Abstract

Experimentally derived biomolecular structures were determined by Nuclear Magnetic Resonance (NMR). The properties of selected peptides and proteins in solution and in membrane mimicking micelles were observed by circular Dichroism (CD), mass spectrometry (MS), and other spectroscopic techniques.

The mDpl(1-30) peptide (30 residues) of the mouse Doppel protein was found to be positioned as an $\alpha$-helix in a DHPC micelle. The same peptide can disrupt and cause leakage in small unilamellar vesicles.

Single D-amino acid isomers of Trp-cage (20 residues), the smallest peptide with a protein-like fold, were analyzed by CD spectroscopy and were found to have different secondary structures and melting temperatures. They were compared against MS measurements specially designed to reveal the secondary structure of proteins.

We studied a novel protein in *E. coli* of unknown structure that is encoded by the putative transcription factor ORF: *ygiT* (131 residues). This protein comprises a helix-turn-helix (HTH) domain in the C-terminus and contains two CxxC motives in the N-terminal domain, which binds Zn. This protein was named 2CxxC. We succeeded in overexpressing and purifying 2CxxC in *E. coli* with enough yield for a $^{13}$C, $^{15}$N uniformly labeled NMR sample. The chemical shift assignment was completed and the NMR structure was calculated in reducing, slightly acidic conditions (1mM DTT, pH 5.5). The determined HTH domain shows good similarity with structures predicted by a homology search, while the N-terminal domain has no other homologous structure in the Protein Data Bank (PDB).

The structure of the paddle region (27 residues) of the HsapBK(233-260) voltage and Ca$^{+2}$ activated potassium channel, in DPC micelles, was determined by NMR. It shows a helix-turn-helix loop, which agrees well with the expected structure and could help to verify the proposed models of the voltage gating mechanism.

The C-repressor (dimer of 99 residues) of bacteriophage P2 was analyzed by NMR. We assigned the chemical shifts and NMR structure determination is under way.