Class IV Adenylyl Cyclase Structure Determination

Adenylyl cyclase (AC) performs vital signaling functions in virtually all living cells. In many human pathogens, such as those responsible for anthrax and whooping cough, AC is a known virulence factor. The importance of AC to all life, and especially its role in disease and in potential biothreat agents, motivates structure and function studies that are required to understand its mechanism of action.

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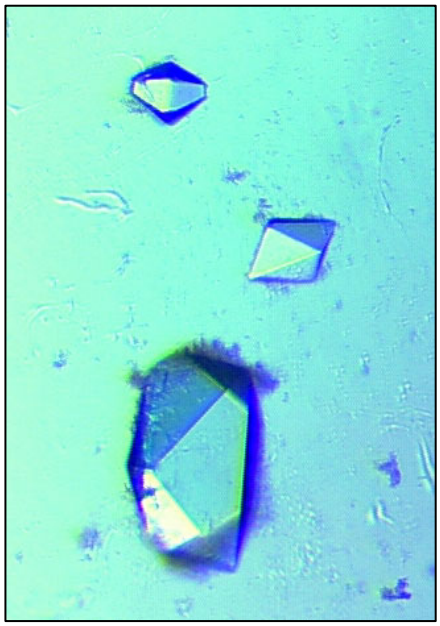
Six classes of adenylyl cyclase (AC) enzymes have been identified, based on their protein sequence and properties. Although all are subjects of active investigation, crystal structures have been reported only for classes II and III. In these cases, for example the anthrax AC belonging to class II and the human AC belonging to class III, structural data have greatly advanced understanding of mechanism with implications for human metabolism and health. The class IV enzymes are found in archaeal organisms and also in some bacteria including the plague-causing *Yersinia pestis*. They are thermostable and are the smallest known AC enzymes (chain length 179 amino acids in *Yersinia pestis*).

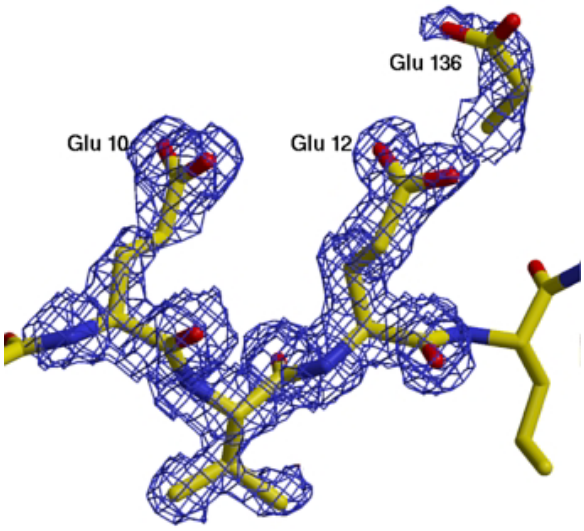
First, we cloned the gene for the class IV AC from *Yersinia pestis*, performed gene expression and enzyme activity measurements, and purified the enzyme. We then crystallized this enzyme.

Shown in Figure 1 are orthorhombic crystals of class IV adenylyl cyclase. The largest crystal pictured is about 0.3 mm long



We then determined its three dimensional structure by using X-ray crystallography as shown in the Figure 2.

Figure 2 is an electron density map at 0.2 nm (or 2.0 Angstrom) resolution and structure in part of the active site.



The enzyme was crystallized in space group $P2_12_12_1$ with two dimers per asymmetric unit. This is the first structure determination of this important form of adenylyl cyclase. A preliminary comparison shows that the *Yersinia* enzyme has a structure that is completely different from the other AC classes.

Future plans include continued refinement of this structure, then a comparison of that structure with the structure of another crystal form differing in pH, and with the other known AC structures (classes II and III).

The resulting data will be correlated with ongoing activity and pathogenicity measurements. The pathogenicity measurements will be in collaboration with an external lab.

This work is expected to lead to several publications of widespread interest as well as further structure-function studies involving activity-targeted mutants.