with identical chromatographic, electrophoretic, and ultraviolet spectral properties was isolated from *E. coli* tRNA.

There have been several reports of the presence of certain bases in transfer ribonucleic acid that can undergo acylation reactions. However, the nature of these bases has not been fully characterized, nor has it been determined whether these are rare bases, or one or more of the four major bases, that are especially reactive because of their conformation in tRNA. With the advent of BD-cellulose[1] columns, which have affinity for nucleic acids with additional hydrophobic groups attached, and particularly for single-stranded nucleic acids, a method for the separation of acylated from unacylated nucleic acids became available (Gillam *et al.*, 1967).

Several groups have used the esters of *N*-hydroxysuccinimide to acylate specific aminoacyl-tRNAs (deGroot *et al.*, 1966; Gillam *et al.*, 1968; Schofield *et al.*, 1970). In the process of preparing acylaminoacyl-tRNAs, Gillam *et al.* (1968)

† From the Department of Pharmacology, State University of New York Downstate Medical Center, Brooklyn, New York 11203. *Received April 19, 1972.* Supported, in part, by Grant GB-5928 from the National Science Foundation.

[1] Abbreviations used are: BD-cellulose, benzoylated DEAE-cellulose; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; A_{260} unit, that quantity of material which, when dissolved in 1 ml of 0.01 N Tris-HCl (pH 8.9), has an absorbance at 260 nm of 1.0; ethanol fraction, that fraction of tRNA which is eluted from BD-cellulose with solutions containing ethanol and not with 0.8 M sodium chloride.