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High-pressure-induced water penetration into IPMDH and pressure adaptation of proteins of deep-sea bacteria

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Introduction

Several organisms have been found at greater ocean depths such as the Mariana Trench, and their proteins are known to be adapted to high-pressure environments. For example, 3-isopropylmalate dehydrogenase (IPMDH) from a deep-sea bacterium *Shewanella benthica* DB21MT-2 (SbIPMDH) is more tolerant towards high-pressure stress than the same enzyme from a land bacterium *S. oneidensis* MR-1 (SoIPMDH). To elucidate the mechanism of pressure adaptation of the protein, we have initiated structural studies on these IPMDHs by the high-pressure protein crystallography (HPPX) method.

Experiment

The high-pressure environment used in this study was generated by a diamond-anvil cell (DAC). While performing HPPX measurements, several difficulties have been noticed. For example, (i) since the pressure medium is liquid, crystals in the DAC are mobile and moving during measurements of the X-ray diffraction. (ii) Recording highly complete data is difficult for crystals belonging to lower symmetry space groups owing to the restricted aperture angle of the DAC, which is often less than 90 degrees.

To prevent the motion of crystals during measurements, the crystals were placed into the sample chamber with a few cigarette-filter fibres tied into a loose knot (Fig. 1). Since the space group of the SoIPMDH–IPM crystals is *C*2, we placed three or four crystals into the pressure cell at one time to collect high-completeness data sets at a given pressure. The different crystals were tiled with different orientations from each other using the knotted fibres (Fig. 1).

Results and Discussion

Structures of SoIPMDH at pressures ranging from 0.1 to 650 MPa were determined at about 2 Å resolution. In the 580 and 650 MPa structures, an additional cleft is generated on the surface of the protein. Three water molecules W697, W698 and W699 appeared inside or near the cleft (Fig. 2). These three waters were not observed in the structures at lower pressures ranging from 0.1 to 410 MPa. The water penetration of W697 into the cleft is supported by hydrogen bonding to Ser266 Oγ. On the other hand, the corresponding residue in deep-sea bacterium SbIPMDH is Ala266. A possible explanation of the different piezo-sensitivity of SbIPMDH could be that a water molecule at the same position as W697 cannot be stabilized in the case of SbIPMDH.

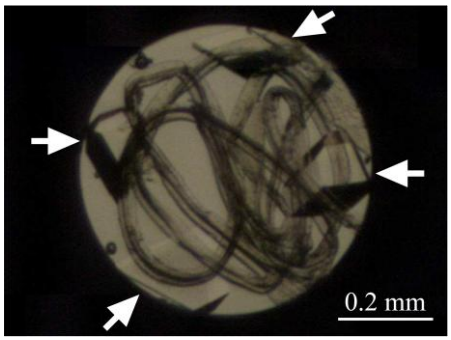
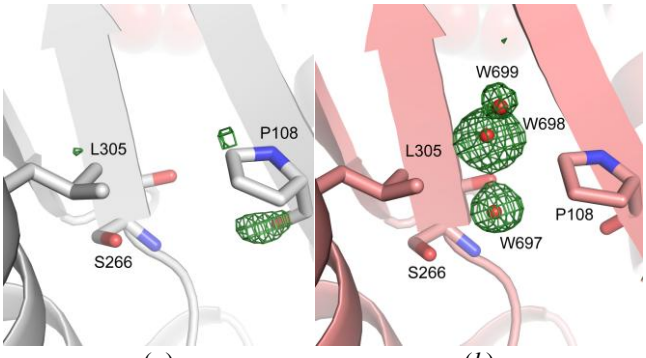


Fig. 1: Photograph of the sample chamber. Four SoIPMDH–IPM crystals (indicated by arrows) are tiled with different orientations from each other and fixed in position using the knotted cigarette-filter fibres.



(*a*) (*b*)

Fig. 2: Generation of a new cleft on the surface of SoIPMDH with water penetration. (*a*) and (*b*) fo-fc map around P108, S266 and L305 is shown as a green mesh contoured at 3.0 sigma under 0.1 and 580 MPa, respectively. Three positive peaks are observed at 580 MPa in *b*, and were assigned as three water molecules, W697, W698 and W699 (represented by red balls).

References

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