

Death-associated protein kinase and ATP analogues complex crystallization phase diagram for neutron crystallography

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1 Introduction

Death-associated protein kinase (DAPK) is a member of the Ca^{2+} /calmodulin-regulated family of serine/threonine protein kinases. One of the roles of the kinase activity of DAPK is the apoptosis in the eukaryotic cell. Neutron protein crystallography provides the structure and dynamic behavior of hydration in proteins including hydrogen atoms. The networks of hydration waters in DAPK with and without ATP have been reported. However, since the hydrogen atoms of water molecules were not included, the discussion of the role of hydration waters was limited.

Neutron Protein crystallography need large single crystal, so crystallization phase diagram of DAPK and evaluation of the crystals were studied.

2 Experiment

Li_2SO_4 was used as a precipitant, and buffer A was prepared using 20mM Tris-HCl (pH8.0) and 150mM NaCl, buffer B was prepared using 20 mM MES (pH6.5) and 150mM NaCl. DAPK protein solution A consists of 1-30mg/ml protein, 20 mM Tris-HCl (pH8.0) and DAPK solution B consists of 5-30mg/ml protein, 20 mM MES (pH6.5). Both protein solutions contain 150mM NaCl, 1mM dithiothreitol (DTT) and 1mM EDTA commonly.

Crystallization was carried out by the sitting drop method, mixing the buffer A/B 5 μL and the protein solution A/B 5 μL , respectively. The incubation temperature was 20°C.

3 Results and Discussion

Phase diagram of DAPK apo-form (pH8.0) is shown in Fig.1. In this figure, blue diamond, red square and green triangle show no crystals obtain, crystals obtain and precipitant, respectively

Phase diagram of DAPK apo-form (pH6.5) is shown in Fig.2. In this figure, blue diamond and red square show no crystals obtain, crystals obtain respectively. Each curve is a crystallization curve.

The crystal of DAPK and ATP analogue (ADP) complex was grown by mixing equal volumes of protein solution (6mg/ml with 4 mM ADP) and 1.65 M Li_2SO_4 , where the buffer was 20 mM MES (pH 6.5) containing 150mM NaCl and 50 mM MgCl_2 .

X-ray diffraction experiments both at Spring8 BL38B1 and at Photon Factory NE3A could show the qualities of the apo-form crystal with 1.60 Å resolution and the DAPK-ADP complex with 1.90 Å resolution.

Fig. 1: Crystallization phase diagram of DAPK apo form (pH8.0)

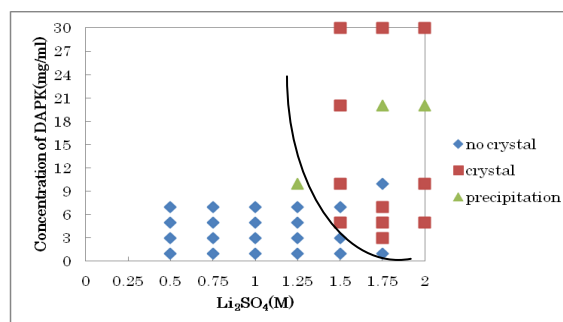
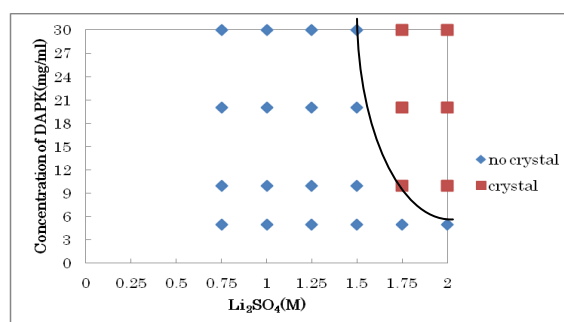


Fig. 2: Crystallization phase diagram of DAPK apo form (pH6.5)



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