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Helix Propensities of Basic Amino Acids Increase with the Length of the Side-chain

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Helix formation in a 17-residue alanine-lysine peptide and analogous peptides with specific lysine→X substitutions, where X is 2,3-diamino-L-propionic acid, 2, 4-diamino-L-butyric acid or L-ornithine, have been examined using circular dichroism measurements. The dependence of helix content on X, its position in the sequence, and the number of lysine→X substitutions are reasonably well described by using the Lifson–Roig theory modified to include N-capping, without explicitly considering charge–helix dipole interactions. The helix propensities for these basic amino acids increase with the length of the side-chain in the rank order 2,3-diamino-L-propionic acid < 2,4-diamino-L-butyric acid < ornithine < lysine. This parallels the increase in helix propensities with side-chain length of other polar and charged amino acids.

Keywords: α-helix stability; basic amino acids; chain-length effects; peptide helices; helix dipole

Introduction

α-Helix formation and stability are determined both by the intrinsic helix-propensities of amino acids and by interactions between their side-chains (Baldwin, 1995). For amino acids with side-chains of similar conformation and type, helix propensities appear to increase as the length of the side-chain increases with the addition of methylene groups. Thus, the following rank order of helix propensities has been observed among non-polar amino acids: isoleucine > valine (Lyu *et al*., 1990, 1991; O'Neil & DeGrado, 1990; Padmanabhan *et al*., 1990; Padmanabhan & Baldwin, 1991; Park *et al*., 1993a; Chakrabartty *et al*., 1994) and norvaline > α-amino-*n*-butyric acid (Padmanabhan & Baldwin, 1991, 1994); and among uncharged, polar amino acids: glutamine > asparagine (Lyu *et al*., 1990; O'Neil & DeGrado, 1990; Park *et al*., 1993a; Chakrabartty *et al*., 1994), uncharged Glu > uncharged Asp (Huyghues-Despointes *et al*., 1993; Park *et al*., 1993b; Scholtz *et al*., 1993), and hydroxybutyl-L-glutamine (HBQ) > hydroxypropyl-L-glutamine (HPQ) > hydroxyethyl-L-glutamine (HEQ) (Lotan *et al*., 1966; Padmanabhan *et al*., 1994).

Charged amino acids affect helix formation by their intrinsic helix-forming propensities and by coulombic interactions of their charged side-chains with each other and with the helix dipole (an intrinsic property of an α-helix arising from the partial alignment of individual peptide dipole moments and/or the non-H-bonded terminal NH and CO groups). These interactions, being coulombic, can be screened by increasing salt concentrations (Huyghues-Despointes *et al*., 1993). Both the nature of the charged residue and its position in the sequence affect helix formation, the effects being strongest when the charged residue is present at the ends and smallest when it is at the centre (Armstrong & Baldwin, 1993; Huyghues-Despointes *et al*., 1993; Scholtz *et al*., 1993). By modelling the charge–helix dipole interaction into the helix-coil transition theory, its contribution to helix formation has been separated from the intrinsic helix propensities and the rank order Glu > Asp has been obtained for the helix propensities of these residues in their charged forms (Huyghues-Despointes *et al*., 1993; Scholtz *et al*., 1993). The helix propensity of the charged residue appears to be unaffected (e.g. Asp) or to increase (e.g. His, Glu) (Armstrong & Baldwin, 1993; Huyghues-Despointes *et al*., 1993; Park *et al*., 1993a,b; Scholtz *et al*., 1993) on titration of the

Abbreviations used: Db or Dab, 2,4-diamino-L-butyric acid; Dp or Dpr, 2,3-diamino-L-propionic acid; O or Orn, L-ornithine; CD, circular dichroism; TFE, trifluoroethanol; r.m.s., root-mean-square; HPLC, high performance liquid chromatography; Nval, norvaline.

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charged side-chain to an uncharged form. Nevertheless, for both the charged and uncharged forms of these acidic amino acids, the helix propensity increases with the length of the side-chain.

We have, in the present study, examined if the helix propensity of a basic amino acid also increases as the length of its positively charged side-chain increases. Specifically, we examined how helix formation in simple, alanine-based peptides is affected by the basic amino acids: 2,3-diamino-L-propionic acid (Dpr), 2,4-diamino-L-butyric acid (Dab), L-ornithine (Orn) and/or lysine (Lys), which have side-chains with one, two, three and four methylene groups, respectively. All the alanine-based peptides that we employed in our study have four basic residues present at positions 2, 7, 12 and 17 in the sequence. Thus, they all have the same overall charge and differ only in how many and where in the sequence a specific, basic amino acid occurs. We find that the dependences of the helix content on the nature, the number, and the position of a specific basic amino acid in the sequence are reasonably well-described using a modified form of the Lifson–Roig theory (Doig *et al*., 1994) at both low and high salt concentrations, even though charge–helix dipole interactions are not explicitly included in our analysis. Our results confirm that intrinsic helix propensities of basic amino acids increase with the length of the side-chain, as observed with other polar and non-polar amino acids.

Results and Discussion

Peptide design

Table 1 lists the peptide sequences used in the present study. The alanine-based reference peptide is made water-soluble by the presence of the four Lys residues as described (Marqusee *et al*., 1989; Padmanabhan *et al*., 1990). These Lys residues present at positions 2, 7, 12 and 17 in the sequence are spaced five residues apart to minimize Lys–Lys charge interactions, and to form non-amphipathic, monomeric α-helices. Monomeric α-helix formation is demonstrated by sedimentation equilibrium measurements of the reference peptide (Padmanabhan *et al*., 1990), and by the fact that thermal helix-to-coil transitions for all the peptides used in the present study (monitored by CD) are reversible and concentration-independent over a tenfold range (10 to 100 μM) in peptide concentration. The helix propensities of Dpr, Dab, Orn and Lys were determined by examining helix formation in peptides corresponding to different Lys → X substitutions (X = Dpr, Dab or Orn) in the reference peptide. This conserves the overall charge on the peptide and the positions of the charged, basic residues in the sequence. Only the side-chains of the basic amino acids contribute to the charges on the peptide as the peptides are all acetylated at the N terminus and amidated at the terminus. An N-terminal Tyr is present in all peptides to determine peptide concentration, as mentioned in Materials and Methods.

Table 1. Peptides and sequences

Peptide[a]	Sequence
Reference	Ac-Y-K-AAAA-K-AAAA-K-AAAA-K-NH_2
1X-2	Ac-Y-X-AAAA-K-AAAA-K-AAAA-K-NH_2
1X-7	Ac-Y-K-AAAA-X-AAAA-K-AAAA-K-NH_2
1X-12	Ac-Y-K-AAAA-K-AAAA-X-AAAA-K-NH_2
1X-17	Ac-Y-K-AAAA-K-AAAA-K-AAAA-X-NH_2
3X-2,7,12	Ac-Y-X-AAAA-X-AAAA-X-AAAA-K-NH_2

[a] X is (1) Dpr: 2,3-diamino-l-propionic acid, side-chain -CH_2-NH_3^+; or (2) Dab: 2,4-diamino-l-butyric acid, side-chain -CH_2-CH_2-NH_3^+; or (3) Orn: l-ornithine, side-chain -CH_2-CH_2-CH_2-NH_3^+.

Helix contents and their dependences on sequence position and on salt and TFE concentrations

The CD spectra (not shown) of all the peptides used in the present study are characteristic of mixtures of helix and coil with two minima (one at 222 nm, and the other between 203 and 207 nm), a maximum around 192 nm, and an isodichroic point at 203 nm (Holzwarth & Doty, 1965). Table 2 lists the helix contents for all the peptides used in the present study under various solution conditions at 0°C and pH 7.0. These helix contents determined from -$[\Theta]_{222}$ values are, as mentioned in Materials and Methods, approximate values because they have not been corrected for the aromatic contributions of the N-terminal Tyr residue (Chakrabartty *et al*., 1993).

Table 2 shows that peptides with three Lys → X substitutions (X = Dpr, Dab or Orn) at positions 2, 7 and 12, and also those with a single Lys → X substitution at the central positions 7 or 12, have lower helix contents than the reference peptide at all salt concentrations, with the rank order of helix propensities being Lys > Orn > Dab > Dpr. Single Lys → X substitutions at positions 2 or 17, which are closer to the ends, cause small or no changes in helix content relative to the reference peptide for X = Dab or Orn. This may be explained by end-fraying effects (Chakrabartty *et al*., 1991; Rohl *et al*., 1992; Rohl & Baldwin, 1994). Unlike Dab or Orn, a single Lys → Dpr substitution decreases helix content significantly at position 2, and increases helix content at position 17 relative to that of the reference peptide. The large effect of a single Lys → Dpr substitution at position 2 may be because, at this position, Dpr, which has a very low helix propensity, strongly destabilizes the more helical N-terminal residues of the asymmetric α-helices formed by these N-acetylated, alanine-based peptides (Doig *et al*., 1994). It is unlikely that these effects are the result of different N or C-capping propensities of Dpr and Lys: these two basic amino acids can serve as hydrogen bond donors only and so both will be expected to have low N-cap propensities, and the C-cap propensities of amino acids do not appear to differ significantly

Table 2. Fractional helicities at different NaCl concentrations and in TFE

Peptide	Fraction helix[a] (NaCl)[b] 0.05 M	0.1 M	1.0 M	2.5 M	Fraction helix[a] (TFE)[c]
Reference	0.63	0.64	0.74	0.72	0.97
1Dpr-2	0.45	0.48	0.56	0.57	0.88
1Dpr-7	0.32	0.32	0.41	0.41	0.90
1Dpr-12	0.33	0.34	0.43	0.43	0.91
1Dpr-17	0.71	0.72	0.81	0.81	0.96
3Dpr-2,7,12	0.04	0.05	0.09	0.13	0.74
1Dab-2	0.52	0.53	0.69	0.70	0.89
1Dab-7	0.44	0.48	0.61	0.61	0.93
1Dab-12	0.47	0.49	0.60	0.61	0.91
1Dab-17	0.64	0.65	0.73	0.73	0.90
3Dab-2,7,12	0.10	0.11	0.26	0.31	0.82
1Orn-2	0.59	0.61	0.72	0.71	0.95
1Orn-7	0.55	0.57	0.67	0.67	0.91
1Orn-12	0.54	0.57	0.68	0.67	0.92
1Orn-17	0.67	0.68	0.76	0.75	0.97
3Orn-2,7,12	0.33	0.36	0.49	0.52	0.90

[a] Fractional helicities (errors $\leqslant \pm 0.03$) were calculated as described (Materials and Methods).
[b] 0°C / pH 7.0.
[c] TFE data were in 9.5 mol% TFE, 0°C, 0.1 M NaCl (pH 7.0).

(Chakrabartty *et al*., 1994; Presta & Rose, 1988; Richardson & Richardson, 1988). The *n*-value of Lys^+ has been measured and found to be small (Chakrabartty *et al*., 1994); the *n*-values of the other basic amino acids studied here have not been measured but, as explained above, they are also expected to be small and therefore have been set equal to the *n*-value for Lys^+. See also the recent measurements of the *n*-values and *c*-values of all the natural amino acids (Doig & Baldwin, 1995). Another explanation for the differences between Dpr and Lys at the end positions may be that charge–helix dipole interactions are stronger with Dpr than with Lys at these end positions (more unfavorable at position 2 and more favorable at position 17), since the side-chain positive charge is more proximal to the helix backbone in Dpr than in Lys. This has been studied by varying the salt concentration, which can affect the coulombic charge–charge and charge–helix dipole interactions.

The helix contents of all the peptides used in the present study increase with salt concentration because this screens the intrinsic helix dipole (Scholtz *et al*., 1991) and also screens charge–helix dipole and charge–charge interactions. The helix content then reaches a maximum around 2 M NaCl and decreases with increasing salt because of Hofmeister effects (Scholtz *et al*., 1991). This general salt effect occurs irrespective of the sequence position at which the substitutions are made. The rank order of helix-forming tendencies and the trends in the effects of the sequence position of Lys, Orn, Dab or Dpr on helix contents remain unchanged, however, with salt concentration (Figure 1a and b; Table 2). These two facts, namely that the rank order of helix-forming tendencies is the same at all positions and at all salt concentrations, suggest that only the intrinsic helix propensities determine this rank order, even at positions 2 and 17, although charge–helix dipole interactions cannot be excluded and may contribute to the results.

For all peptides, the helix content increases with increasing trifluoroethanol (TFE) concentration to a maximum between 90% and 95% helix at TFE $\geqslant$ 10 mol% (Figure 2a to c). (The maximum helix contents at TFE $\geqslant$ 10 mol% are lower for 3Dpr and 3Dab, presumably because the presence of three residues of low helix propensity, Dpr or Dab, prevents maximal helix stabilization by TFE: Table 2.) Since the rank order of helix-forming tendencies and the trends in the effects of single Lys $\rightarrow$ X substitutions on helix formation are not altered by changes in salt concentration, and since the maximal helix stabilization by TFE is nearly identical for all peptides, we next examined if intrinsic helix propensities could account for the observed effects of Lys $\rightarrow$ X substitutions on helix formation.

Helix propensities and position effects on helix contents examined using the modified Lifson–Roig theory

The Lifson–Roig theory, modified to include the N-capping effects (Doig *et al*., 1994), was used to determine the apparent helix propensities of the charged, basic amino acids and to examine their position effects on helix formation. Use of modified Lifson–Roig theory allows the inclusion of the strong capping effect of the N-acetyl group, which is an important factor in causing the residues at the N-terminal end to be more helical than those at the C-terminal end (Doig *et al*., 1994). The helix propensities of the basic amino acids in their uncharged forms were not determined, since the peptides used in the present study can aggregate under conditions where the basic side-chains are neutralized (Marqusee *et al*., 1989). We have not modelled in our analysis charge–helix dipole

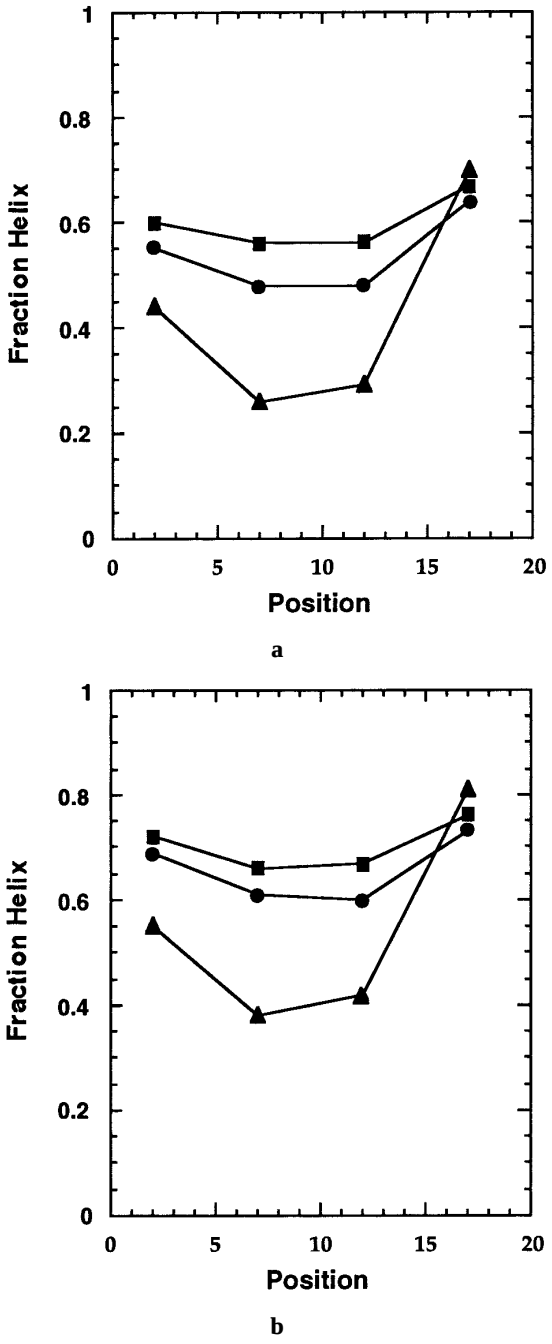


Figure 1. Fraction helix of peptides at 0°C and pH 7.0 *versus* position of X in the sequence: a, 0.1 M NaCl; b, 1.0 M NaCl. X = Dpr, (▲); X = Dab, (●); X = Orn, (■). The lines are drawn to aid viewing and have no theoretical significance.

interactions which, being coulombic, are sensitive to salt concentration. Instead, we estimated the apparent helix propensities at low (0.1 M NaCl) and at high salt concentrations (1.0 M NaCl). These values could then be used to infer if the change in the length of the charged side-chain from Lys to Dpr has any significant effect on the coulombic interactions that affect helix formation.

The helix contents at a given salt concentration of the reference peptide and the peptides 1X-7 and 1X-12 were simultaneously analyzed to determine the apparent helix propensities at that salt concentration. Peptides 1X-7 and 1X-12,. where X is at the central positions, 7 and 12, were chosen because, at these central positions, contributions from charge interactions to helix stabilization will be least (Armstrong & Baldwin, 1993; Huyghues-Despointes *et al*., 1993; Scholtz *et al*., 1993) and therefore there will be little, if any, difference in the magnitudes of charge interactions between Lys and X as a consequence of their different side-chain lengths. Table 3 lists the values of the helix propensities (w_X) for X = Lys, Orn, Dab or Dpr, and the destabilization that results per Lys → X substitution ($\Delta\Delta G^0 = -RT \ln(w_X / w_K)$). The different *w*-values obtained a 0.1 M and 1.0 M NaCl (Table 3) reflect the different net contributions from coulombic interactions at these two salt concentrations: higher salt concentrations screen the intrinsic helix dipole and also screen unfavorable charge interactions among side-chains, thereby leading to higher values of helix propensity. At both the low (0.1 M) and the high (1.0 M) salt concentration, $\Delta\Delta G^0$ values are very nearly identical; this indicates that the magnitudes of the charge interactions are essentially unaffected by the different side-chain lengths of Lys and X.

Helix propensities obtained from peptides 1X-7 and 1X-12 were then used to predict the helix contents for peptides 1X-2 and 1X-17 at the two salt concentrations. Charge–helix dipole interactions are significantly greater, and consequently more sensitive to salt, at these end positions, 2 and 17, than at the central positions 7 and 12 (Armstrong & Baldwin, 1993; Huyghues-Despointes *et al*., 1993; Scholt *et al*., 1993). If the different side-chain lengths of Lys, Orn, Dab and Dpr cause significant differences in their charge interactions, then the apparent helix propensities determined from the helix contents for peptides 1X-7 and 1X-12 would predict poorly the helix contents of peptides 1X-2 and 1X-17, especially at low salt. The predicted values are in good agreement, however, with their measured values at both high and low salt: the r.m.s. deviation between the predicted and measured helix contents is 4%, which is close to the experimental error of $\leqslant$3% in the measured helix contents (Figure 3a and b). This agreement is again consistent with the charge interactions involving Lys, Orn, Dab and Dpr not differing significantly from one another simply as a result of their different side-chain lengths. The effect of Lys → X substitutions on helix contents, therefore, arises primarily from the different, intrinsic helix propensities of Lys, Orn, Dab and Dpr.

Possible reasons for the increase in helix propensity with chain length of the basic amino acids

The basic question about these results is: why does the helix propensity increase with chain length for the basic amino acids? The following points

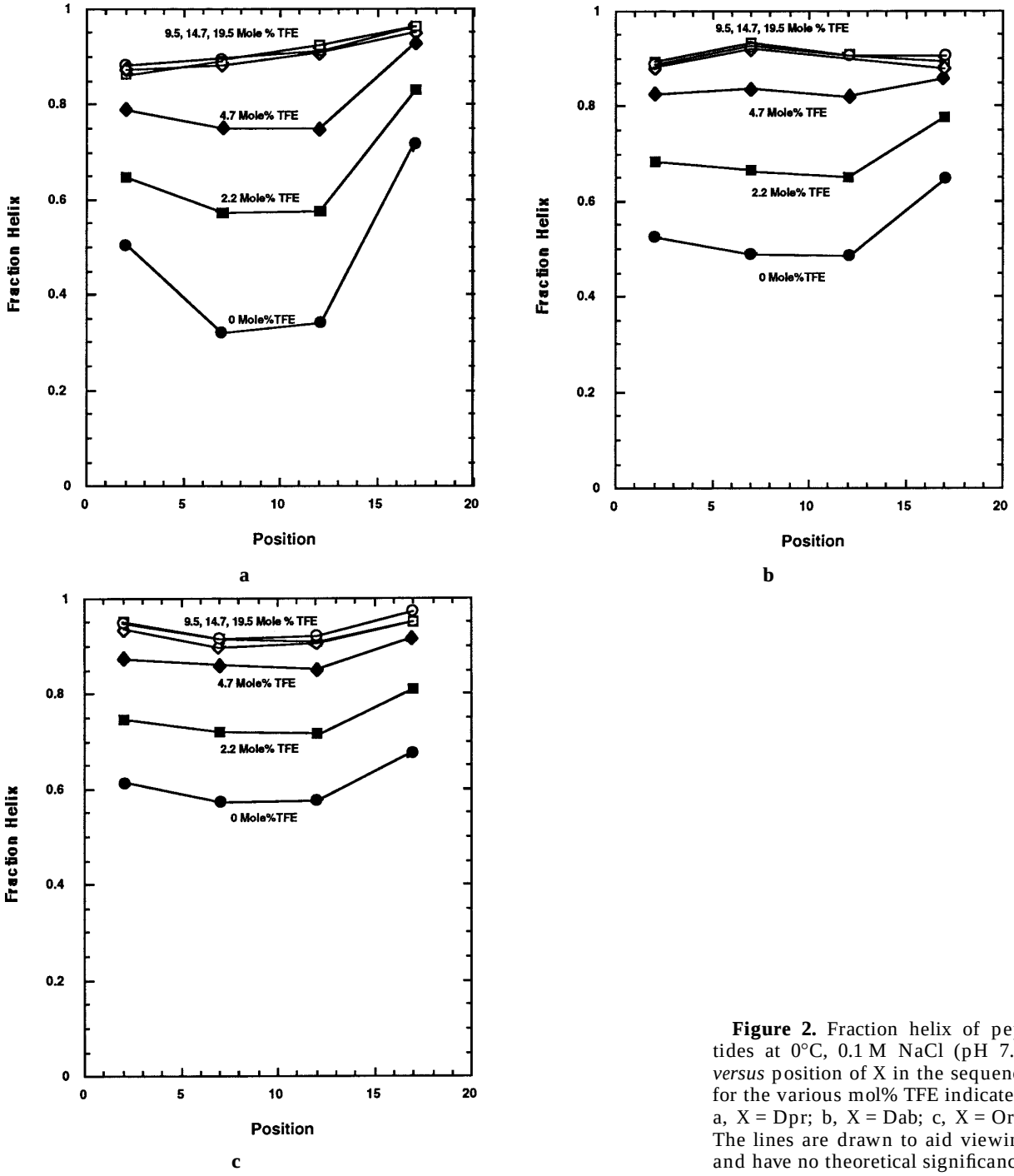


Figure 2. Fraction helix of peptides at 0°C, 0.1 M NaCl (pH 7.0) *versus* position of X in the sequence for the various mol% TFE indicated. a, X = Dpr; b, X = Dab; c, X = Orn. The lines are drawn to aid viewing and have no theoretical significance.

from Table 4 provide a possible answer. (1) The basic amino acid with the shortest side-chain (Dpr, $-CH_2NH_3^+$) is strongly helix breaking ($w = 0.08$; the dividing line between helix breaking and helix forming is $w = 1$). The w-values of the basic amino acids increase with chain length but the longest one studied here (Lys, $-(CH_2)_4NH_3^+$) has $w = 0.90$ and is slightly helix-breaking. (2) In contrast, the straight-chain, non-polar amino acid which corresponds to Dpr (Abu, $-CH_2CH_3$) has the much higher value $w = 0.84$ (compared to $w = 0.08$) and is only slightly helix-breaking. The w-values of the non-polar side-chains increase slowly with chain length: the longest one in Table 4 (Nle, $-CH_2)_3CH_3$) has $w = 1.20$. (3) Although the data for the other polar side chains are fragmentary, they resemble the basic amino acids more than the non-polar amino acids. The first member of each set is a fairly strong helix breaker ($w = 0.31$ for Asn, Asp^- and Asp^0) and there is a substantial rise in helix propensity shown by the next member of each set ($w = 0.56$ for Gln, 0.45 for Glu^- and 0.66 for Glu^0).

Therefore, although other factors undoubtedly enter in, it seems clear that an amino acid residue

Table 3. Apparent helix propensities (w) of Dpr, Dab and Orn

	0.1 M NaCl		1.0 M NaCl	
X	w_X	$\Delta\Delta G^{0a}$ (kcal mol^{-1})	w_X	$\Delta\Delta G^{0a}$ (kcal mol^{-1})
Lys	0.68	0.00	0.90	0.00
Orn	0.45	0.22	0.56	0.25
Dab	0.25	0.54	0.34	0.52
Dpr	0.07	1.23	0.08	1.31

Determined by fitting the helix contents of the reference peptide and peptides 1X-7 and 1X-12 as described in Materials and Methods. Temperature=273 K, pH 7.0.

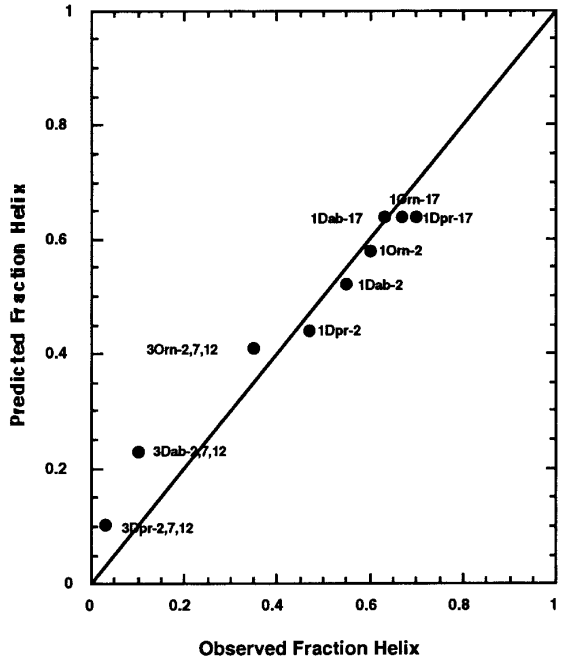
[a] $\Delta\Delta G^0 = -RT \ln(w_X / w_K)$.

with a polar group close to the peptide backbone is likely to be helix-destabilizing and that lengthening the side-chain of a basic amino acid increases its helix propensity for this reason. A main reason for this behavior is hydrogen bonding of the polar side-chain to peptide CO or NH groups in the backbone, which can interfere with α-helix formation. Side-chain H-bonding to the backbone has been demonstrated repeatedly in NMR studies (see, for example, Bundi & Wüthrich, 1979) and in X-ray studies of protein structures. Of the other factors that affect helix propensities of natural amino acids, two factors that are likely to be relevant here are the loss in side-chain conformational entropy on forming the helix (see Creamer & Rose, 1994; Blaber *et al*., 1994; Lee *et al*., 1994; Chakrabartty *et al*., 1994; Doig & Sternberg, 1995, and references therein) and the possibility of making non-polar interactions between the stem of the side-chain and -CH_3 groups of alanine residues in the helix.

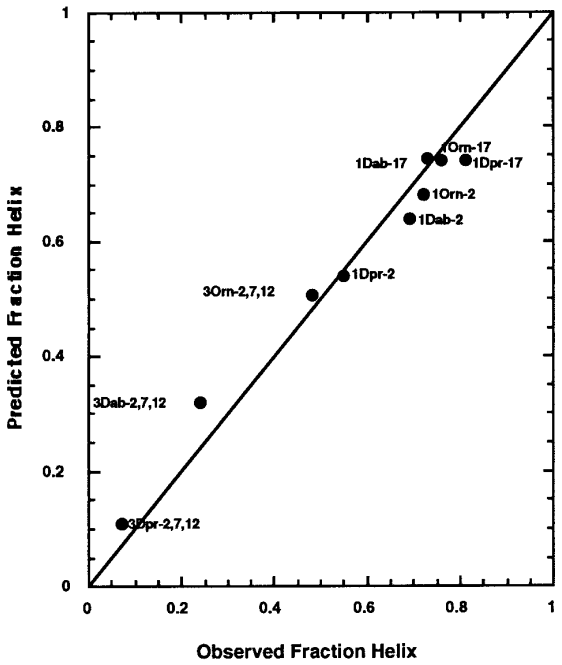
Materials and Methods

Peptide synthesis

Peptides containing Dpr, Dab and three Orn were synthesized by solid phase methods on a Biosearch/Milligen 9500 or 9600 automatic synthesizer or in a manual synthesis vessel, on *p*-methylbenzhydrylamine (polystyrene/1% divinyl benzene) resin. The *N*-α-tert-butyloxycarbonyl (Boc) diaminopropionic (Dpr) and diaminobutyric (Dab) with carbobenzoxy (Z) side-chain protection were purchased from Bachem Bioscience, Inc., as the dicyclohexylamine (DCHA) salts. Prior to coupling, the DCHA salt was removed with an equivalent of H_2SO_4 (Stewart & Young, 1984). *N*-α-Fluorenyloxycarbonyl (Fmoc) amino acids were used for all other reside couplings to minimize the loss of Z side-chain protection of Dab and Dpr that would occur during repetitive cycles of TFA removal of the Boc protecting group. The Fmoc group was removed with piperidine. 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) active esters were used for coupling Dab, Dpr and Fmoc amino acids. Boc amino acids were double-coupled with diisopropylcarbodiimide through residue eight, where appropriate; the remaining residues were introduced as TBTU active esters. Recouplings were performed if the qualitative Kaiser test was not negative. The tryosine derivative used was dichlorocarbobenzoxy-L-tyrosine. The N-terminal was acetylated with acetic anhydride. After HF cleavage (using anisole as the scavenger) and desalting on a G15 Sephadex in 50% (v/v) acetic acid, the peptides were purified by reverse-phase HPLC on Vydac C-18 resin in gradients of acetonitrile containing 0.1% TFA. Purity was ascertained by HPLC; amino acid



a



b

Figure 3. Goodness of fit of experimental fraction helix to the values predicted using the modified Lifson–Roig theory for the peptides indicated. The predicted values for these peptides were determined using the Lifson–Roig parameters determined by fitting the fraction helix of the reference peptide and peptides 1X-7 and 1X-12 as described in the text. The straight line corresponds to a 1:1 correspondence of the experimental and predicted data. a, 0.1 M; b, 1.0 M salt.

Table 4. Helix propensities of amino acids with differing side-chain lengths in alanine-based peptides

X	Side-chain	w_X[a]	$-RT \ln w_X$ (kcal mol^{-1})	Reference[b]
Lys^+	$-(CH_2)_4-NH_3^+$	0.90	0.06	a
Orn^+	$-(CH_2)_3-NH_3^+$	0.56	0.31	a
Dab^+	$-(CH_2)_2-NH_3^+$	0.34	0.54	a
Dpr^+	$-CH_2-NH_3^+$	0.08	1.37	a
Glu^-	$-(CH_2)_2-COO^-$	0.45[c]	0.43	b
Asp^-	$-CH_2-COO^-$	0.31[c]	0.63	c
Glu^0	$-(CH_2)_2-COOH$	0.66[c]	0.23	b
Asp^0	$-CH_2-COOH$	0.31[c]	0.63	c
Gln	$-(CH_2)_2-CO-NH_2$	0.56	0.31	d
Asn	$-CH_2-CO-NH_2$	0.31	0.63	d
HBQ	$-(CH_2)_2-CO-NH-(CH_2)_4-OH$	0.91	0.05	e
HPQ	$-(CH_2)_2-CO-NH-(CH_2)_3-OH$	0.83	0.10	e
HEQ	$-(CH_2)_2-CO-NH-(CH_2)_2-OH$	0.70	0.19	e
Nle	$-(CH_2)_3-CH_3$	1.20	–0.10	f
Nval	$-(CH_2)_2-CH_3$	1.14	–0.07	f
Abu	$-CH_2-CH_3$	0.84	0.09	f
Ile	$-CH-(CH_3)CH_2-CH_3$	0.44	0.45	d
Val	$-CH-(CH_3)CH_3$	0.23	0.80	d

HBQ, hydroxybutyl-L-glutamine; Nval, norvaline.

[a] 0°C, pH 7.0, 1 M NaCl.

[b] a, This study; b, Scholtz *et al*. (1993); c, Huyghues-Despointes *et al*. (1993); d, Chakrabartty *et al*. (1994); e, Padmanabhan *et al*. (1994); f, calculated from data of Padmanabhan & Baldwin (1994).

[c] 0°C, pH 7.0, 0.01 M NaCl.

composition was confirmed by analysis on a Beckman 6300 analyzer after hydrolysis for 22 hours at 110°C in 6 N HCl.

Peptides containing a single ornithine were synthesized on a Milligen 9050 automated synthesizer using Rink (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy) resin from Advanced ChemTech and the free Fmoc-L-Orn(Boc) (*N*-α-9-fluorenylmethoxy carbonyl-*N*-ϵ-*t*-butyloxycarbonyl-L-ornithine) from Star Biochemicals, activated by HOBT (1-hydroxybenzotriazole, Sigma) and BOP (benzo-triazolyltris (dimethylamino) phosphonium hexafluorophosphate-Biosearch). Peptides were acetylated at the N terminus using acetic anhydride and then cleaved from the benzhydrylamine resin with 95:5 trifluoroacetic acid / anisole mixture for two to four hours to yield peptides amidated at the C terminus. Crude peptides were purified by reverse phase fast protein liquid chromatography (f.p.l.c., Pharmacia) using a C_{18} resin with gradients of 10 to 40% acetonitrile:water (0.1% trifluoroacetic acid). Peptide identity was confirmed using fast atom bombardment or plasma desorption mass spectrometry.

Experimental measurements

Peptide concentrations were determined in 6 M guanidium hydrochloride by the absorbance at 275 nm of the N-terminal Tyr present in all the peptides (ϵ_{275} = 1450 M^{-1} cm^{-1}: Brandts & Kaplan, 1973). Circular dichroism (CD) measurements were made on an Aviv 60DS spectropolarimeter equipped with a Hewlett-Packard 89100A temperature control unit. The spectropolarimeter was calibrated with (+)-10-camphorsulphonic acid (Chen & Yang, 1977). CD spectra of peptides at 0°C, 0.1 M potassium fluoride, 1 mM potassium phosphate (pH 7.0), 40 to 60 μM peptide, were obtained using a 1 mm pathlength cuvette, 0.2 nm step size, one second average time and averaged over four scans. Mean molar residue ellipticity measurements at 222 nm, ($[\Theta]_{222}$ deg·cm^2 $dmol^{-1}$) in 1 M NaCl (pH 7.0) (1 mM each of sodium phosphate, sodium citrate, sodium borate 1 mM NaPCB) were made in 10 mm pathlength cuvettes (10 to 30 μM peptide) or in 1 mm pathlength cuvettes (>50 μM peptide). Trifluoroethanol (TFE) titrations were carried out at 0°C, 0.1 M NaCl (pH 7.0) as described (Nelson & Kallenbach, 1986).

Helix contents were determined from $[\Theta]_{222}$ measurements using $[\Theta]_{222}$ values of +640 and $-40{,}000\{1-(2.5/r)\}$ where $r = 17$, the number of amino residues in the peptide, for 0% and 100% helix contents, respectively (Scholtz *et al*., 1991). Most of our results were obtained prior to the observation of a contribution from the aromatic, N-terminal Tyr present in these peptides to the CD signal at 222 nm (Chakrabartty *et al*., 1993). The dependence of the magnitude of this aromatic contribution on the helix content of the peptide or on the position of Tyr in the sequence is not known, although the latter contribution is partially accounted for in our peptides by keeping the position of Tyr in the sequence constant. Consequently, it has not been possible to correct for this aromatic contribution to the $[\Theta]_{222}$ values and the helix contents reported for the peptides in this paper are approximate.

Analysis of helix contents using modified Lifson–Roig helix-coil theory

The Lifson–Roig theory (Lifson & Roig, 1961) modified to include end-capping effects (Doig *et al*., 1994) was used to analyze the measured fractional helicities. This modified theory describes the fractional helicity of a peptide by five parameters: N, the peptide chain length; w, the helix propagation; v, the helix nucleation; n and c, the N and C-terminal capping. w, v, n and c are unique for each amino acid, being measures of the propensities for helix propagation, nucleation, and N and C-terminal capping and independent of sequence. (w and v are related to the Zimm–Bragg parameters s and σ (Zimm &

Bragg, 1959) as: $s = w/(1 + v)$ and $\sigma = v^2/(1 + v)^4$: Qian & Schellman, 1992). We assume $v = 0.048$ and $c = 1$ for all residues, $w = 1.61$ for Ala, and $n = 1$, 0.4 and 9.7 for Ala, Lys and the acetyl group, respectively, as reported (Chakrabartty *et al*., 1994). We also assume, for the calculations in this paper, the same low N-cap propensity, $n = 0.4$, for all the basic amino acids used in the present study, since basic amino acids which can only serve as hydrogen bond donors are statistically unfavorable at N-cap positions, in contrast to hydrogen bond acceptors (Presta & Rose, 1988; Richardson & Richardson, 1988). Since the C-cap propensities of various amino acids do not appear to vary significantly, they were all set to one (Chakrabartty *et al*., 1994). The fractional helicities of the peptides indicated were simultaneously fitted to the modified Lifson–Roig model using a least-squares procedure based on a binary search algorithm on a Silicon Graphics Personal Iris computer. The w-values reported for the basic amino acids in this study are apparent values, since charge interactions including those of the helix dipole (Scholtz *et al*., 1993) have not been modelled in our analysis and it was not possible to make corrections for the aromatic contribution of the N-terminal Tyr to the CD signal (Chakrabartty *et al*., 1993).

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