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PS-03.09.02 X-RAY STRUCTURE OF THE [ALA-84 → VAL] MUTANT OF HUMAN TUMOR NEUROSH FACTOR. By P. Sahudjian1, T. Parin2,3, X. Van Ostade2, J. Tournier2, W. Pier2 and J. Navara3. 1Chimie Biomoléculaire (URA 1450 du CNRS) UFR-Biologie, 93012-BOBIGNY CEDEX, France and 2LURE, Bât 209, Université Paris Sud 91405 ORSAY, 3Laboratory for Molecular Biology, State University of Genti, Leuven, Belgium. 4Laboratoire de Physique, Faculdé de Pharmacie, 92900-CHATENAY MALABRY, France.

In order to understand the reactivity of hTNF, the inactive mutant [Ala 84 Val] was prepared by mutagenesis and crystallized. This very conservative single mutation almost completely abolishes the cytotoxic activity on L929 cells and further information was expected from its three dimensional structure analysis. The crystals belong to the trigonal class, space group $P_{3}1_2_1_2$ with two trimers (6 molecules) in the asymmetric unit. The parameters are roughly equivalent to that of the native protein and the structures are isomorphous. This case served as a test for a new molecule replacement method (AMoRe) in which the spherical harmonic coefficients are not yet evaluated but accurately determined by solving their integral forms. The structure, proved to be resistant to classical methods, was easily solved by this way, using the tetragonal form coordinates. The structure, collected at the DCM synchrotron in Orsay, were used in the structure refinement (XPLOR and PROLSQ techniques). The active site of hTNF, which is located at the monomer interfaces, shows significant differences and the three cavities thus delimited by the trimerization are not equivalent. The differences observed between the native and the mutant structures will be presented.

View of the Ca tracing, down the local three-fold axis. The interresidue movements which access the hTNF active site are labeled by full arrows. They all are located at the bottom of the trimer and on both sides of the active sites formed upon trimerization.

PS-03.09.03 X-RAY CRYSTALLOGRAPHIC STUDIES OF LEUKEMIA INHIBITORY FACTOR

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The aim of the work is to determine the tertiary structure of murine Leukemia Inhibitory Factor (LIF) expressed in E. coli using the techniques of X-ray crystallography.

LIF is a member of a group of polypeptide regulatory factors which includes interleukin-6 and interleukin-11. It is a molecule capable of such disparate effects as inducing the differentiation of the clonal growth cells of a mouse myeloid leukemia cell line (Tomida, Yamamoto-Yamaguchi & Hozumi, 1984) and preventing the the differentiation commitment in normal embryonic stem cells (Williams, et al. 1988).

The conditions under which recombinant murine LIF crystalline were determined by V. Stanton in early 1991. The crystals are monomorphic and diffract to 2A. Diffraction data have been collected using the synchrotron at the Photon Factory in Tsukuba (Japan), a Xentronics multi-wire area detector and a MAR detector. Twelve heavy atom derivatives have been prepared and phased by multiple isomorphous replacement is proceeding.


PS-03.09.04 LARGE MOTIONS IN SIGNAL TRANSDUCTION ASSOCIATED WITH CALMODULIN AND INITIAL RECEPTORS FOR ACTIVE TRANSPORT AND CHEMOTAXIS.

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Calmodulin (CaM) is the principal calcium-dependent transducer and regulator of a variety of eukaryotic cellular processes such as carbohydrate metabolism, cell division, cytoskeletal function, gene expression, ion channel regulation, and neurotransmitter synthesis and release. In many of these processes, CaM activates a plethora of intracellular enzymes, and the CaM-binding domains in several of them have been shown to reside in a region consisting of approximately 18-residue peptide segment. There is little identity in the amino acid sequences of these domains. Synthetic peptides based on these sequences have been shown to bind calmodulin in a calcium-dependent manner with the same 1:1 stoichiometry and tight affinity as the native protein. To understand the atomic details of the mechanism of action of CaM, our laboratory has determined three X-ray structures of calmodulin — two of the calcium-activated, unbound forms and one in a form bound to a synthetic target peptide. In one of the largest conformational changes ever observed for a protein, the binding of calmodulin to the target peptide causes a 5-residue turn of the central $alpha$ helix of the native structure to unwind and expand with the consequence that the two domains come close together in a cis orientation and wrap around the target peptide. To emphasize this previously unrecognized and key feature of the linker function in molecular recognition and activation, we introduced the term "expansion joint". Thus calmodulin activates enzyme activity by gripping and pulling the peptide segment out of sterically hindering position.

Peripheral binding proteins serve not only as initial ligand receptors for active transport and chemotaxis, but also play a key role in initiating signal transduction in both processes. Ligand-induced conformational changes in essential signaling transduction. We have shown by X-ray crystallography that binding of maltodextrins to the receptor causes a combined large hinge-bending and slight twist motions between the two domains, bringing the domains close together and enclosing the ligand bound in the vNleN between the two domains. It is this "closed" ligand-bound form that is presumably recognized by the membrane-bound components, triggering the signaling mechanism that triggers transport or chemotaxis.


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