J|A|C|S
COMMUNICATIONS

Published on Web 04/17/2002

An Unnatural Amino Acid that Induces β-Sheet Folding and Interaction in Peptides

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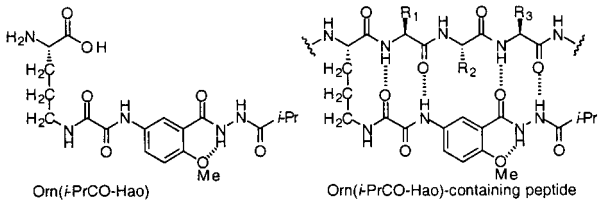
Received January 24, 2002

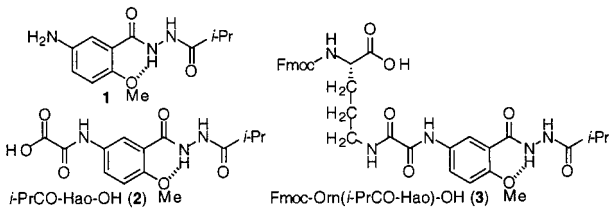
Peptides generally do not participate in the sort of strong and specific β-sheet interactions that occur widely among proteins.[1] While β-sheet formation between proteins is often involved in exquisite protein−protein interactions, β-sheet formation between peptides is generally associated with the formation of insoluble aggregates of ill-defined structure. Although a variety of elegant strategies to coax peptides to fold into well-defined β-sheetlike structures have now been developed, few strategies for achieving strong and specific β-sheet interactions between peptides have been reported.[2,3] Our (J.S.N.) research group has recently established that compounds that mimic the structures of protein β-sheets can dimerize in a highly specific fashion and has shown the facility with which amino acid building blocks that mimic the hydrogen-bonding functionality of a peptide β-strand can be used to generate peptides that dimerize by β-sheet formation.[4,5] Here, we introduce a unique amino acid that can readily be incorporated into peptides to make them fold into β-sheetlike structures that dimerize through β-sheet interactions.

This new amino acid, Orn(*i*-PrCO-Hao), consists of an ornithine residue with the β-strand-mimicking amino acid Hao[5] attached to its side chain. When Orn(*i*-PrCO-Hao) is incorporated into a peptide, or appended to its *N*-terminus, the Hao group hydrogen bonds to the three subsequent residues to form a β-sheetlike structure. The $CH_2CH_2CH_2NH$ side-chain of the ornithine residue allows the Hao oxalamide carbonyl group to form a hydrogen-bonded ten-membered ring with the amino group of the subsequent residue, like a β-turn in a β-hairpin. Chart 1 illustrates the structure of Orn(*i*-PrCO-Hao) and that of a peptide containing it.

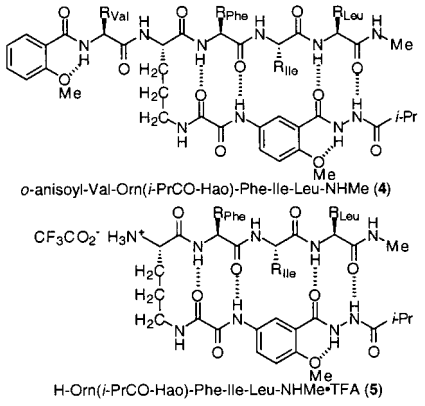
Chart 1



The amino acid Orn(*i*-PrCO-Hao) is readily used in peptide synthesis as its Fmoc derivative, which is conveniently prepared from hydrazide **1**.[6] Condensation of hydrazide **1** with ethyl oxalyl chloride, followed by hydrolysis of the ethyl ester group with NaOH and passage of the reaction mixture through acidic ion-exchange resin, yields *i*-PrCO-Hao-OH (**2**). Coupling of *i*-PrCO-Hao-OH and Boc-Orn-O-*t*-Bu with EDC and HOBt, followed by cleavage of the Boc and *tert*-butyl ester groups with TFA and Fmoc protection with Fmoc-OSu, yields Fmoc-Orn(*i*-PrCO-Hao)-OH (**3**).



Fmoc-Orn(*i*-PrCO-Hao)-OH behaves like a regular amino acid in peptide synthesis and was uneventfully incorporated into the peptide *o*-anisoyl-Val-Orn(*i*-PrCO-Hao)-Phe-Ile-Leu-NHMe (**4**) through standard automated Fmoc solid-phase peptide synthesis using PS-PEG-indole-NHMe[7] resin, with DIC and HOAt[8] as the coupling agent for Fmoc-Orn(*i*-PrCO-Hao)-OH and *o*-anisic acid.[9] A second synthetic strategy was developed to facilitate the preparation of peptides with *N*-terminal Orn(*i*-PrCO-Hao) residues, which avoids the need for the preparation of Fmoc-Orn(*i*-PrCO-Hao)-OH. In this strategy, Boc-Orn(Fmoc)-OH is used as the penultimate amino acid in the peptide synthesis, and *i*-PrCO-Hao-OH (**2**) is used as the final amino acid. *N*-Terminal Orn(*i*-PrCO-Hao) peptide H-Orn(*i*-PrCO-Hao)-Phe-Ile-Leu-NHMe•TFA (**5**) was prepared in a fashion similar to that of **4**, using DIC and HOAt[8] as the coupling agent for *i*-PrCO-Hao-OH.



^{1}H NMR spectroscopic studies establish that peptide **4** forms a dimeric β-sheetlike structure in $CDCl_3$ solution. Most notably, ^{1}H NMR transverse-ROESY (Tr-ROESY)[10] studies in $CDCl_3$ solution (3.9 mM, 298 K) show five prominent NOEs between the *i*-PrCO-Hao unit and the Phe, Ile, Leu, and NHMe groups of the peptide strand indicative of a folded β-sheetlike structure and three prominent NOEs between the *N*- and *C*-terminal groups of the peptide (*o*-anisoyl-Val and Leu-NHMe) consistent with a dimeric structure. Chart 2 illustrates the structure of the dimer and shows these NOEs. Particularly compelling among these data is a strong

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10.1021/ja025699i CCC: $22.00

Chart 2

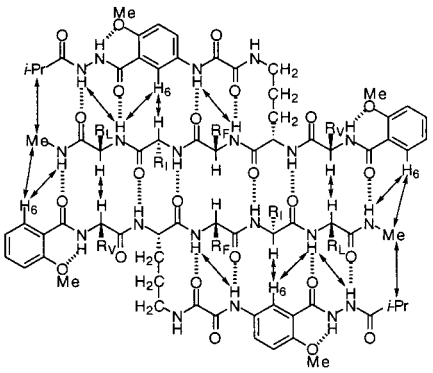
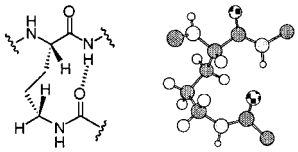


Chart 3



interstrand NOE between the proton at the 6-position of the aromatic group of Hao and the Ile α-proton and a strong intersheet NOE between the Val and Leu α-protons.

The $^3J_{HN\alpha}$ coupling constants of the peptide strand of peptide **4** are large (8.8−9.8 Hz), and the interresidue NOEs between the NH and α-protons of the amino acids are strong, reflecting a β-sheetlike conformation of the peptide strand.[11−13] Also consistent with β-sheetlike structure, the chemical shifts of the α-protons of the amino acids in **4** are shifted 0.71−1.54 ppm downfield of values typical of random coil conformations.[14] Consistent with the hydrogen bonding associated with folding and dimerization in chloroform solution, the NH groups of the peptide strand appear at 8.3−9.4 ppm, which is substantially downfield of the characteristic chemical shift of peptide NH groups that are not hydrogen-bonded (ca. 6 ppm). Also consistent with a folded structure in which the Ile side-chain of the peptide is close to the aromatic ring of the Hao unit, the Ile δ- and γ-methyl groups appear unusually upfield at 0.54 and 0.68 ppm, and one of the Ile γ-methylene protons appears unusually upfield at 0.75 ppm, while the other appears at 1.23 ppm.

The ^{1}H NMR studies also suggest that the ornithine unit adopts a well-defined turn conformation. Particularly prominent in the Tr-ROESY spectrum of **4** is a *very strong* NOE between the α-proton of Orn and one of its two diastereotopic δ-protons. This proton appears dramatically downfield of the other diastereotopic δ-proton (4.39 ppm vs 3.09), indicating that these two protons reside in very different environments. No NOE is seen between the α-proton of Orn and the other diastereotopic δ-proton, confirming the difference in environments. These two protons also exhibit widely different coupling patterns, with the downfield δ-proton resonance resembling a quartet or a triplet of doublets with three large (10−12 Hz) coupling constants, and the upfield δ-proton resembling a broad doublet with one large coupling constant. Molecular modeling gives a turn structure consistent with these NOE data and shift data, in which the *pro-S* δ-proton is in contact with the Orn α-proton and is shifted downfield by proximity to the adjacent carbonyl group of the Hao group and by proximity to the carbonyl of the adjacent Val residue. Chart 3 illustrates this turn structure.

Analogous ^{1}H NMR studies of peptide **5** indicate that this TFA salt folds but does not dimerize in CD_3OD solution. Most notably, Tr-ROESY studies in CD_3OD solution (10.1 mM, 298 K) show an NOE between proton at the 6-position of the aromatic group of Hao and the Ile α-proton, indicating folding. NOEs between the Phe and Leu α-protons, which would be expected in the antiparallel β-sheet dimer are absent. NOEs between the *i*-PrCO and MeN protons cannot be detected, due to overlap of the *i*-PrCO methine and MeN resonances, but are detected in 1:1 CD_3OD-$CDCl_3$, in which these resonances do not overlap. Consistent with proximity between the Ile side-chain and the aromatic ring of the Hao unit, the Ile δ- and γ-methyl groups appear upfield at 0.70 and 0.74 ppm, respectively, and one of the Ile γ-methylene protons appears upfield at 0.85 ppm, while the other appears at 1.37 ppm. That these NOEs are weaker than those of **4** and the upfield shifting is more modest suggests that the extent of folding of **5** in CD_3OD is somewhat less than that of **4** in $CDCl_3$. Consistent with a less well-defined ornithine turn structure, the diastereotopic δ-protons of **5** in CD_3OD are less separated than those of **4** in $CDCl_3$, appearing at 3.64 and 3.22 ppm.

Collectively, these synthetic and spectroscopic studies establish that the amino acid Orn(*i*-PrCO-Hao) induces β-sheet structure and interactions in peptides in suitable organic solvents. Unlike our Hao amino acid, which acts as a *prosthetic* to replace three residues of the peptide strand, the Orn(*i*-PrCO-Hao) amino acid acts as a *splint* that helps enforce a β-sheetlike structure without replacing the residues and their side chains. This feature of Orn(*i*-PrCO-Hao) is important, because it allows the creation of β-sheet structure with minimal perturbation of the peptide sequence. Another important feature, which has allowed us to screen and analyze one-bead-one-compound combinatorial libraries of peptides containing Orn(*i*-PrCO-Hao) for biological activity, is that Orn(*i*-PrCO-Hao) behaves like a regular α-amino acid in Edman sequencing.[15] We will describe this work in due course.

Acknowledgment. We thank the NIH and NSF for grant support (GM-49076 and CHE-9813105).

Supporting Information Available: Synthetic procedures and spectral data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA025699I