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Structure determination of proteins and their complexes by multidimensional NMR spectroscopy

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In recent years NMR spectroscopy has become a powerful tool for studying the three-dimensional structure of proteins in solution. This development was made possible by the introduction of two-dimensional NMR techniques, and has been recently enhanced by the development of three- and four-dimensional NMR spectroscopy. Structure determination by NMR is based on information on a large number of proton-proton distances and dihedral angles obtained by appropriate NMR measurements. The NMR-derived distance and dihedral angles constraints are used in distance geometry combined with simulated annealing calculations to generate the three-dimensional structure of the studied molecule. Dipolar coupling measurements of weakly aligned proteins in a magnetic field provide additional information on the orientation of the bond vectors in the macromolecules. This new technique enables faster and more accurate structure determination. Multidimensional NMR techniques currently allow structure determination of proteins up to 50 kDa.

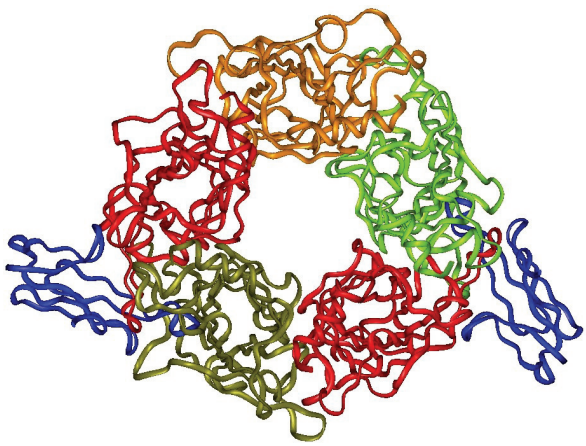

Fig. 1 *A model of the AChR hetero-pentamer in complex with two bungarotoxin molecules. The model is based on the NMR structure of bungarotoxin in complex with an α-AChR(182-202) peptide and the crystal structure of a mollusk acetycholine binding protein.*

NMR Structure of alpha-Bungarotoxin in Complex with an Acetylcholine Receptor Peptide Reveals the Basis for Species Specific Resistance to the Toxin and alpha-Neurotoxins Inhibition of Acetylcholine Binding

The acetylcholine receptor (AChR) is a ligand gated cation channel activated by the neurotransmitter acetylcholine. Located in muscle and neuronal membranes, the AChR translates the chemical signal of acetylcholine binding into an electrical one, leading to intercellular transmission. The alpha-subunit of the acetylcholine receptor (alpha-AChR) contains a binding site for the snake venom derived antagonist, alpha-bungarotoxin (alpha-BTX). The structure of the AChR has not been solved to date due to its hydrophobic nature.

Using 2D 1H-NMR spectroscopy we solved the three-dimensional structure of a peptide corresponding to alpha-AChR residues 182-202 in complex with alpha-BTX. Our structure correlates the observed changes in toxin susceptibility with naturally occuring mutations of alpha-AChR in different species. Based on the structure of a homologous molluscan acetylcholine binding protein, a model of the extracellular domain of the AChR was constructed. The docking of alpha-BTX into this model was obtained by superimposing the loop formed by AChR residues 184-200 in complex with alpha-BTX on the corresponding loop in the molluscan protein. It is found that the acetylcholine binding-sites at the interface of alpha/gama- and alpha/delta subunits of the receptor are occupied by the side-chain of toxin residue Arg-36. This arginine, conserved amongst all alpha-neurotoxins, mimics the choline moiety which is competitively inhibited by alpha-BTX. These findings coincide with previous mutagenesis studies and illustrate the inhibition mechanism of AChR by alpha-neurotoxins.

The Human Interferon Receptor: NMR-Based Modeling and Mapping of interferon -alpha2 Binding Site

The human interferon receptor (IFNAR) mediates the antiviral and antiproliferative activities of type I interferons (IFNs). This receptor is comprised of subunits IFNAR1 and IFNAR2, the latter exhibiting nanomolar affinity to IFNs. The extracellular domain of IFNAR2 (IFNAR2-EC), a soluble 25 kDa IFN-binding polypeptide, and its complex with IFN-alpha2, were studied using multidimensional NMR. The global fold of IFNAR2-EC was deduced from the NMR data and was utilized to improve

the alignment of IFNAR2-EC against homologous receptors and model its structure. The changes in chemical shift of IFNAR2-EC induced upon IFN binding were used to map the binding site for IFN on IFNAR2-EC. The proposed binding domain encompasses all residues implicated by mutagensis studies in IFN binding, and suggests adjacent residues cooperate in forming the binding surface. D2O-exchange experiments indicate that binding of IFN-alpha2 induces a global tightening upon the immunoglobulin scaffold of IFNAR2-EC. This increase in receptor rigidity may play an important role in initiating the intracellular stage of the IFN signaling cascade.

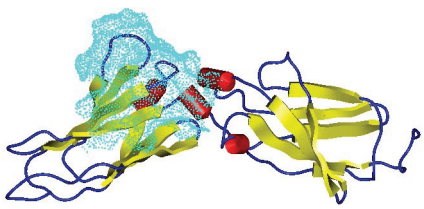

Fig. 2 *NMR-based homology model of IFNAR2-EC. NMR-derived secondary structure elements were used to align IFNAR2-EC against two related receptors and model its three-dimensional structure. Chemical shift changes induced by IFN-α binding (light blue surface) map the ligand binding site.*

Acknowledgements

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Selected Publications

Weliky, D. P, Bennett, A. E., Zvi, A., Anglister, J., Steinbach, P. J., & Tycko, R. (1999) Solid-state NMR evidence for an antibody-dependent conformation of the V3 loop of HIV-1 gp120. Nature Struct. Biol. 6, 141-145.

Tugarinov, V., Zvi, A., Levy, R. & Anglister, J. (1999). A cis proline turn linking two β-hairpin strands in the solution structure of an antibody-bound HIV-1IIIB V3 peptide. Nature Struct. Biol. 6, 331-335.

Sharon, M., Oren, Z., Shai, Y. & Anglister, J. (1999). 2D-NMR and ATR-FTIR Study of the Structure of a Cell-Selective Diastereomer of Melittin and Its Orientation in Phospholipids. Biochemistry 38, 15305-15316.

Tugarinov, V., Zvi, A., Levy, R., Hayek, Y., Matsushita, S., and Anglister, J. (2000). NMR structure of an anti-gp120 antibody complex with a V3-peptide reveals a surface important for co-receptor binding. Structure 8, 385-395.

Samson, A.O., Chill, J.H., Rodriguez, E., Scherf, T. and Anglister, J. (2001). NMR mapping and secondary structure determination of the major acetylcholine receptor α-subunit determinant interacting with α-bungarotoxin. Biochemistry,40,5464-5473.