Crystallization of the Mg-releasing intermediates of kinesin ATPase

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Method Article

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Abstract

Introduction

X-ray crystallography of kinesin enzymes in the unstable intermediate states is challenging because an uniform shape of protein is required for its crystallization. Using nucleotide analogs is one of the frequently-used techniques to solve this problem. We previously used ATP and ADP-phosphate analogs to solve the crystal structures of kinesin ATPase before and just at the point of ATP hydrolysis \(1-3). However, since there are no analogs suitable for the Mg- and ADP-releasing processes, crystallization of these processes has been perceived to have many challenges and difficulties. The protocol outlined below describes how we have succeeded in crystallizing kinesin in the Mg-releasing intermediate states. Albeit this protocol will not be a panacea for all enzymes, we hope this procedure might be helpful in crystallizing some other enzymes in this transitional state.

Procedure

1. Dialyze purified KIF1A solution against buffer A \(10 mM MOPS pH 7.0, 100 mM NaCl, 1 mM DTT, 5 mM EDTA, 1 mM NaN3 and 20% \(w/v) sucrose) using Slide-A-Lyzer dialysis cassette \(Pierce) for more than an hour. 2. Concentrate the dialyzed solution to 40-80 mg/ml using Amicon Ultra centrifugal filter device \(Millipore). 3. Add 2-, 4-, or 10-fold excess apyrase to the concentrated KIF1A solution, keep on ice for 30 min and centrifuge at 20,000 g to eliminate aggregates. 4. Mix the 2 µl apyrase-mixed KIF1A solution with 2 µl reservoir buffer, and dispense it in hanging drops over 500 µl well solutions containing the reservoir buffer. Reservoir buffer includes 29-31% \(w/v) PEG4000, 100 mM Tris-HCl pH 8.5, 200 mM sodium acetate, 1 mM NaN3, 1 mM DTT and 3% \(w/v) xylitol. 5. Harvest the crystals using cryoloop \(Hampton Research) every 24 hours from the day when the crystal appears \(around day3) to the day when the crystal disappears \(around day7), and flash-freeze them immediately in the cold nitrogen stream at -175 °C.

Critical Steps

Step 1: Protein should be pure. EDTA induces the release of magnesium and NaN3 stabilizes the EDTA-treated KIF1A. Step 2: The concentration of KIF1A should be more than 40 mg/ml. Below this concentration, crystallization does not occur. Step 3 to 5: Since a slow chemical reaction of Mg-release or ADP-release proceeds in crystals, crystals may appear in the hanging-drop about three days after crystallization and disappear after around seven days. The critical parameters on crystallization are protein concentration, apyrase concentration, concentration of PEG4,000 and the timing of harvesting the crystals.

Anticipated Results

Crystals in the early intermediate state of Mg-release most likely are obtained by harvesting the crystals relatively shortly after crystallization \(three or four days after crystallization) from the drop including 2-fold excess apyrase. Additionally, the crystals in the late intermediate state of Mg-release may be obtained by harvesting it relatively later \(six or seven days after crystallization) from the drop including 10-fold excess apyrase. The crystals in the mid-intermediate state of Mg-release may be obtained under the condition in-between.

References

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