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DETERGENT ORGANISATION IN CRYSTALS OF MONOMERIC OUTER MEMBRANE PHOSPHOLIPASE A

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The 12 Å resolution structure of the detergent in crystals of monomeric outer membrane phospholipase A (OMPLA) has been determined using neutron diffraction. Five large single OMPLA crystals were soaked in stabilizing solutions containing different ratios H_2O/D_2O, thus obtaining distinct neutron scattering contrasts. From the diffraction data at these contrasts, the structure of the detergent micelles was determined. The hydrophobic β -barrel surfaces of the protein molecules are covered by rings of detergent. These detergent belts are fused to neighbouring detergent rings forming a continuous three-dimensional network throughout the crystal. The thickness of the detergent layer around the protein varies from 7 to 20 Å. The enzyme's active site is positioned just outside the hydrophobic detergent zone and is thus in a proper location to catalyze the hydrolysis of phospholipids in a natural membrane. The dimerization face of OMPLA is covered with detergent, but the detergent density is weak near the exposed polar patch suggesting that burying this patch in the enzyme dimer interface may be favorable. Furthermore, this study presents the first example a third type of membrane protein crystal of which the detergent structure is determined and therefore is valuable for a more complete understanding of membrane protein crystallization.

Keywords: MEMBRANE-PROTEINS, DETERGENT, NEUTRON-DIFFRACTION

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MECHANISM OF PYROPHOSPHATASE CATALYSED HYDROLYSIS OF PYROPHOSPHATE BY X-RAY AND NEUTRON DIFFRACTION

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Phosphoryl transfers catalyzed by several different enzymes are very essential reactions for any living cell. The most-thoroughly studied phosphoryl transfer enzyme is soluble inorganic pyrophosphatase (PPase). It catalyses the hydrolysis of pyrophosphate to two orthophosphates. The focus of our studies has been yeast S. cerevisiae PPase (Y-PPase).
By X-ray diffraction we have already determined structures for four complexes during the catalytic cycle, some of them at ultra high resolution. By combining all available information we have been able to develop a detailed model for the catalytic mechanism of PPase. In it, the attacking nucleophile is an hydroxide ion coordinated by two metal ions. In addition, Asp117 coordinates the proposed nucleophile and this interaction may be via a low barrier hydrogen bond. However, although we may be able to determine other structures during catalytic cycle by X-ray diffraction, we will not be able to determine the actual positions of essential hydrogen atoms - we can just predict where they should be. But by neutron diffraction we can in principle determine the hydrogen positions.
We have successfully produced, purified and crystallized the perdeuterated Y-PPase required in neutron diffraction studies. A large crystal volume is required and we have been able to grow PPase crystals as large as 3 mm^3. The large crystals are as well-ordered as the small ones, and diffract X-rays to almost 100 pm resolution. Using the LADI instrument at ILL we have determined that crystals with a volume of about 1 mm^3 diffract neutrons to better than 300 pm resolution.

Keywords: PHOSPHORYL TRANSFER, NEUTRON CRYSTALLOGRAPHY, DEUTERATION

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COMPARATIVE PROTEIN STRUCTURE MODELING OF GENES AND GENOMES

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Structural genomics aims to determine or accurately predict 3D structure of most proteins (1). This aim will be achieved by a focused, large-scale determination of protein structures by X-ray crystallography and NMR spectroscopy, combined efficiently with accurate protein structure prediction (2). Comparative protein structure modeling will be discussed in this context. To allow large-scale modeling, we automated fold assignment, sequence-structure alignment, comparative model building, and model evaluation (3). These steps were implemented mostly in our MODELLER package, which is available on the web at http://guitar.rockefeller.edu/. The modeling pipeline has been applied to all of the approximately 750,000 protein sequences in the TrEMBL database, resulting in models for segments of approximately 430,000 proteins. These models are stored in the MODBASE database (4), accessible over the web at http://guitar.rockefeller.edu/modbase. Several examples of how comparative modeling can be useful in the biological analysis of individual proteins as well as whole genomes will be described.

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COMPUTATIONAL STRUCTURAL GENOMICS OF DOMAIN COMBINATIONS

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On average, over half of the proteins in a proteome and about 45% of the amino acids can be assigned a domain of known three-dimensional structure using powerful multiple sequence comparison methods. One of the most effective methods to identify homologies between sequences and structures currently available is the library of SAM T99 Hidden Markov Models and their assignments to proteomes in the SUPERFAMILY database.
Structural assignments provide information about protein function and are useful guides towards proteins that may belong to families or folds of unknown structure. The set of structural assignments for an organism also give us insight into the organisation and evolution of proteomes in terms of domain duplications and domain combinations. Thus we now know that at least two thirds of the proteins in an organism are multi domain proteins, consisting of more than one domain. Therefore, the interaction between domains is an important factor in determining protein function, and there is much less structural information at this level than for individual domains. Our current knowledge of linear patterns of domain combinations and the geometry of domain combinations will be described, as well as what fraction of all domain combinations is covered by structures in the Protein DataBank

Keywords: COMPUTATIONAL STRUCTURAL GENOMICS, DOMAIN COMBINATIONS, STRUCTURAL ASSIGNMENTS