

and neck of the opposing subunit was used as restraints in the process of fitting the ABD and CaM in the molecular envelope.

Keywords: actin-binding protein, crystallization strategies, SAXS

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Structural and biochemical characterization of actin binding by dystrophin and utrophin

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Duchenne and Becker muscular dystrophies (DMD & BMD) are muscle-wasting disorders caused by mutations in the X-linked dystrophin gene. Utrophin is an autosomal homolog to dystrophin that has been shown to functionally compensate for dystrophin in cultured muscle cells and in vivo in the muscular dystrophy (mdx) mice model and hence may prove to be useful as a therapeutic replacement for dystrophin in DMD and BMD. Both proteins belongs to the spectrin superfamily of proteins, which also includes spectrin and α -actinin. These proteins are characterized by N-terminal actin binding domains and C-terminal variable domains separated by numerous spectrin like repeats. The spectrin repeats are triple-helical coiled structures that can bind a variety of ligands, including F-actin, as is the case for some repeats found in dystrophin. The aim of this research is to examine the interaction of both dystrophin and utrophin with actin by structural and biochemical methods and compare their actin binding properties. Studies have shown that the N-terminal spectrin repeats of utrophin are required for a high affinity interaction of the actin-binding domain with F-actin. It is unclear whether these repeats have an intrinsic affinity for F-actin and in the current study we are determining the actin-binding properties of the two N-terminal spectrin repeats using X-ray crystallography and co-sedimentation assays. The crystal structure of first spectrin repeat of utrophin has been determined to 1.8 Å and exhibits the characteristic three helix structure as observed for spectrin, α -actinin, plakin.

Keywords: actin binding, Duchenne muscular dystrophy, becker muscular dystrophy

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Crystallization and crystal analysis of ATP synthase

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ATP synthase (FoF1) is responsible for ATP production in living cells, and is a membrane protein located in the energy conversion membrane. ATP synthase consists of a channel Fo portion (100,000 dalton, ab2c8-12) and a large soluble catalytic F1 portion (380,000 dalton, $\alpha\beta\gamma\delta\epsilon$). The unique rotational catalysis mechanism of F1 includes rotation of the rod-like γ subunit, which is thought to control the conformations of the three catalytic β -subunits in a cyclic manner.

We have purified and crystallized ATP synthase from a thermophilic bacterium PS3. Among detergents tried, dodecyl-maltoside was the best, though decyl-maltoside was the close second. Used columns were those of an ion-exchange and gel-filtration types. Initial tiny crystals from PEG6000 are now replaced by crystals that allowed diffraction analysis, after extensive and systematic crystallization condition search. ATP synthase in our crystals contains all the subunits. Those crystals diffracted to a 7 Å resolution at synchrotron. Our initial analysis had been hampered by incorrect beam position parameters supplied and a high mosaicity of crystals (nearly 4 degree). Though the first problem was got rid of with relative ease, the second problem was very difficult. Our current refined procedures for processing the data made us to think that the problem can be overcome if we could get accurate cell parameters since other possible problems have been ruled out in the analyses. As we realized that PS3 ATP synthase was difficult for x-ray study, we have searched for better thermophilic bacterial sources from hot springs. We have got some bacterial strains that may be suited to structural study of ATP synthase but other proteins as well.

Keywords: ATP synthase, crystallization, crystal analysis

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Structural view of the ATPase cycle of a myosin that moves backward

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Myosins are molecular motors that use the energy obtained from the hydrolysis of ATP to move along actin filaments. Among myosin family, class VI myosins are very intriguing because of their atypical motility properties. First, dimeric myosin VI is capable of taking multiple steps (processive movement) of 30-36 nm along actin filament. These steps are surprisingly large considering that myosin VI has a rather small lever arm and they cannot be explain by the structural transitions that occur within the myosin motor of other classes. Secondly, this motor produces force towards the minus end of actin filaments, which is the opposite direction of all other characterized myosins. In order to understand the molecular basis of these features, we would like to describe the structure of this myosin in different states of its ATPase cycle. During the ATPase cycle, myosin goes through states of strong and weak affinity for the actin filament. To this day, we solved the structure of three states of the cycle, one state that mimics the state of strong affinity for actin (at the end of the movement on the filament) and two states before the force production. One of them represents the starting point for movement on actin, the pre powerstroke state. The analysis of the structures from the beginning and the end of the powerstroke allows us to understand how myosin VI moves in the opposite direction (toward the minus-end of actin filaments) due to a unique insertion between the motor domain and the lever arm. These structures also allowed us to understand the origin of the large size of the myosin VI lever arm swing (powerstroke). Unexpectedly, we found that a conformational change occurs in the converter which allows an optimized movement of the lever arm during the stroke.

Keywords: myosin, conformational change, motility