Supplementary Material Available: Tables of crystal and intensity collection data, atomic coordinates, bond distances and angles, and anisotropic displacement parameters and an ORTEP drawing for $W(PMe_3)_6$ (4 pages); listing of observed and calculated structure factors (2 pages). Ordering information is given on any current masthead page.

Observation of a Cis Amide Isomer within a Linear Peptide

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Recently we initiated an examination of the conformational preferences of bombolitins, a series of peptides isolated from bumble bee venom.[1] Bombolitins exhibit biological profiles within membrane environments similar to those of mastoparan,[2] melittin,[3] and crabrolin,[4] in spite of the low sequence homology. All of these peptides are related in the amphiphilic nature of the α-helices that they can form. In order to investigate the structures and determine a possible mechanism for activity, we are studying the peptides in the presence of SDS micelles as a mimic for a biological membrane.[5,6] During the NMR investigation of one of the bombolitins, bombolitin I, we observed the presence of a cis amide bond between the first and second residues, Ile[1] and Lys[2]. To our knowledge this is the first report of a cis amide within a linear peptide without a proline.[7]

Bombolitin I is a heptadecapeptide with the following sequence:

I-K-I-T-T-M-L-A-K-L-G-K-V-L-A-H-V-NH_2

The proton NMR resonances of the peptide were assigned from phase-sensitive COSY, DQ correlated spectroscopy, and HOHAHA experiments.[8,9] The sequential assignment of the residues was achieved by NOESY spectra.[10]

In aqueous solution bombolitin I is random; all of the NOEs are indicative of conformational averaging. This is also borne out with circular dichroism studies.[6] The nominal backbone NOEs observed throughout the peptide indicate that all of the amide linkages are in a trans arrangement. In contrast, in the presence of SDS above the critical micellar concentration (CMC), the peptide adopts a well-defined α-helix. Characteristic NOEs [e.g., NH(i) → NH(i+1) and C$^\alpha$H(i) → C$^\beta$H(i+3)][11] are quite conclusive; a long stretch of helix extending from residue 2 to at least residue 14 is present. This finding is in agreement with CD results.[6]

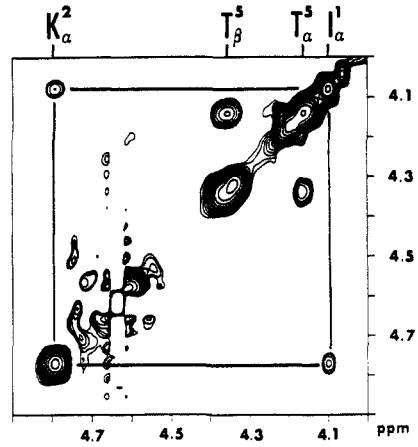

Figure 1. The aliphatic region of a pure-phase absorption NOESY spectrum (mixing time 200 ms) at 400 MHz of bombolitin I (2.6 mM with 320 mM SDS-d_{25}) in ^2H_2O. The NOE demonstrating the cis ω-bond between Ile[1] and Lys[2] [C$^\alpha$H(1) → C$^\alpha$H(2)] is illustrated. The other connectivity present arises from an intraresidue interaction.

In addition to the characteristic NOEs, there is one observation that is highly unusual. The strong Ile[1] C$^\alpha$H → Lys[2] C$^\alpha$H NOE demonstrates a cis arrangement about the Ile[1]–Lys[2] amide bond.[12] This result is illustrated in Figure 1. The two diagnostic peaks for a cis amide bond are C$^\alpha$H(i) → C$^\alpha$H(i+1) and NH(i) → C$^\alpha$H(i+1).[11,12] Only the former is observed here due to fast exchange of the free amino group of Ile[1].

The proton NMR spectrum of bombolitin I in the presence of SDS exhibits a single set of resonances for each amino acid. No trace of the trans amide isomer was detected. Considering also the CD results that indicate that the peptide is completely bound to micelles,[6] we conclude that a single conformation is assumed by bombolitin I upon association with SDS.

The cis ω-bond must arise from the association of the peptide with the micelles and the amphiphilic nature of the α-helix formed within this environment. Although our results do not allow for insight into the interaction of the peptide with the micelles, a favorable array would have the α-helix on the surface of the micelle with the hydrophobic face directed into the surfactant and the hydrophilic face into the aqueous solution. Only with a cis arrangement can the side chains of isoleucine and lysine point toward opposite sides with Ile[1] interacting with the hydrocarbons of the micelles and Lys[2] with the water.

This study clearly indicates that the membrane can have a dramatic effect on the conformation of a peptide and underlines the importance of examining peptides within environments similar to the conditions wherein they exhibit activity. Peptide–membrane interactions can be energetically more important than the steric constraints usually associated with the cis arrangement (i.e., cis 2–3 kcal/mol higher in energy than trans). This result supports the role of membranes as mediators in the interactions of peptides with their receptors.

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