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COMMUNICATIONS

# Straight-chain Non-polar Amino Acids Are Good Helix-formers in Water

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For comparison with earlier data on naturally occurring non-polar amino acids (Ala, Leu, Phe, Val, Ile), the comparative helix-forming tendencies have been measured for non-polar amino acid residues that have unbranched side-chains, with an ethyl, propyl or butyl group, and also for methionine. The substitutions are made in a 17-residue alanine-based peptide. The results show that straight-chain non-polar amino acids have high helix-forming tendencies compared to $\beta$-branched non-polar amino acids. Restriction of side-chain conformations in the helix, with a corresponding reduction in conformational entropy, is the likely explanation. There is a small increase in helix-forming tendency as the side-chain increases in length from ethyl to butyl, which suggests that a helix-stabilizing hydrophobic interaction is being detected.

*Keywords:* $\alpha$-helix propensity; non-polar side-chains

We reported that $\beta$-branching (in Val and Ile) or $\beta$-substitution with a bulky group (in Phe) causes a non-polar amino acid to be helix destabilizing in water (Padmanabhan *et al.*, 1990), in agreement with previous studies of helix formation by homopolymers in organic solvents (Blout, 1962). On the other hand Ala, which has the smallest side-chain except for Gly, and Leu, which is not branched at $C^{\beta}$, are good helix-formers in water. These results are based on substituting Ala by specific non-polar amino acids in a 17-residue (Ala, Lys) peptide that forms a monomeric $\alpha$-helix in water (Padmanabhan *et al.*, 1990). The sequences of the peptides studied have been designed to avoid helix stabilization by specific interactions such as salt bridges.

In contrast, host–guest studies of random copolymers (Sueki *et al.*, 1984; Wojcik *et al.*, 1990) find that $\beta$-branching or $\beta$-substitution is not important in determining $\alpha$-helix formation in water, since Ile has a higher helix propensity than Ala or Leu, while the values for Phe, Leu and Ala are similar. The parameters of the helix–random coil transition are measured in host–guest experiments (Sueki *et al.*, 1984; Wojcik *et al.*, 1990), and the helix propensity is identified with $s$, the helix propagation parameter as defined in the Zimm–Bragg theory of helix–coil transitions (Zimm & Bragg, 1959). The host amino acid used in host–guest experiments is helix-forming, while being water-soluble but non-ionizing, hydroxybutyl or hydroxypropyl-L-glutamine. Side-chain interactions involving the host hydroxybutyl or hydroxypropyl-L-glutamine residues may stabilize the host helix and influence the results of host–guest experiments; this effect may explain the contradiction between the host–guest results and the studies of short (Ala, Lys) peptides (Marqusee *et al.*, 1989). Substitution experiments in other short, helix-forming peptides (Merutka *et al.*, 1990; Lyu *et al.*, 1990) and in a dimeric coiled-coil helix (O'Neil & DeGrado, 1990) yield scales of relative helix propensity for non-polar amino acids that are similar to the one we reported (Padmanabhan *et al.*, 1990). The explanation suggested for our results is that the $\alpha$-helix backbone is intrinsically stable in water for amino acid residues with a $\beta CH_2$ (or $\beta CH_3$) side-chain (Marqusee *et al.*, 1989; Scholtz *et al.*, 1991), but that the side-chain conformations of $\beta$-branched or $\beta$-substituted amino acids are restricted in the helix, so that the side-chain conformational entropy is reduced in the helix, compared to the random coil (Padmanabhan *et al.*, 1990).

Helix substitution experiments are reported here for non-polar, straight-chain amino acids that contain an ethyl, propyl or butyl side-chain. These



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**Table 1**

*Helix-forming tendencies of non-polar amino acids*

| Peptide | Sequence† | $-[\theta]_{222}$‡ | Side-chain |
|---|---|---|---|
| 3Ala§ | Ac-YKAA **A** AKAA **A** AKAA **A** AK-$NH_2$ | 25,800 | $-CH_3$ |
| 3Val§ | Ac-YKAA **V** AKAA **V** AKAA **V** AK-$NH_2$ | 5600 | $-CH(CH_3)_2$ |
| 3Leu§ | Ac-YKAA **L** AKAA **L** AKAA **L** AK-$NH_2$ | 26,300 | $-CH_2CH(CH_3)_2$ |
| 3Phe§ | Ac-YKAA **F** AKAA **F** AKAA **F** AK-$NH_2$ | 7600 | $-CH_{2\phi}$ |
| 3Ile§ | Ac-YKAA **I** AKAA **I** AKAA **I** AK-$NH_2$ | 13,400 | $-CH(CH_3)CH_2CH_3$ |
| 3Met | Ac-YKAA**M**AKAA**M**AKAA**M**AK-$NH_2$ | 20,800 | $-CH_2CH_2SCH_3$ |
| 3Abu‖ | Ac-YKAA**aB**AKAA**aB**AKAA**aB**AK-$NH_2$ | 20,300 | $-CH_2CH_3$ |
| 3Nval‖ | Ac-YKAA**nV**AKAA**nV**AKAA**nV**AK-$NH_2$ | 26,100 | $-CH_2CH_2CH_3$ |
| 3Nle‖ | Ac-YKAA**nL**AKAA**nL**AKAA**nL**AK-$NH_2$ | 28,200 | $-CH_2CH_2CH_2CH_3$ |

† All peptides have an N-terminal tyrosine residue and are acetylated at their N-terminal ends and amidated at their C-terminal ends. Stock peptide concentrations were determined by measuring tyrosine absorbance in 6 M-gluanidium ($\varepsilon_{275} = 1450\ M^{-1}\ cm^{-1}$; Brandts & Kaplan, 1973).

‡ The mean residue ellipticity at 222 nm in units of deg. $cm^2\ dmol^{-1}$, measured at 0°C, pH 7·0, in 1 M-NaCl and 1 mM each of sodium citrate, sodium phosphate, sodium borate at peptide concentrations of 15–20 μM, in a 1 cm pathlength cuvette. The value of $-[\theta]_{222}$ for 100% helix has been estimated at 33,000 from titration with trifluoroethanol (Padmanabhan *et al.*, 1990), but a somewhat higher value is likely from studies of the chain-length dependence of $-[\theta]_{222}$ for the helix (G. Merutka, W. Shalongo & E. Stellwagen, unpublished results; Scholtz *et al.*, 1991). Thermal unfolding curves were measured at 2 concentrations that differ 5-fold and were found to be superimposable, indicating that the peptides were monomeric (cf. Marqusee *et al.*, 1989). The contribution of the phenylalanine side-chain to $[\theta]_{222}$, as measured in free phenylalanine, is negligible in the peptide 3Phe.

§ From Padmanabhan *et al.* (1990).

‖ aB, α-amino-*n*-butyric acid; nV, norvaline; nL, norleucine.

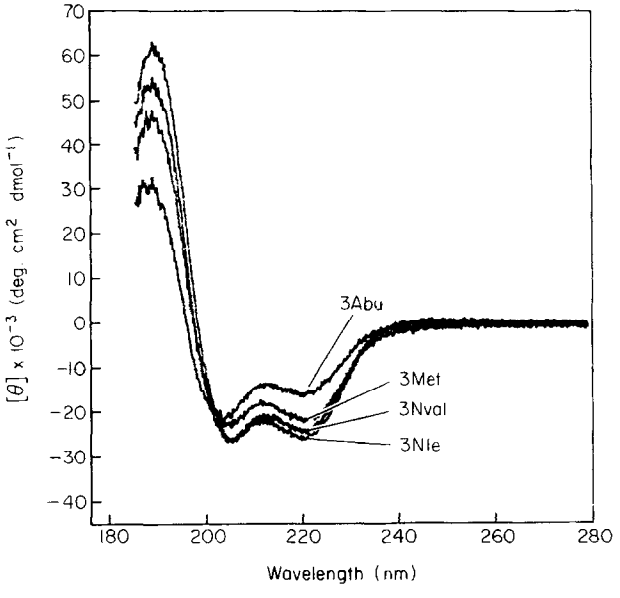


**Figure 1.** c.d. spectra of peptides at 0°C, pH 7·0, in 0·1 M-KF, 1 mM-potassium phosphate, at peptide concentrations of 40 to 50 μM in a 1 μM path length cuvette. Peptides were synthesized on a Milligen 9050 automated synthesizer, using pentafluorophenyl esters of Fmoc (9-fluorenylmethoxycarbonyl) amino acids from Milligen, as described (Padmanabhan *et al.*, 1990; Marqusee *et al.*, 1989). Peptide purification procedures have been described (Padmanabhan *et al.*, 1990; Marqusee *et al.*, 1989). Purification was achieved by fast protein liquid chromatography (f.p.l.c.) reverse phase chromatography on a $C_{18}$ resin, using a gradient of 10% to 40% acetonitrile in 0·1% trifluoroacetic acid; after purification, each peptide gave a single peak on f.p.l.c. chromatography. The pentafluorophenyl ester of Fmoc-L-norleucine was available from Milligen, and the synthesis of peptide 3Nle followed the previous procedure. Peptides 3Abu and 3Nval were synthesized from the free Fmoc amino acids activated by HOBT (Sigma) and by BOP (Biosearch Research Chemicals). (HOBT, 1-hydroxybenzotriazole; BOP, benzotriazolyltris(dimethylamino)phosphonium hexafluoro-phosphate.) Fmoc-α-amino-*n*-butyric acid was synthesized by Peninsula Labs and Fmoc-L-norvaline was prepared by the procedure of Stewart & Young (1984), and checked by nuclear magnetic resonance. Peptide 3Met was cleaved from the resin under nitrogen in the presence of methionine as a scavenger, and the peptide was treated with 1 M-dithiothreitol at 37°C for 3 days to reduce any sulfoxide (Houghton & Li, 1979) before purification. 3Met samples were checked for sulfoxide by f.p.l.c. before each experiment. The primary-ion molecular weight of each peptide was determined by fast atom bombardment mass spectrometry and agreed with the predicted value within 0·1 dalton. c.d. spectra were recorded on an AVIV 60DS instrument as described (Padmanabhan *et al.*, 1990).

correspond to the amino acids α-amino-*n*-butyric acid (Abu†), norvaline or 2-amino-pentanoic acid (Nval), and norleucine or 2-amino-hexanoic acid (Nle). The amino acid methionine has also been studied. The results are shown in Table 1, together with the peptide sequences. Helix content is measured by circular dichroism (c.d.) in 1 M-NaCl, to minimize electrostatic repulsion between $Lys^+$ residues. c.d. spectra of the peptides in 0·1 M-KF are given in Figure 1. The spectra show the two minima at 222 and 207 nm and the maximum at about 190 nm characteristic of mixtures of α-helix and random coil (Holzwarth & Doty, 1965). The results shown in Figure 1 differ slightly from those of

† Abbreviations used: Abu, α-amino-*n*-butyric acid; Nval, norvaline (2-amino-pentanoic acid); Nle, norleucine (2-amino-hexanoic acid); c.d., circular dichroism.

Table 1 because of the different salt (KF) and peptide concentration (0·1 M) used in obtaining c.d. spectra (0·1 M-KF). Table 1 shows that all the amino acid residues with a $-CH_2CH_2R$ side-chain, including Met, are significantly better helix-formers in this peptide system than are Val, Ile and Phe. A slight but interesting dependence of helix-stabilizing effect on chain length can be seen in Table 1. First, the helix context of 3Abu (ethyl) drops below that of 3Ala (methyl). Presumably, this results from some reduction in the conformational entropy of the ethyl side-chain in the helix as compared to the random coil. Then the helix content rises again for 3Nval (propyl) and 3Nle (butyl). This suggests that some type of helix-stabilizing interaction, probably a hydrophobic interaction, can be detected with the longer side-chains, even though most of the residues in the helix are Ala. The substituted side-chains cannot interact with themselves in the helix, as they are separated by five residues.

In summary, the nature of the substitution at the $C^{\beta}$ atom appears to be the major determinant of the relative helix-forming tendencies of non-polar amino acid residues. For straight-chain amino acid residues, the helix-forming tendency shows a small increase with increasing length of the side-chain, possibly caused by hydrophobic interactions. Our data also suggest that the high helix-forming tendency of alanine results from minimal restriction of its side-chain rotamer conformations.

It is important now to quantify these substitution experiments by measuring values of $s$, the helix propagation parameter, for Ala and for the substituted amino acids, and also to test the proposal that the properties of the host helix explain the difference between the results found in this system and the host–guest results (Marqusee *et al.*, 1989).

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