

From peptide hormone to ETEC toxin: Structure based design of a pharmaceutically relevant expression system

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Enterotoxigenic *Escherichia coli* (ETEC) infection accounts for the majority of cases of acute secretory diarrhea. The causative agents are enterotoxins secreted by ETEC, among them the heat-stable enterotoxin STh. STh is a 19 amino acid peptide containing three disulfide bonds that stimulates fluid secretion in the bowel by binding to the intestinal guanylyl cyclase C (GC-C). Since GC-C agonists have pharmacologic potential as treatment for both chronic constipation and colorectal carcinoma it is crucial to develop methods for the synthesis of STh and related peptides. Here we present a strategy for recombinant expression of STh that relies on the use of the prosequence of human uroguanylin to support proper folding and disulfide bond formation, a method that was designed based on the structures of the prohormones based on solution NMR, still the only method that allows determination of biomolecular structures in atomic resolution in physiological environments. The chimeric protein prouro-STh consisting of the N-terminal propeptide of uroguanylin and the C-terminal STh peptide was expressed in *E. coli* and an efficient purification protocol was developed. Trypsin digestion of this protein released the enterotoxin which could be obtained in high purity. NMR and mass spectrometry confirmed the identity and homogeneity of the toxin, and its biological activity was confirmed by a cell-based *in vivo* assay. The expression scheme introduced here represents a cost-efficient and scalable way of STh production.

Biography

Rösch completed his doctorate in biophysics with the theoretical and practical combination of nuclear magnetic resonance and biochemical techniques at the Max-Planck-Institute for Medical Research and the U of Heidelberg, Germany, in 1978. After his structural study of phosphokinases with phosphorus and proton NMR spectroscopy at University of Pennsylvania as a postdoc, he rejoined the Max-Planck-Institute for Medical Research in Heidelberg, where he continued work on NMR of proteins. This led to an advanced understanding of the mechanisms of phosphotransferases. Dr. Rösch is chairing the Dept of Biopolymers at U Bayreuth, Germany, since 1990, and he serves as a director of the Research Center for Bio-Macromolecules at the same university since 2007. His current research focusses are the study of transcription processes in pro- and eucaryotes, the study of allergens, and the study of receptor lig and interactions by spectroscopic methods, mainly NMR spectroscopy. In addition, his group recently started work on analysis of nutritional and pharmaceutical products by NMR, and he cofounded ALNuMed Ltd as a spin-off of the U of Bayreuth. Dr. Rösch and his coworkers at the Research Center for Bio-Macromolecules were recently granted a cutting-edge 1-GHz NMR-spectrometer, the second of its kind worldwide.

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