CRYSTALLOGRAPHIC STUDY OF AN ANTI-MN/CA IX MONOCLONAL ANTIBODY M75 FAB FRAGMENT

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MN/CA IX is a cell surface protein, strongly associated with certain types of human carcinomas. The predicted protein of cloned MN/CA IX cDNA consists of the signal peptide, proteoglycan-related sequence, carbonic anhydrase domain, trasmembrane segment and a short intracellular tail. Until now, molecular basis of involvement of MN/CA IX in carcinogenesis has remained unclear. Structural study of an MN/CA IX binding monoclonal antibody M75, complexed with its epitope peptide may contribute toward elucidation of the role of MN/CA IX.
MN/CA IX is a cell adhesion molecule, its carbonic anhydrase (CA) is enzymaticaly active. Monoclonal antibody M75 reacts excellently with MN/CA IX. The cells adhere to immobilized MN/CA IX and mAb M75 abrogates attachment of cells to MN/CA IX (Závada et al., 2000). Using synthetic oligopeptides, the epitope of mAb M75 was localized in the proteoglycan domain of MN/CA IX, in the region of a tandem repeat and identified as amino acids PGEEDLP.
We obtained crystals of free Fab M75 and Fab M75 complexed with different epitope peptide. The data set for Fab M75 was collected at 'home source' to 2.1 Å resolution. The Fab M75 structure was solved by molecular replacement, using Fab Bv04-01 (PDB code 1NBV) as search model. Zavada J, Zavadova Z, Pastorek J, Biesova Z, Jezek J, Velek J. Human tumor-associated cell adhesion protein MN/CA IX: identification of M75 epitope and of the region mediating cell adhesion. (2000) Br J Cancer. 82:1808-13.

Keywords: FAB FRAGMENT, CARBONIC ANHYDRASE, CRYSTAL STRUCTURE

STRUCTURAL STUDIES OF THE ENZYMES OF THE LELOIR PATHWAY

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Metabolism of galactose necessitates the conversion of galactose to glucose 1-phosphate. This is catalyzed by the enzymes of the Leloir pathway. Four enzymes are responsible for this interconversion, and impairment of any one of these can lead to a diseased state known as galactosemia. β-(D)-galactose is first converted into the alpha-anomer by galactose mutarotase. The resulting alpha-(D)-galactose is then converted into galactose 1-phosphate by galactokinase. Galactose 1-phosphate is subsequently uridylylated by galactose 1-phosphate uridylyl transferase, which adds the uridyl group from a molecule of UDP-glucose. The resulting UDP-galactose is then converted into UDP-glucose by UDP-glucose 4-epimerase. It is the transferase that effectively liberates the resultant glucose 1-phosphate for metabolism during glycolysis. What is evident, however, is that UDP-glucose is not consumed in the conversion of galactose to glucose, since it is regenerated from UDP-galactose by the epimerase. The reversibility of the epimerase reaction is essential for the synthesis of galactosyl moieties in polysaccharides and glycoproteins if there is a minimal amount of galactose in the diet. Structures of the enzymes in the Leloir pathway will be described.

Keywords: **ENZYMES MECHANISMS GALACTOSEMIA**

MACROMOLECULAR CRYSTAL GROWTH BY MEANS OF MICROFLUIDICS

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We have performed a feasibility study in which we show that chip-based, microfluidic (LabChip®) technology is suitable for protein crystal growth. This technology allows for accurate and reliable dispensing and mixing of very small volumes while minimizing bubble formation in the crystallization mixture. The amount of (protein) solution remaining after completion of an experiment is minimal, which makes this technique efficient and attractive for use with proteins, which are difficult or expensive to obtain. The nature of (LabChip®) technology renders it highly amenable to automation. Protein crystals obtained in our initial feasibility studies were of excellent quality as determined by X-ray diffraction.
Subsequent to the feasibility study, we designed and produced the first (LabChip®) device specifically for protein crystallization in batch mode. It can reliably dispense and mix from a range of solution constituents into two independent growth wells. We are currently testing this design to prove its efficacy for protein crystallization optimization experiments.
In the near future we will expand our design to incorporate up to 10 growth wells per (LabChip®) device. Upon completion, additional crystallization techniques such as vapor diffusion and liquid-liquid diffusion will be accommodated. Macromolecular crystallization using microfluidic technology is envisioned as a fully automated system, which will use the 'tele-science' concept of remote operation and will be developed into a research facility for the International Space Station as well as on the ground.

Keywords: **PROTEIN CRYSTAL GROWTH AUTOMATION MICROGRAVITY RESEARCH**

MICRO-ARRAY CHIP FOR HIGH THROUGHPUT PROTEIN CRYSTALLOGRAPHY

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Since protein crystallization is influenced by a number of factors, development of a method for high throughput protein crystallization is one of the most important steps for accomplishment of structural genomics. Several crystallization robots with micro-litter droplet are now commercially available. Even a nano-droplet robot has been developed. However, such robots are space consuming and very expensive. Moreover, the speed of setting crystallization droplets with such robotic system is not quick enough. We have been developing a unique device for high throughput screening of protein crystallization condition with nano-volume. The device is micro-array chip utilizing the fiber type DNA chip technology.
The prototype chip has 48 different crystallization conditions integrated on a slide glass. Each precipitant solution of 50 nL is kept in a hollow fiber of the arrayed chip separately. One should just put protein solution onto the chip manually with any single auto-pipetter. Protein and the precipitant solutions are mixed in the micro cell on the chip, and crystallization screening will be performed. Only 50 nL for each crystallization condition, total of about 5 micro-L protein solution, including loss, is required for screening of 48 conditions.
Using the chip, we can perform many screening conditions without any robotic systems. Our unique screening procedure will make it possible to set up more than 100 different crystallization conditions within ten seconds. In principle, more than 1,000 screening can be performed on slide glass size chip.

Keywords: **PROTEIN CRYSTALLIZATION**