intermediate which does not belong to any of Cas2 family and toxin-antitoxin system.

Keywords: HP0315, VapD, endoribonuclease

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Crystal Structure of the dimerization domain of human filamin A

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By crosslinking actin filaments, filamins play important roles in regulating the dynamics of the actin cytoskeleton which plays a central role in many cell functions such as the maintenance of cell shape, cell division, adhesion, motility, signal transduction and protein sorting. Consistent with this, mutations in human filamin genes are associated with a wide range of developmental abnormalities and defective neuronal migration. And filamins as integrators of cell mechanics and signalling by interacting with transmembrane receptors and cytosolic signaling proteins.

In humans, three filamin isoforms have been identified: filamin A, filamin B, filamin C. Of these, filamin A (FLNa) is the most abundant and widely expressed. Heterozygous null FLNa alleles result in defective neuronal migration causing periventricular heterotopia, while certain FLNa missense mutations cause familial cardiac valvular dystrophy and putative gain-of-function mutations result in a spectrum of congenital malformations generally characterized by skeletal dysplasias.

Human vertebrate filamins are homodimers of two 280kDa subunits, and each subunit contains an N-terminal actin binding domain consisted of two calponin homology domains followed by 24 tandem repeat domains (FLNa1-24) that are interrupted by flexible hinge regions between FLNa15 and FLNa16 and FLNa23 and FLNa24. Dimerization through FLNa24 is crucial for the actin-crosslinking function of filamins.

We report the structure of FLNa domain 24 (FLNa24), and compare the structure with FLNc24 and discuss how dimerization is formed in FLNa24.

Keywords: filamin A

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Protein crystallization with zeolite

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X-ray crystallography is one of the most powerful techniques for the three-dimensional structural determination of proteins. The high-resolution crystal structures allow us to study in great detail the relationship between structure and function of proteins. However, production of high-quality protein crystals is still a major bottleneck in structural biology [1]. Although the sparse matrix crystallization screening is widely used in protein X-ray crystallography, coarse and unadjustable samplings of this screening often fail in obtaining high quality of crystals especially in the case of proteins with poor crystallizability. In order to overcome this difficulty, a new effective methodology for improving crystal quality is needed.

Recently, we have reported an advanced crystallization method using synthetic zeolite molecular sieves (MS) as hetero-epitaxic nucleants by which a directed crystal nucleation on the material surface occurred in a variety of proteins, thereby providing new crystal forms with a substantial improvement of diffraction quality in some cases [2], [3].

In this study, the hetero-epitaxic nucleant method using MS was applied to the sparse-matrix crystallization screening of 20 protein samples, which provided formation of larger single crystals with better diffraction quality as compared with the conventional screening. In current protein crystallography, available sample amount for crystallization trials is limited especially when the expression level of the target protein is low. In such cases, a minimum set of sparse matrix crystallization screening in the presence of MS may be the most effective way to achieve the diffraction quality of protein crystals.

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Crystallization of macromolecules in selenate to solve the phase

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Previously, the crystal structures of two model proteins were determined by the single anomalous diffraction method using scattering from selenium. Selenium was incorporated to the protein during crystallization substituting sulfate (ammonium- or sodium-), one of the most used precipitants in protein crystallization with selenate (sodium-).

The crystal structures of porcine pancreatic elastase (PPE) and proteinase K (ProtK) were solved at 1.6 Å and 0.94 Å respectively taking advantage of anomalous scattering from one SeO_4 site. A single data set was recorded slightly above the selenium absorption K edge at 12.67 keV for PPE while atomic resolution data were recorded at higher energy, 14.50 keV in the case of ProtK. In both examples, high quality experimental electron density maps were computed and the structures were solved solely relying on SAD phases from the selenate ion. In these two samples, the selenate ion occupies the exact same site as the sulfates in the reported native structures.

Additionally, a soaking experiment indicates that proteinase K crystallized in sulfate-free solution can be soaked in a cryo-solution containing SeO_4 and that the structure can be solved using Se-SAD phasing. Crystallographic data were recorded on a sulfate-free and a sulfate sample at low energy (7.1 keV and below) to confirm the absence and presence of SO_4 from S-SAD experiments. Those low energy experiments were also used to better estimate absorbed dose per sample at different energies.

Out of 63000 protein crystal structures currently available, 8500 contain at least one sulfate ion which represents more than 13 % of the total X-ray crystal structures. This quick, easy and cost-effective substitution method offers an adequate alternative to heavy atom search or seleno-methionine labeled protein production in structural biology and structural genomic. It requires minimum additional work in the work flow, exactly a single complementary step during the production of crystals.

Finally, this method was successfully applied to solve the structure of a “quasi” new structure.

Comparisons low/high energies experiments relying on S/Se atoms will also be discussed.

Keywords: sulfate, selenate, phasing