Table I. Best-Fit Parameters for Eq 5 from a Nonlinear Least-Squares Method[13]

thiol	k_1, mM^{-1} s^{-1}	k_{-1}/k_2, mM	K, mM
2ME	113 ± 4	0.053 ± 0.005	4.64 ± 0.55
3MPA	127 ± 13	0.100 ± 0.021	1.58 ± 0.40

of new phthalaldehyde-like fluorogenic reagents,

Figure 1 shows the dependence of k_{obsd} on 2ME and 3MPA concentrations (line A and B, respectively) for the formation of the isoindole product. In both cases, the initial increase in k_{obsd} is followed by a gradual decrease as the thiol concentration increases. Under similar conditions, with the exception that 3MPA concentration was kept constant ([3MPA] = 0.186 mM) and the OPA concentration was varied, a plot of k_{obsd} vs. [OPA] showed good linear correlation (correlation coefficient $r > 0.97$) with best-fit slope = 70,4 ± 5.6 mM^{-1} s^{-1}. The dependence of k_{obsd} on [Ala] was also examined in mixtures in which thiol was in excess ([2ME] = 2.0 mM or [3MPA] = 1.83 mM). Good linear correlations were observed in these studies with $r > 0,99$ in all cases; linear regressions gave slopes of 75.5 ± 0.8 and 60.2 ± 1.9 mM^{-1} s^{-1} for 2ME and 3MPA, respectively.

According to the kinetic model described above, OPA reacts with Ala to form an intermediate I which further reacts with the thiol to give the fluorescent isoindole product. In addition, OPA also reacts reversibly with the thiol to form an adduct L, with apparent dissociation constant K. Applying the steady-state approximation for the concentration of I and under the condition where [OPA] < [thiol], the rate expression for the formation of P is given by

$$d[P]/dt = \frac{k_1[OPA][thiol][Ala]}{k_{-1}/k_2 + [thiol]} \frac{K}{K + [thiol]} \quad (3)$$

where [OPA] = total OPA concentration. Under the condition where [Ala] << [OPA] and [thiol] (as in the case with the analytical reaction),

$$d[P]/dt = k_{obsd}[Ala] \quad (4)$$

where

$$k_{obsd} = \frac{k_1[OPA][thiol]}{k_{-1}/k_2 + [thiol]} \frac{K}{K + [thiol]} \quad (5)$$

The data shown in Figure 1 were fitted to eq 5 by a nonlinear least-squares method[13] and the results are shown in Table I. The k_1 values are identical within the error limits, which is in agreement with the proposed mechanism because the k_1 step should be thiol-independent. The reverse/forward partition ratio (k_{-1}/k_2) for 3MPA is approximately 2-fold larger than that for 2ME. Since the k_{-1} step respresents the breakdown of I to give the reactants, it should also be thiol-independent; therefore, it follows that k_2(2ME)/k_2(3MPA) ~ 2, which implies that 2ME is more effective in trapping the intermediate I under these conditions. This may very well be the result of a larger fraction of 2ME mercaptide anion than 3MPA anion at pH 9.3 because of the higher basicity of 3MPA (pK = 10.2-10.3)[14,15] relative to that of 2ME (pK = 9.4-9.5).[15,16]

The observed rate suppression at high thiol concentration is probably due to the formation of one or more OPA-thiol adducts which decrease the free OPA concentration. The addition reaction of water or thiol to an aldehyde to give the hydrate or hemithioacetal is well-known,[16-20] and for this system, the proposed adduct L may be the cyclic hemithioacetal 2 (Scheme I). Similar thiol rate suppression was observed by Trepman and Chen[11] in their study of the reaction of OPA with alanine and 2ME, but the rate suppression was attributed to the formation of an OPA-$(2ME)_2$ adduct, and a mechanism involving the reaction of the amino acid with an OPA-2ME adduct to give the isoindole product was proposed. The formation constant for the OPA-2ME adduct was determined spectrophotometrically by these workers to be approximately 164 M^{-1} at pH 9.0 which corresponds to a dissociation constant of 6.1 mM. However, examination of the profile of log k_0 vs. log [2ME] presented in the same report shows a change in slope from zero to -1 at [2ME] ~ 6 mM, suggesting that the OPA-2ME adduct does not lead to the isoindole product. Although our kinetic results do not exclude the formation of other intermediates or more complex pathways that are kinetically indistinguishable, the kinetic model presented in this report is consistent with data recorded in this work and also with data observed by Trepman and Chen.[11]

In conclusion, analysis of the kinetic results presented above suggests (a) a mechanism for the formation of the isoindole product involving the reaction of an OPA-amine intermediate, probably an imine, with the thiol to give the fluorescent isoindole product, and (b) the formation of the OPA-thiol adduct decreases the free OPA concentration and brings about a reduction in the rate of formation of the isoindole product.

Registry No. OPA, 643-79-8; Ala, 56-41-7; 2ME, 60-24-2; 3MPA, 107-96-0.

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Design of DNA-Binding Peptides: Stabilization of α-Helical Structure by DNA

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Several lines of evidence have been recently converging to demonstrate the fundamental importance of α-helical elements in determining the binding specificity of sequence-specific DNA-binding proteins. Attention was first focused on the role of the α-helix when the crystal structures of λ repressor,[1] cro,[2] and *E. coli* CAP[3] were each found to contain a protruding bihelical unit that appeared to be complementary to the surface of B DNA. Amino acid sequence homologies tentatively identified 20-30 other DNA-binding proteins that contained a similar bihelical unit.[4] Structure determinations by X-ray on cocrystals of DNA with phage 434 repressor[5] and with the enzyme *Eco*RI[6] have now shown that an α-helix from each protein does indeed lie in the DNA major groove, presumably making sequence-specific contacts. In size and shape the α-helix (typical cylindrical radius 4-6 Å) is complementary to the B DNA major groove (cylindrical radius 6 Å), where the base pairs differ in hydrogen bond donor/acceptor patterns and hydrophobicity. Similar conclusions about the role of α-helical elements were supported by genetic analyses of DNA-binding proteins and have recently been extended using the

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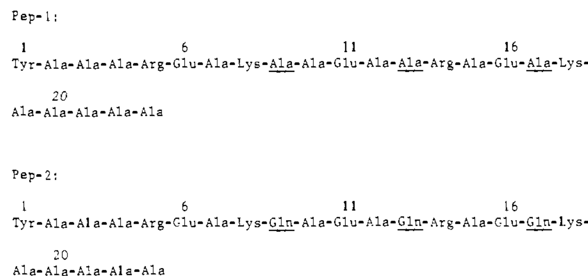

Figure 1. Amino acid sequence of Pep-1 (top) and Pep-2 (bottom). Underlined residues differ in the two peptides.

methods of site-directed mutagenesis. The DNA-binding specificity of 434 repressor was recently changed to that of phage P22 repressor by replacing a single α-helix in 434 repressor with a helix from the P22 protein.[7] The evident role of a small 10–20 amino acid helical segment in determining the binding specificity of larger proteins has fostered interest in the use of peptides to model protein–DNA interactions. Helical fragments of larger proteins have been studied,[8,9] and recently a novel peptide with helical potential was cloned and reported to protect DNA from restriction enzyme digestion.[10] The ultimate goal of our research efforts is to extend the use of peptides in the study of protein–DNA interactions by designing sequence-specific DNA-binding helical peptides.

In this first phase of our studies we undertook to determine the secondary structure, when actually bound to DNA, of a peptide designed to have helical potential. Such information would provide a foundation for the systematic design of sequence-specific DNA-binding peptides and could justify the use of peptides in modeling the interactions between DNA and helical regions of proteins. In contrast to the conformation of a protein segment that is stabilized by surrounding tertiary structural contacts, the conformation of a peptide is more labile and more dependent upon local intermolecular forces. A peptide might have significant helical structure in some solvents and yet could adopt a different secondary structure when bound to a macromolecule such as DNA. In addition, monomeric peptides with helical potential frequently adopt nonregular conformations in hydrogen-bonding solvents such as water.

For the present studies we sought to design an idealized DNA-binding peptide that would have a high helical potential,[11,12] fit longitudinally into the B DNA major groove when α-helical, and be monomeric and water soluble. The amino acid sequence chosen, Pep-1, is shown in Figure 1. We predicted that this peptide would bind four to six base pairs of DNA primarily thru nonspecific electrostatic interactions between Lys or Arg residues and phosphate oxygens.

Pep-1, synthesized by the Merrifield solid-phase method[13] and purified by gel filtration (Sephadex G25SF) followed by C18 (Altex) reversed-phase HPLC, showed the expected amino acid composition upon acid hydrolysis. The primary structure was confirmed by automated Edman sequencing.

Circular dichroism (CD) spectroscopy of Pep-1 revealed a predominantly random coil structure with 13% α-helicity in 10 mM sodium phosphate buffer, pH 7.5.[14] However, this value increased to 65% α-helicity in the presence of 80% trifluoroethanol (TFE), a solvent that promotes intramolecular hydrogen bonding. The strong intrinsic ellipticity of DNA and the steep slopes of the DNA spectra precluded the acquisition of accurate difference CD spectra of peptide bound to DNA; an alternate method was required for analysis of this interaction.

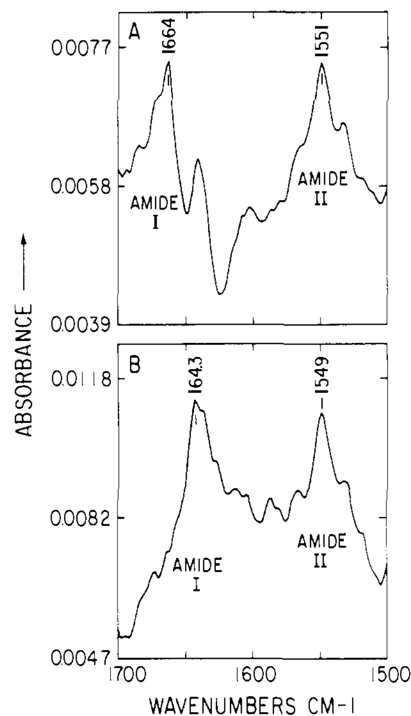

Figure 2. FT-IR spectra of Pep-1 in the absence (A) and presence (B) of DNA.

The conformation of peptide in peptide–DNA mixtures was determined by Fourier transform infrared (FT-IR) spectroscopy. Spectra were acquired (IBM IR/85 spectrometer) on 0.5 mM Pep-1 and DNA–Pep-1 mixtures ([Pep-1] = 0.5 mM; λ phage DNA from Bethesda Research Laboratories; [base pairs] = 1.08 mM). The stoichiometry of peptide and DNA was chosen on the basis of DNA cleavage experiments, described below, which indicated a strong peptide–DNA interaction at these relative concentrations. All samples were examined in buffered aqueous solution (16 mM Tris-Cl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.75) in an internal reflectance cell (Barnes), after a 30-min incubation at 37 °C. The spectrum of water was subtracted from all sample spectra, that of DNA from the peptide–DNA spectra. DNA caused a 21-cm^{-1} (data acquired with 8-cm^{-1} resolution) shift to lower energy in the Pep-1 amide I absorption maximum (1664 to 1643 cm^{-1}; Figure 2). This effect is consistent with literature values for a significant increase in α-helicity[15,16] and also corresponds with the results of FT-IR experiments in which TFE was used to induce α-helicity in Pep-1. In these measurements the effect of 80% TFE was an 11-cm^{-1} shift (data acquired with 4-cm^{-1} resolution) in the amide I absorption maximum (1647 to 1636 cm^{-1}; spectra acquired on an Analect 6260 spectrometer). These data suggest that Pep-1 is predominantly α-helical when bound to DNA.

In view of our FT-IR findings, we decided to include a second peptide in further studies. Since hydrogen bonds between protein and DNA are believed to contribute significantly to the increased affinity that results in sequence-specific binding,[17,18] we decided to modify the design of Pep-1 so as to include potential hydrogen-bonding residues. Three alanines in Pep-1 were replaced with glutamines in Pep-2 (Figure 1). Molecular modeling studies with Pep-2 suggested that glutamines, at the positions chosen, could form five to six nonspecific hydrogen bonds with the bases in the major groove of virtually any DNA sequence. Pep-2 was synthesized and purified as was described for Pep-1. CD spectroscopy

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of Pep-2 in pH 7.5 phosphate buffer with 0% TFE revealed 13% α-helicity, identical with Pep-1. However, Pep-2 had an even greater helical potential than Pep-1, as the α-helicity increased to 81% in 80% TFE.

DNA binding was further characterized by assessing the efficacy of the peptides in protecting DNA from digestion by the restriction endonucleases *Hin*dIII and *Eco*RI. Linearized plasmid PBR322 DNA was digested and analyzed by methods similar to those reported by others.[19] Preincubation of peptide with DNA (30 min at 37 °C) prior to the initiation of digestion caused a decrease in subsequent DNA cleavage. Greater protection was seen with Pep-2 than with Pep-1, presumably because of the glutamine-mediated hydrogen bonds. Thus, Pep-2 at 20 μM caused a 50% reduction in HindIII DNA cleavage; 20 μM Pep-1 did not inhibit *Hin*dIII cleavage. Pep-2 at 120 μM caused a 50% reduction in *Eco*RI DNA cleavage; 120 μM Pep-1 caused a 20% reduction in *Eco*RI cleavage.

We have shown that a suitably designed peptide may adopt a predominantly α-helical conformation when bound to DNA, in spite of having minimal regular secondary structure in solution. Futhermore, hydrogen-bonding residues appear to increase the affinity of the helical peptides for binding to DNA, as has been postulated for helical regions of DNA-binding proteins. These results provide a necessary base for the rational design of sequence-specific DNA-binding peptides and demonstrate the potential utility of peptides in modeling the interactions between DNA and helical regions of endogenous proteins.

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Reactions of Coordinated Ligands: Coupling of Two Diphosphenes (P=P) at a Metal Center To Give a Coordinated Phosphametallocyclopentane. Synthesis and X-ray Structure of (t-BuPP-t-Bu)Ni(t-BuP-t-BuP-t-BuP-t-BuP): A Square-Planar Ni(II) Geometry Imparted by Sterically Demanding Ligands

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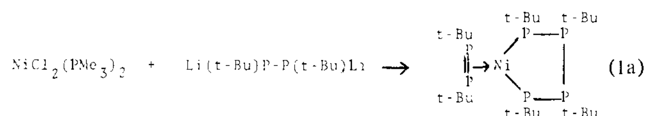
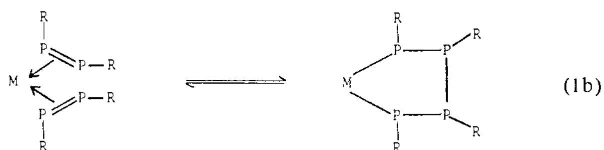
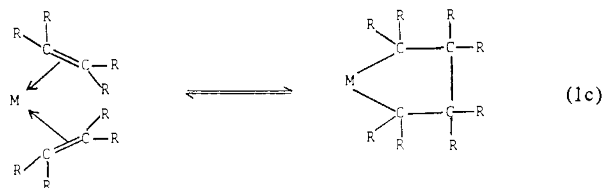
There has been much recent interest in multiple bonding among the heavier main group 5 elements.[1] Structural details have been reported for unsupported P=P,[1,2] P=As,[3] and As=As[4] double bonds and some transition-metal complexes.[5] Some initial reactivity studies have also been reported.[5,6] We report here the synthesis and X-ray crystal structure of the unusual complex (t-BuPP-t-Bu)Ni(t-BuP)$_4$ (**1**) (eq 1a) which is unique for the



(1a)



(1b)



(1c)

following reasons: (a) **1** contains the phosphametallocyclopentane unit Ni-t-BuP-t-BuP-t-BuP-t-BuP which demonstrates the first example of a coupling reaction between two diphosphene (P=P) units at a metal center (eq 1b).[7] Such a reaction would be analogous to metallacyclopentane formation for alkenes (eq 1c).[8] (b) **1** is the first example of a mononuclear η^2-bonded diphosphene transition-metal complex that does not have Cp, CO, or PR$_3$ as supporting ligands.[9] Mononuclear η^2-bonded P=P systems are

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