```
  1  MTEYK LVVVG AGGVG KSALT IQLIQ NHFVD
 31  EYDPT IEDSY RKQVV IDGET CLLDI LDTAG
 61  QEEYS AMRDQ YMRTG EGFLC VFAIN NTKSF
 91  EDIHQ YREQI KRVKD SDDVP MVLVG NKCDL
121  AARTV ESRQA QDLAR SYGIP YIETS AKTRQ
151  GVEDA FYTLV REIRQ H
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Figure 1. Primary structure of human H-*ras* (1-166) protein (calculated isotopically averaged molecular weight, 18 853.3).

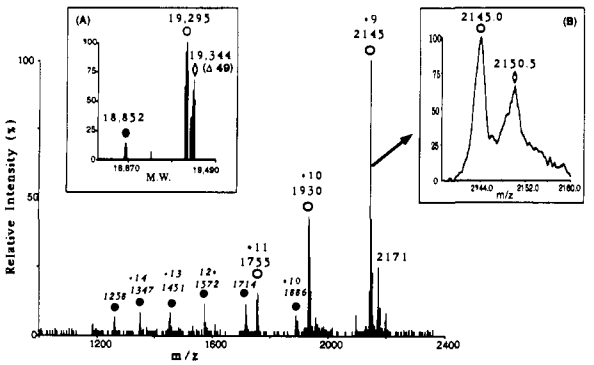

Figure 2. Ion-spray mass spectrum of *ras*:GDP complex at pH 5.8 (2 μg/μL in 2 mM NH_4OAc buffer), with the deconvoluted spectrum (inset A) and part of the narrow scan from m/z 1700 to 2400 (inset B). Open (○) and full (●) circles denote ion signals corresponding to the *ras*:GDP complex and the free *ras* protein, respectively.

tivity of this protein in GDP binding assays and GTPase assays was comparable to published data.[16-18]

The *ras*:GDP sample solution was analyzed by ion-spray MS using a Sciex API III triple quadrupole mass spectrometer. An aqueous solution of *ras*:GDP (2 μg/μL in 2 mM NH_4OAc buffer, pH 5.8) was infused at 5 μL/min through the ion-spray interface.[19] Several scans (10-20) from m/z 300 to 2400 at a scan rate of 2 s/scan were summed to yield the final profile spectrum.

The ion-spray mass spectrum of *ras*:GDP showed two envelopes of peaks corresponding to multiply protonated ions of the *ras*:GDP complex and the *ras* protein (Figure 2). The first envelope comprising intense signals at m/z 1755, 1930, and 2145 corresponding to the +11, +10, and +9 charge states of the *ras*:GDP protein-ligand complex, respectively, yields an average mass of 19 295 Da (Figure 2, inset A), thus confirming the 1:1 stoichiometry of the *ras*:GDP complex (calculated MW 19 293.5 Da). The much less abundant ions at m/z 1258, 1347, 1451, 1572, 1714, and 1886 representing the +15 to +10 charge states of the *ras* protein, respectively, provide an average mass of 18 852 Da (Figure 2, inset A); this spectrum also reveals the presence of a third component with a molecular mass 49 Da higher than that of the *ras*:GDP signal, probably due to the attachment of two magnesium atoms. This additional component was clearly resolved in the narrow scan mass spectrum (Figure 2, inset B).

The stability of the *ras*:GDP complex is highly dependent on the solvent system employed in the ion-spray analysis. Our studies indicate that dissociation of the *ras*:GDP noncovalent complex is brought about either by adding methanol or by lowering the pH of the *ras*:GDP aqueous solution or both. For example, denaturation of the *ras*:GDP complex is not brought on until the pH of the aqueous solution is lowered to ca. 3.5, whereas addition of 10% methanol at that pH induces extensive dissociation of the complex.

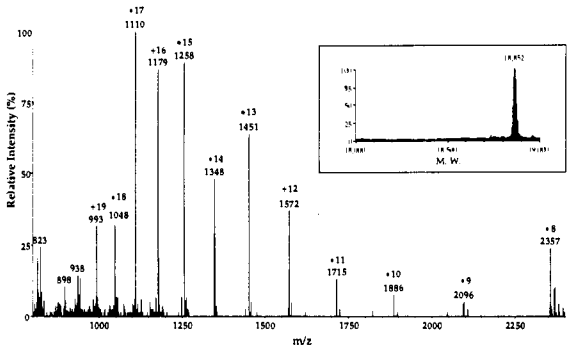

Figure 3. Ion-spray mass spectrum of the apo-*ras* protein at pH 2.7 (4 μg/μL in 1:1 methanol/water containing 2 mM NH_4OAc and 5% AcOH), with the deconvoluted spectrum shown in the inset.

In the analysis of the *ras*:GDP sample by infusion of a 1:1 methanol/H_2O solution containing 2 mM NH_4OAc and 5% acetic acid (pH 2.7), the complete denaturation of the *ras*:GDP complex was evidenced by the appearance of the multiply charged peak envelope of the free *ras* protein comprising the +12 up to +19 charge states (average mass 18 855 Da). This is in good agreement with the multiply charged peak envelope observed in the ion-spray mass spectrum of the apo-*ras* protein (not bound to GDP) shown in Figure 3. In addition, a second distribution centered around the +11 charge state was also present in the above mass spectrum of the *ras*:GDP complex (pH 2.7), yielding an average mass of 18 853 Da. This probably arises from another "tighter" conformational state of the *ras* protein in solution, identical to the one assumed by the free *ras* protein in Figure 2, wherein a smaller number of basic amino acids is exposed for protonation. Similar observations have been made in the electrospray analysis of bovine cytochrome *c*.[20]

In conclusion, we have successfully applied ion-spray mass spectrometry toward the detection of the GDP-bound noncovalent complex of the oncogenic human H-*ras* protein, and we believe this method could be extended for the study of the GTP-bound *ras* protein, as well as complexes of the *ras* protein with other pharmaceutically interesting compounds that could occupy the place of the nucleotide.

Position-Dependent Stabilizing Effects in α-Helices: N-Terminal Capping in Synthetic Model Peptides

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Received March 16, 1992

The role of individual side chains in stabilizing or destabilizing α-helices has been analyzed by statistical approaches[1] and by host-guest experiments on model peptides or polypeptides.[2] While each NH group of a peptide in the "middle" of a helix is H-bonded

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Table I. Helix Content and Change in Free Energy of Helix Formation on Substitution of Lyutides

lyutide	$-[\theta]_{222(obsd)}$[a]	f, %[14]	$-[\theta]_{222(calcd)}$[b,14]	σ ($\times 10^{-4}$)[c]	$\Delta\Delta G(N_{cap})$[d]	$\Delta\Delta G$(mid)[e]
S3	16000 ± 480	50	16050	5.4	−0.74	
N3	14150 ± 740	44	14280	4.0	−0.58	
G3	10580 ± 680	33	10600	2.2	−0.25	
A3	7700 ± 670	24	7980	1.4	0	
S10	9300 ± 310	29	9600			0.55
G10	5560 ± 310	17	5750			0.98

[a] The observed mean residue ellipticity at 222 nm, in deg·cm²/dmol. CD measurements were performed as described.[2c,f,14] The values reported correspond to samples of ca. 30 μM concentration at 4 °C. [b] The calculated mean residue ellipticity at 222 nm.[14] [c] Nucleation constant, used to calculate [θ] value in previous column. [d] Change in free energy of helix formation at N_{cap} relative to Ala: $\Delta G(N_{cap}) = -RT \ln \sigma$ (kcal/mol). [e] Free energy change of helix formation on substitution in the middle of helix relative to Ala: $\Delta G_{Ala} = -0.43$ kcal/mol.[5,14]

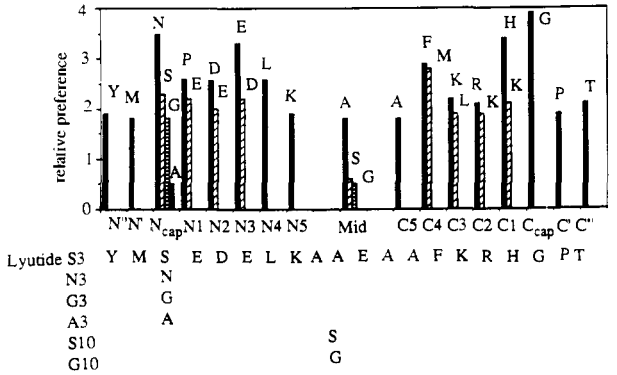

Figure 1. Relative preference values for each amino acid in the helical sequences of 42 proteins (Table I, ref 4) with the sequences of lyutides. Peptides were synthesized as previously described.[2c,f] Single-letter abbreviations for these amino acids are as follows: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; H, His; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; R, Arg; S, Ser; T, Thr; Y, Tyr.

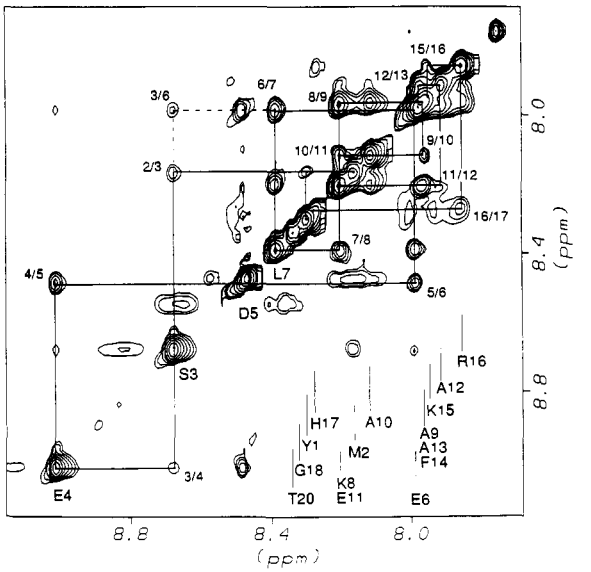

Figure 2. ^{1}H NOESY experiment on lyutide S3, showing sequential connectivity in NOEs between adjacent peptide NH groups along the chain. Assignments are indicated in the single-letter codes for amino acids given in the legend to Figure 1. A GN 500 spectrometer was used, at 10 °C, pH 5.7, in 15% D_2O. The mixing time τ_m[10a] was 350 ms.

to a CO group four residues away, the first four NH donors and the last four CO acceptors in a helix cannot be satisfied by main chain peptide groups.[3-6] The structure of helices in proteins reveals that certain side chains interact with the backbone to satisfy this unfulfilled H-bonding potential.[3-5] We demonstrate here that specific side chains stabilize isolated α-helices differently at the N_{cap} position (the interfacial residue demarcating the helix N-terminus) relative to the middle using a new series of designed helical peptides (lyutides) with the sequences shown in Figure 1. The top panel in the figure summarizes the side chains that occur most frequently at different positions in helices from proteins.[4] The parent lyutide sequence includes one of these side chains at each of its corresponding positions. These peptides represent consensus helical sequences at each position, except that the middle four residues in lyutide S3 contain an additional glutamic acid for solubility and to permit a salt bridge interaction with the lysine at C3.[7]

Five substituted peptides were synthesized with the sequences shown in Figure 1. The presumed N_{cap} Ser 3 was replaced by Asn, Gly, and Ala, and a site in the middle region of the chain, A10, was replaced by Ser and Gly. The strategy is to compare the Ser 3 and Asn 3 molecules—the two most frequent N_{cap} side chains—with Gly and Ala, respectively the least and most probable amino acid side chains in the middle positions of protein helices (proline excluded). Asp was not selected for substitution at N_{cap} because its charge can interact with the α-helix N-terminus.[8] The lyutides form α-helical structures in aqueous solution, as shown by their characteristic CD spectra, with double minima at 222 and 208 nm and maxima at 195 nm, indicating partial helix and coil contributions.[9] The CD signal at 222 nm[2c] shows no dependence on concentration (up to 350 μM); the structure in these molecules is thus intramolecular. Figure 2 shows the network of short i, $i+1$ NH–NH distances extending from residue 2 to residue 17 in the molecule, indicating that the region of the molecule that includes S3 is indeed α-helical.[10]

The order Ser > Gly > Ala for helix stabilization that we observe at the N_{cap} position (Figure 1) differs from that in the middle of an α-helix, where Ala > Ser > Gly.[2] Our order agrees with that found in substitutions at capping positions in proteins.[5,8,11] Apart from Gly, each side chain that occurs with high frequency at N_{cap} can accept a main chain NH proton.[3,4] Our results are consistent with stabilization of the helical structure in isolated model peptides by this mechanism. However, Gly cannot act in the same way as Ser or Asn. Gly might interfere less than the bulkier Ala side chain with solvation of the NH groups at this end of the chain.[5] Alternatively, the greater conformational freedom of Gly might allow the preceding main chain CO group to H-bond with neighboring backbone or side chains, as in Schellman's mechanism for Gly capping at the C-terminus.[3,4,13]

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Quantitative evaluation of the N_{cap} effect requires introducing position-dependent σ values into models of the helix–coil equilibrium.[6,14] A rough estimate can be obtained by assuming that the N_{cap} interaction affects σ, the helix nucleation constant, rather than s, the helix propagation constant,[6] and fitting a new σ value to the CD data.[14] Substitutions in the middle of the sequence are taken to reflect changes in s values, rather than σ.[14] Table I shows that Ala stabilizes α helix more than Ser by 0.55 kcal/mol in the middle, as in other models,[2b,c] while Ser is 0.74 kcal/mol more stable than Ala at the N_{cap} position. The availability of σ and s values specific for the N_{cap}, C_{cap}, and middle positions of α-helices should lead to improved predictions of helix structure.[1]

Acknowledgment. This research was supported by grants from the NIH (GM 40746) and the NSF (DMB 88-15998). P.C.L. is an NRSA postdoctoral fellow of the NIH (GM 14803). N.J. was supported by a MOST summer research fellowship.

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Aberrantly Methylated DNA: Site-Specific Introduction of *N*7-Methyl-2′-deoxyguanosine into the Dickerson/Drew Dodecamer

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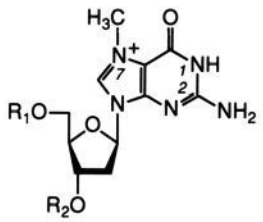
Whereas the products of enzymatic DNA methylation are essential for proper genetic function, most products of nonenzymatic DNA methylation are genotoxic.[1] Adducts arising from aberrant methylation are believed to present a persistent challenge, since most organisms maintain high concentrations of a methylating agent[2,3]—*S*-adenosyl-L-methionine—in the solution containing their DNA.[4] The predominant adduct, *N*7-methyl-2′-deoxyguanosine (m^7dG),[5,6] remains poorly understood at the



m^7dG: R_1, R_2 = H or DNA
m^7dGTP: $R_1 = HP_3O_9$, R_2 = H

molecular level, in part because it has heretofore not been incorporated into DNA site-specifically. M^7dG is not only encountered frequently as a biological lesion but is also widely used as a probe for specific protein–DNA interactions;[7,8] this has intensified the need for its chemical and structural characterization in DNA. Here we report the site-specific insertion of m^7dG into a prototypical duplex oligodeoxynucleotide, the Dickerson/Drew dodecamer.[9]

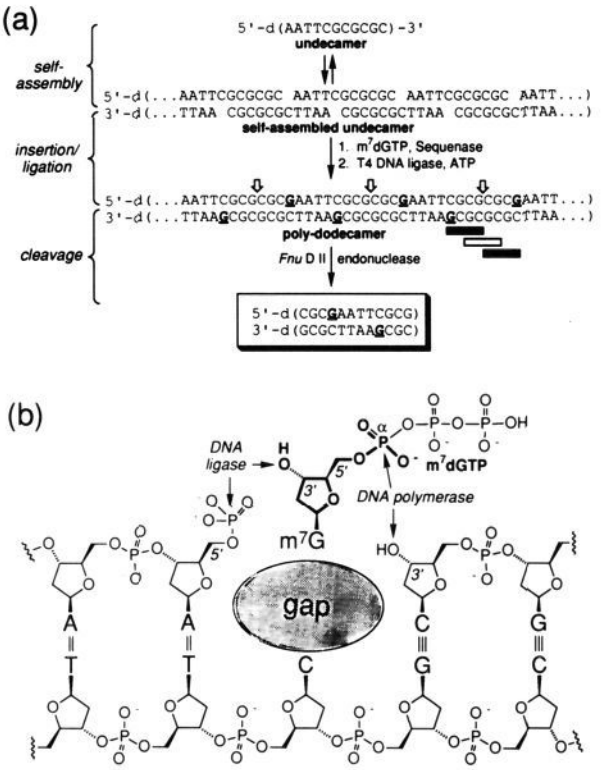


Figure 1. Synthesis of a Dickerson/Drew dodecamer specifically modified with m^7dG. (a) Reaction scheme. Arrows denote sites at which blunt-ended cleavage yields the desired product. Boxes directly under sequence denote overlapping *Fnu*DII sites: unmodified site (unshaded); m^7dG-containing sites (shaded). All oligonucleotides shown in this scheme and elsewhere in the text possess a 5′-phosphoryl substituent; 3′-phosphoryl substituents, if any, are shown explicitly. (b) Structure of the single-nucleotide gaps into which an m^7dG nucleotide unit (bold structure) is inserted enzymatically. The orientation of the strands is reversed from that shown in part a. Arrows denote atoms that are joined by the enzymes indicated.

Table I. Thermal Denaturation of Duplex Oligonucleotides[a]

2 ssDNA ⇄ dsDNA

sequence	T_m (°C)	$\Delta G°$	$\Delta H°$	$T\Delta S°$
5′-d(CGCGAATTCGCG)	63.0	−14.7	−66.8	−52.1
5′-d(CGCGAATTCGCG)	58.5	−14.9	−74.5	−59.6

[a] $\Delta G°$, $\Delta H°$, and $T\Delta S°$ are in units of kcal mol^{-1}. T = 298 K.

An undecamer, 5′-d(AATTCGCGCGC), was designed to self-assemble (by base-pairing) into a multimeric array with single-nucleotide gaps (Figure 1a). The gaps possess all of the functionality required for enzymatic insertion of a single m^7dG residue: 3′-hydroxyl and 5′-phosphate groups, to participate in phosphodiester linkages with an m^7dG nucleotide unit (in bold); and a cytosine (on the opposite strand) to Watson–Crick pair with m^7dG (Figure 1b). Incubation of 5′-d(AATTCGCGCGC) with m^7dGTP[10] and modified T7 polymerase[11] ("Sequenase") afforded the dodecamer 5′-d(AATTCGCGCGCG) (G = m^7dG). This was ligated to yield a polydodecamer (Figure 1a) containing repeating units of the desired product; all that remained was to cleave the

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