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Structural study of the pressure adaptation of proteins from deep-sea bacteria

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In recent years, significant development in the high-pressure macromolecular crystallography (HPMX) using a diamond anvil cell (DAC) has been performed especially by Prof. R. Fourme's group in combination with shorter wavelength X-ray of synchrotron radiation [1]. We are also trying to establish HPMX experimental environment at the Photon Factory, Japan [2]. HPMX is a unique method that provides high-resolution structural informations under pressure including hydration waters at a molecular surface and an internal cavity. One of the important applications is studying functional sub-states of biological macromolecules, and we are attempting to elucidate a mechanism of pressure tolerance of proteins from several organisms living in deep seas such as the Mariana Trench. For example, 3-isopropylmalate dehydrogenase (IPMDH) from the deep-sea bacterium Shewanella benthica DB21MT-2 is much more tolerant to the pressure stress than its counterpart from the land bacterium S. oneidensis MR-1 (So-IPMDH), even though these two enzymes share about 85% amino-acid identity. Crystal structures of So-IPMDH have been determined at about 2 Å resolution under pressures ranging from 0.1 to 650 MPa. Waters penetrating into the internal cavity at the dimer interface and squeezing into a molecular surface cleft opposite the active site are observed at above 410 MPa and 580 MPa, respectively [3]. The bottom of the cleft of So-IPMDH is characterized by the presence of Ser266 at the bottom, which is able to form a hydrogen bond to the squeezed water molecule. On the other hand, IPMDHs from deep-sea bacterium favors an alanine at the same position (Ala266). As expected, no water penetration is observed there at the same pressure range for the S266A mutated So-IPMDH, and the mutation develops tolerance to the pressure. In addition, some results of the high-pressure structure analysis of other proteins, and pressure-induced phase transitions in some protein crystals will also be mentioned.

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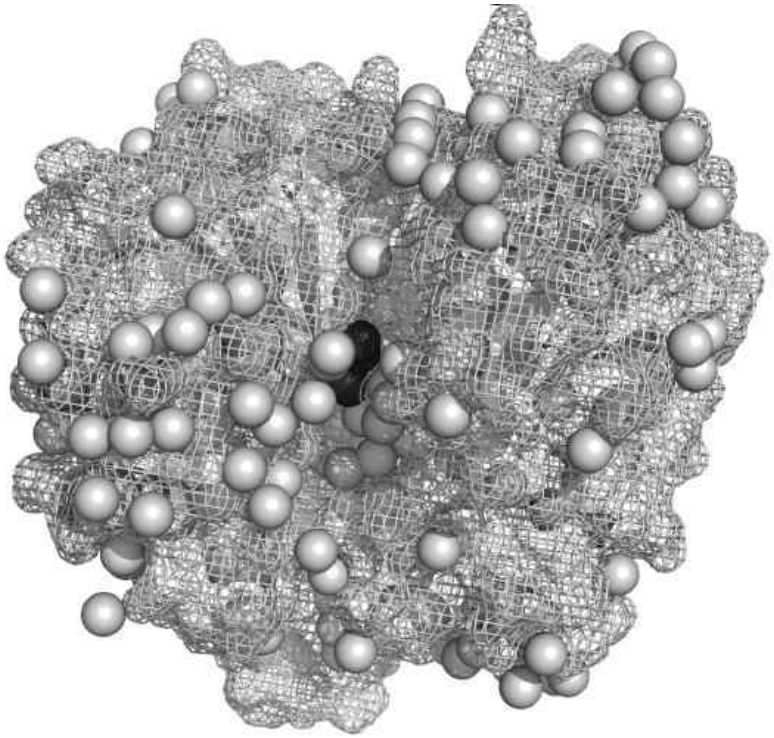

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