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CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION ANALYSIS OF PROSTAGLANDIN F2a SYNTHASE FROM *TRYPANOSOMA BRUCEI*

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Parasitic protozoa Trypanosoma causes diseases in both human and domestic animals. In human, the disease is characterized by clinical symptoms such as fever, headache, body pain, sleepiness, and immunosuppression. Bioactive substances produced during the infection cause these symptoms, whose molecular mechanisms remain to be elucidated. Prostaglandins (PGs) of 2-series are metabolites of arachidonic acid formed by the oxidative cyclization of the central five carbon atoms within this fatty acid. It is well documented that PGD_2 is one of the endogenous sleep-promoting substances. PGD_2 and PGE_2 play important roles in clinical and pathological manifestations (such as somnolence, immunosupression acute fever, shivering, headache) in mammals. Also, it is often believed that host cells solely produce prostaglandins, which mediate inflammatory responses during infection with parasitic protozoa. Trypanosoma produces PGD_2, PGE_2, and $PGF_{2\alpha}$ ($PGF_{2\alpha} > PGE_2 > PGD_2$), and releases them in the medium and out of parasitized red blood cells. We crystallized the NADP-dependent prostaglandin $F_{2\alpha}$ synthase from T. brucei used by the hanging-drop vapour-diffusion method. The crystal was tetragonal system, space group $P4_12_12$, with unit-cell parameters a = b = 112.3, c = 140.0 Å. The X-ray diffraction data were collected from a crystal to a resolution of 2.8 Å using our home facility at room temperature.

Keywords: PROSTAGLANDIN F2A SYNTHASETRYPANOSOMA SLEEPING SICKNESS

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STATISTICAL ANALYSIS OF PROTEIN-DNA COCRYSTALLIZATION

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Over the past years a large number of high-quality crystal structures of protein-DNA complexes have been determined by many investigators. However, the crystallization of protein-DNA complexes presents some limitations compared with that of proteins alone. The approach to crystallizing complexes between proteins and DNA requires many more considerations. In this case, important additional parameters in the crystallization experiment are the sequence and length of the synthetic DNA-oligonucleotide as well as the choice of the overhanging base pairs that may facilitate contacts in the crystal.

Nevertheless, with several hundred complexes crystallized so far we have learned basic guidelines for the crystallization of new protein-DNA complexes. Here we will present useful ideas for their crystallization, ranging from the preparation and purification of the two components to strategies that could help to crystallize them more readily.

Keywords: **PROTEIN DNA COMPLEXES CRYSTALLIZATION**

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EVALUATION OF ORGANIC SALTS AND AMINO ACIDS AS PRECIPITANTS FOR PROTEIN CRYSTALLIZATION

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The sodium salt of malonic acid was shown to be a more successful crystallization reagent for protein crystallization than the inorganic precipitant salts often used as precipitants [1]. We tested a selection of salts of other organic acids as precipitants in protein crystallization. Five organic salts, two amino acids and mixtures thereof were applied on a set of thirteen proteins which are commonly used as test cases for protein crystallization. Crystallization setups were done in the modified microbatch method. Some screens were done in duplicate in a sitting drop vapor diffusion experiment. Each of oxalate, succinate and malonate did crystallize six or more proteins out of our test set of thirteen, with oxalate being the most successful agent. Benzoate, glutamate or arginine were less successful crystallization agents. Mixtures of salts did not produce more crystallization hits than the salts alone.

References:

[1] A.McPherson, A comparison of salts for the crystallization of macromolecules, Protein Science (2001) 10,418-422.

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STRUCTURE OF SUBSTRATE-FREE DIOL DEHYDRATASE REVEALS ACTIVATION MECHANISM OF ADENOSYLCOBALAMIN

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We will discuss on activation mechanism of adenosylcobalamin based on crystal structures of diol dehydratase in the presence and absence of substrate. Diol dehydratase catalyzes the conversions of 1,2-diols to the corresponding deoxy aldehydes, which requires adenosylcobalamin and monovalent cation as a cofactor. Adenosylcobalamin has a cobalt-carbon (Co-C) bond whose cleavage is required to initiate enzymatic reaction. We have determined crystal structures of substrate-bound diol dehydratase with cyanocobalamin and with adeninylpentylcobalamin. These structures indicate the state after activation of adenosylcobalamin. To elucidate its activation mechanism upon substrate binding, substrate-free form of the enzyme is required. We crystallized diol dehydratase in the absence of substrate and determined its crystal structure. Crystals of substrate-free enzyme were obtained from the similar condition previously reported except that its protein solution was prepared without substrate. Diffraction data were collected at the BL41XU beamline at SPring-8, Japan. The crystal diffracted to 1.85 Å resolution. The structure was determined by the molecular replacement method and refined at 1.85 Å resolution. The overall fold of substrate-free form is similar to the substrate-bound form. There are two significant differences at the active site. At the substrate binding-site, two water molecules occupy the site instead of 1,2-propanediol. Based on superimposition of the substrate-free structure on the substrate-bound structure, the length of the Co-C bond is estimated at 2.8 Å in substrate-free form and 3.3 Å in substrate-bound form, respectively. This means that the Co-C bond is elongated in 0.5 Å to cleave the bond upon substrate binding.

Keywords: **ADENOSYLCOBALAMIN DIOL DEHYDRATASE COBALT CARBON BOND**