Chemical Engineering Doctoral Defense

The study of cyanobacterial bicarbonate transporters and Applications of microcrystal electron diffraction

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Abstract

Cyanobacteria contribute to more than a quarter of the primary carbon fixation worldwide. They have evolved a CO_2 concentrating mechanism (CCM) to enhance photosynthesis because inorganic carbon species are limited in the aqueous environment. Bicarbonate transporters SbtA and BicA are active components of CCM, and the determination of their structures is important to investigate the bicarbonate transport mechanisms. *E. coli* was selected as the expression host for these bicarbonate transporters, and optimization of expression and protein purification conditions was performed. Single particle electron cryomicroscopy (cryo-EM) or protein crystallography was carried out for each transporter. In this work, SbtA, BicA and SbtB, a regulator protein of SbtA, were heterologously expressed in *E. coli* and purified for structural studies. SbtB was highly expressed and two different crystal structures of SbtB were resolved at 2.01 Å and 1.8 Å, showing a trimer and dimer in the asymmetric unit, respectively. The yields of SbtA and BicA after purification reached 0.1 $\pm$ 0.04 and 6.5 $\pm$ 1.0 mg per liter culture, respectively. Single particle analysis showed a trimeric conformation of purified SbtA and promising interaction between SbtA and SbtB, where the bound SbtB was also possibly trimeric. For some crystallization experiments of these transporters, lipidic cubic phase (LCP) was used. In the case of LCP, often times the crystals grown are generally too tiny to withstand radiation damage from the X-ray beam during an X-ray diffraction experiment. As an alternative approach for this research, the microcrystal electron diffraction (MicroED) method was applied to the LCP-laden crystals because it is a powerful cryo-EM method for high-resolution structure determination from protein microcrystals. The new technique is termed as LCP-MicroED, however, prior to applying LCP-MicroED to the bicarbonate transporters, methods needed to be developed for LCP-MicroED. Therefore the model protein Proteinase K was used and its structure was determined to 2.0 Å by MicroED. Additionally electron diffraction data from cholesterol and human A_{2A} adenosine receptor crystals were collected at 1.0 Å and 4.5 Å using LCP-MicroED, respectively. Other applications of MicroED to different samples are also discussed.

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Zoom Link: https://asu.zoom.us/j/83660931167