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# C-terminal helix capping propensities in a polyalanine context for amino acids bearing nonpolar aliphatic side chains

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**Abstract**—Relative C-capping propensities for nonpolar amino acids and the primary amide, which control helicity for many small peptides, have been determined by a new method. Practical consequences of the observed propensities and their temperature dependences are discussed. © 2001 Elsevier Science Ltd. All rights reserved.

Introduced as new concepts thirteen years ago by Presta and Rose,[1] the helicity enhancing effects of peptide N and C-caps are well-documented. The mechanisms of helicity enhancement are understood for caps that carry charges. N or C-terminal amino acids or other functions bearing charged side chains interact electrostatically with N or C-terminal dipoles created by the oriented amide residues of the helix backbone.[2] A cationic side chain stabilizes a helix if sited at a C-terminus, an anionic side chain stabilizes if sited at a N-terminus. A variety of caps that bear uncharged, polar side chain functions can also strongly stabilize helices. These include the natural amino acids asparagine, serine, and threonine, known in globular proteins to form side-chain-to-main-chain hydrogen bonds, as well as acyl caps bearing polar H-bond acceptors such as sulfones and sulfoxides.[3] These functions may H-bond and stabilize the first or last three helical backbone amide functions of a helical conformation. As noted in Fig. 1, owing to their positions at helix termini these amides lack a full complement of intrahelical hydrogen bonds.

*N*-glycyl, *N*-acetyl and *C*-$NH_2$ caps generate large helix stabilizations that are less well understood. The N-acetyl cap increases fractional helicities of medium-sized peptides by around 70%, and similar increases are observed if an N-terminal amino acid residue is replaced by glycyl.[4] How do such simple functionalities act to enhance helicity? As developed later in this report, they appear to lack features that are common to most amino acids and that reduce the efficiency of amide caps. The structural simplicity of Ac, $NH_2$, and Gly appears to be responsible for their capping efficiencies.

In 1995 Doig and Baldwin proposed a set of relative helix-capping parameters for the natural amino acids. These were calculated largely from CD-derived helicity changes for X-site substitutions in two generic model peptides: H-X-$AKA_4KA_4KA_2$GY-$NH_2$ for N-caps and

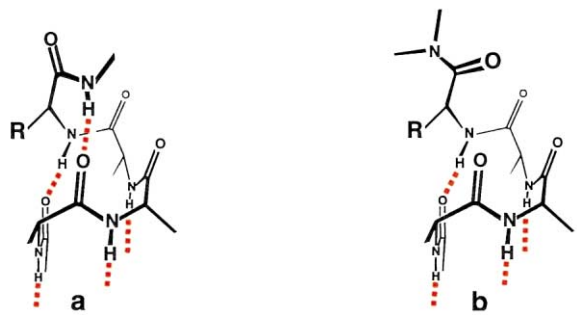


**Figure 1.** Representations of the C-terminus of a polypeptide α-helix that terminates at or before a residue X with side chain R. Hydrogen bonds are shown as red dotted lines. Each C-terminus has three carbonyl oxygens that lack intramolecular H-bonds and presumably interact with water molecules. (a) Residue X (side chain R) assumes a conformation that allows it to form a C-terminal H-bond with the helix, lengthening it by one residue. If the C-terminal secondary amide is replaced by a tertiary amide, this conformation is not populated. (b) Residue X (side chain R) assumes a conformation in which it cannot lengthen the helix and instead serves as its C-cap. C-terminal tertiary amides allow this and similar conformation to be populated.

*Keywords*: α-helix; helix capping; circular dichroism; peptides.

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Ac-YAKA$_4$KA$_4$KA$_2$G-X-NH$_2$ for C-caps.[5] In addition to the expected capping efficiencies of Ac, Gly, and NH$_2$, a significant variation of relative C-capping propensities was noted for the amino acids that bear simple alkyl side chains. However, for these and for other hydrophobic amino acid residues, the authors noted that their Lifson–Roig-derived analyses frequently assigned meaningless negative capping propensities. A useful perspective would be provided by C-capping data obtained from other contexts. For practical reasons, the capping residues of both test peptides used by Doig et al. were sited at ($i$, $i$±2) separations from lysine residues, which may introduce a context bias. Moreover, the test peptides were not terminated by amide functions but by free amines or carboxylic acid functions. These may not provide optimal models for through-bond inductive effects or through-space electronic effects of α-amides.

As a general context for C-capping studies we sought a polyalanine-derived model peptide that maintains a polyamide backbone sequence throughout and adapted our recently introduced spaced, solubilized polyalanines.[6] We selected the model sequence WK$_4$In$_2$'LG-Hel-A$_8$-NH$_2$ for the primary C-terminal amide and the sequences WK$_4$Inp$_2$'LG-Hel-A$_8$-X-Inp-NH$_2$ for candidate amino acids X. (In these sequences, Hel is our previously characterized strongly helix-stabilizing N-terminal cap[7], Inp is 4-carboxypiperidine, an achiral proline analogue, and 'L is *tert*-leucine). For these sequences the N-terminal region provides a UV reporter, W; solubilizers, K$_4$, and an isolation element, In$_2$'L. The C-capping test region of these peptides is G-Hel-A$_8$-X-Inp, and its helicity is taken as proportional to $-[\theta]_{222}$.[6]

The choice of a tertiary carboxamide at the X-Inp junction requires comment. An amino acid may assume three roles within a partially helical peptide sequence. It may appear within either helical or nonhelical regions; or it may act as a helix cap, defining a boundary between these regions. It can only define a C-cap boundary if its carboxamide NH residue fails to participate in helical H-bonding, or equivalently, if as seen in Fig. 1 its $\phi$ and $\psi$ dihedral angles assume nonhelical values. A tertiary amide lacks the NH required for participation, and moreover the steric bulk of its two alkyl groups forces $\phi$ to assume positive values, which lie outside the helical range but allow the other major peptide conformations.[8]

As a working hypothesis we regard the helix C-capping propensity of the boundary residue X to be largely determined by the H-bond donor capacity of its α-NH. This donor capacity is expected to vary with changes in C-region solvation. It also should increase slightly if neighboring electronegative atoms are present, and it should decrease significantly if neighboring strong H-bonding acceptors compete with the helix C-terminus for its affinity. Following suggestive modeling results of Jorgensen et al. for nucleotide and imide H-bond formation,[9] we view the conformation at the α-carbon of X as largely defining the competition of neighboring H-bonding donors for its α-NH. This competition is represented schematically by the two-state equilibrium of Fig. 2b,c. Fig. 2b represents a group of $\phi$, $\psi$ conformations at the α-carbon of X for which its CO and α-NH interact minimally. For these, the intrinsic H-bonding donor capacity of the α-NH is not attenuated and the helix is maximally stabilized. Fig. 2c represents a group of conformations of X for which the distance between O and H of the CO and α-NH groups is short, and stabilizing electrostatic effects compete with and attenuate the helix H-bonding affinity of the α-NH. This model predicts that the mole fraction of C$_5$ and related extended conformations of residue X will correlate inversely with its helical C-capping propensity. Calculations of conformational energetics for alanine peptides identify C$_5$ and PII conformations as populated,[10] although a decrease of population with temperature is suggested by CD spectra for simple unordered peptides.[11] The degree of hydration of the C-terminal helix region should also decrease with temperature. Taken together these effects suggest that residues X

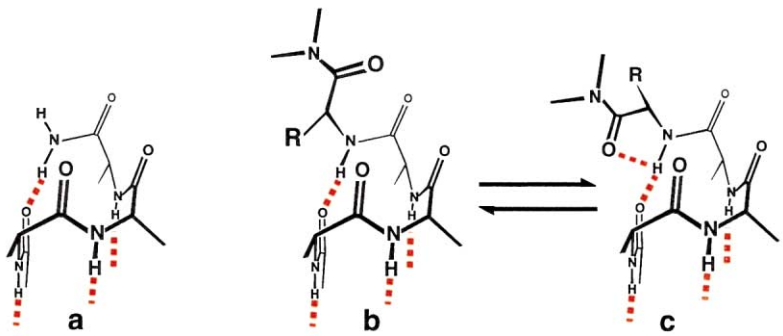


**Figure 2.** C-capping functions for a peptide α-helix. (a) The peptide is terminated by a primary amide. Lack of crowding is expected to facilitate hydration of this peptide C-terminus. (b) This conformation of the amino acid X that C-caps the helix positions its side chain R close to the amide NH of the residue, which is expected to have minimal electrostatic or H-bonding interactions with solvent or amide carbonyls, maximizing its donor H-bonding affinity within the helix. (c) This C$_5$ conformation of the amino acid X that C-caps the helix positions its amide carbonyl oxygen in proximity to its NH, minimizing its donor H-bonding affinity within the helix. The equilibrium between conformations of types b and c is expected to depend on the nature of side chain R and should shift to the left for R = H, and to the right for R = *tert*-butyl.

that bear rigid, bulky alkyl side chains that are biased toward extended conformations should show the smallest and least temperature dependent C-capping propensities. Owing to its unique conformational permissiveness, glycine is expected to favor the helix-stabilizing conformation Fig. 2b, and among the amino acids, it should show the largest helical C-capping propensity. The simple primary amide C-cap, Fig. 1a, should also show a large C-capping propensity, but since it is also expected to be unusually hydrated, predicting its temperature dependence is difficult.

Table 1 reports experimental values for $[\theta]_{222}$ at three temperatures for the primary amide and for the six C capping amino acids Gly, Ala, Leu, Val, Ile, and $^t$Leu. The 2°C ellipticites for Ala, Leu, Val and Ile are essentially identical. Unlike the result of Doig et al., this implies that relative to Ala the C-capping propensities of Leu, Val, and Ile are all 1.0; the natural amino acids with alkyl side chains are equally efficient as C-caps. Relative to Ala the value for Gly is ca. 10% higher and that for $^t$Leu is ca. 10% lower. From the standpoint of the model advanced in Fig. 2, these represent the conformational extremes among α-amino acids, and the direction of the effect that is observed is completely consistent with that model. By contrast the ellipticity for the primary amide is 30% larger than that for alanine, implying a strong C-capping propensity for this function. From our observations with other peptide pairs, this effect seems to be general.[13]

Striking temperature effects are seen for the ellipticity values of Table 1. As predicted, Val and $^t$Leu, the derivatives with the least degree of conformational freedom for the alkyl side chain and the strongest biases toward extended backbone conformations exhibit very small temperature dependences. The ellipticity for the primary amide is exceptionally temperature dependent; as a result, for the examples of Table 1 this functionality shows the largest relative helical C-capping propensity at 2°C and the smallest at 60°C. In the context of our model assumptions, this result is best explained by a large temperature dependence for the hydration state of this function. This result has an important practical implication. Temperature dependent helicity changes for peptide models that incorporate primary amide C-caps may be highly atypical and unrepresentative of temperature effects expected for peptides of biological origin.

**Table 1.** Values of $[\theta]_{222,res}$[a] in water for $WK_4In_2{}^tLG\text{-}Hel\text{-}A_8\text{-}(C\text{-}cap)$[12]

| C-cap | $[\theta]_{222,res}$ 2°C | $[\theta]_{222,res}$ 25°C | $[\theta]_{222,res}$ 60°C | %[b] |
|---|---|---|---|---|
| $NH_2$ | −19.9 | −14.6 | −7.9 | 60 |
| Gly-Inp | −16.7 | −13.6 | −12.2 | 27 |
| Ala-Inp | −15.2 | −12.6 | −11.2 | 26 |
| Leu-Inp | −15.2 | −12.4 | −11.2 | 26 |
| Val-Inp | −14.8 | −12.9 | −12.9 | 13 |
| Ile-Inp | −15.5 | −13.5 | −12.1 | 22 |
| $^t$Leu-Inp | −13.9 | −12.7 | −12.8 | 9 |

[a] Per residue ellipticities in (deg cm$^2$ dmol$^{-1}$)×10$^{-3}$.
[b] % Change from $[\theta]_{222,res}$ 2°C→$[\theta]_{222,res}$ 60°C.

In this first study we have introduced a new principle in the form of a general context for deriving relative helical C-capping propensities from ellipticity data. Application of our approach to a larger class of amino acids, extension to helical N-capping propensities, and Lifson–Roig modeling to calculate numerical values for the relative helical capping propensities are all in process and will be reported subsequently.

## Acknowledgements

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12. Peptides were synthesized, characterized and subjected to CD-analysis as previously reported.[6] Satisfactory EI-mass-spectrometry data were obtained for all compounds.
13. We made similar observations with $AcHel(A_4K)_4A_2\text{-}{}^tLInp_2W$ and $AcHel(A_4K)_4A_2\text{-}NH_2$.