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CRYSTAL STRUCTURE OF β-CINNAMOMIN, A TOXIC PROTEIN FROM PHYTOPHTHORA CINNAMOMI

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Fungi from Phytophthora (P.) and Pythium (Py.) species are among the most aggressive plant pathogens, as they invade many economically important crops and forest trees. In particular, the decline disease of cork oak and holm oak (Quercus suber and Quercus ilex), in Portugal and Spain, has been associated with the infection by P. cinnamomi [1]. Most of these fungi secrete large amounts of 10 kDa proteins, called elicitins that can act as elicitors of plant defense mechanisms [2]. Elicitins were recently classified as a new class of Sterol Carrier Proteins, as they are able to transfer sterol molecules between plant plasma membranes. The crystallographic structure of the highly necrotic recombinant basic cinnamomin (β-CIN) from P. cinnamomi has been determined at 1.8 Å resolution, using the molecular replacement method. β -CIN has the same overall structure as β-cryptogein, an elicitin secreted by P. cryptogea, although it shows a different surface electrostatic potential distribution. The protein was expressed in *Pichia pastoris* and crystallized in the triclinic space group (a=31.69, b=36.99, c=44.09, α=76.86^o, β=84.41^o and γ=80.26^o) with two monomers in the asymmetric unit [3]. The interface formed by these two monomers resembles that from β -CRY dimer, although with less interactions [4].

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Keywords: β CINNAMOMIN, ELICITIN, STEROL CARRIER PROTEIN

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CRYSTAL STRUCTURE OF HUMAN AUTOCRINE MOTILITY FACTOR

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Autocrine motility factor (AMF), a tumor-secreted cytokine, stimulates cell migration *in vitro* and metastasis *in vivo*. AMF is genetically identical to the extracellular cytokines neuroleukin (NLK) and maturation factor (MF) and, interestingly, to the intracellular enzyme phosphohexose isomerase (PHI). The crystal structures of the inhibitor-free open form and the inhibitor (E4P, erythrose 4-phosphate)-bound closed form of human AMF have been determined at 1.9 Å and 2.4 Å resolution, respectively. Upon E4P binding, local conformation changes (open to closed) occur around the inhibitor-binding site. The E4P-bound structure shows that the location of the inhibitor (of cytokine activity) binding site of human AMF is very similar to those of the inhibitor (of enzymatic activity) binding sites of PHIs. The present study clearly shows that there is structural overlap of the regions responsible for the enzymatic and cytokine functions of AMF/PHI and suggests two scenarios for the inhibition mechanism of cytokine activity of AMF by the carbohydrate phosphate. One likely scenario is that the compound could compete for AMF binding with the carbohydrate moiety of the AMF receptor (AMFR), which is a glycosylated seven-transmembrane helix protein. The other scenario is that the local conformation changes upon inhibitor binding may affect the AMF-AMFR interactions. Since the E4P is one of the smallest compounds having AMF inhibitor activity, knowledge of the present crystal structure would provide an insight into the lead compound design of more effective AMF inhibitors.

Keywords: ANGIOGENESIS CYTOKINE METASTASIS

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CONFORMATIONAL DYNAMICS OF A MYOGLOBIN TRIPLE MUTANT OBSERVED BY TIME-RESOLVED LAUE CRYSTALLOGRAPHY

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Myoglobin is a paradigmatic model to study the internal fluctuations of proteins and their implications in controlling function and accessibility to active sites. We have used single-bunch Laue diffraction to study with nanosecond time resolution conformational changes occurring in crystals of a triple-mutant of sperm whale myoglobin (L29Y, H64Q, T67R; denoted Mb-YQR) upon rupture of the Fe-CO bond by laser photolysis. Outstanding crystal quality, high photolysis level, optimisation of the ESRF ID09 beamline and efficient data processing gave complete data sets to 1.55 Å resolution from 3ns to 3ms after photolysis. As already observed for wild-type myoglobin, CO dissociation induces an immediate displacement of the iron out of the heme plane as well as bending of the heme pyrrole ring C towards the distal pocket. However, several features unique to Mb-YQR were discovered. Immediately after dissociation, Y29 swings towards the CO binding location to fill the vacant space. Strikingly, the rotation of Q64 to establish a hydrogen bond with Y29 extends to the microsecond timescale, dragging helix E towards its position in the deoxy state of the protein. On this timescale, other significant motions of residues and water molecules are identified on the distal site, whereas a transient occupation of the xenon 1 cavity is observed on the proximal site, presumably due to CO still trapped in the matrix. These results significantly advance our understanding of the conformational relaxation dynamics of myoglobin. They provide structural evidence of their extended time course, as discovered in the past by time resolved spectroscopy.

Keywords: MYOGLOBIN, CONFORMATIONAL DYNAMICS, LAUE CRYSTALLOGRAPHY

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X-RAY SNAPSHOTS OF QUINONE COFACTOR BIOGENESIS IN BACTERIAL COPPER AMINE OXIDASE

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Copper-containing amine oxidase catalyzes the oxidative deamination of various biogenic primary amines to the corresponding aldehydes, ammonia, and hydrogen peroxide. The enzyme also contains a covalently bound organic cofactor, 2,4,5-trihydroxyphenylalanine (topa) quinone, which is formed by post-translational modification of a specific tyrosine residue in the presence of cupric ion and molecular oxygen (called biogenesis). To elucidate the mechanism of the topa quinone biogenesis, we analyzed intermediate structures during the biogenesis in phenylethylamine oxidase from Arthrobacter globiformis. Apo enzyme crystals were anaerobically soaked in copper solution and freeze-trapped for determination of the initial structure of the topa quinone biogenesis. To see the structure in the following stage, we started the reaction by exposing the copper-bound crystals to the air, and freeze-trapped them immediately and sufficiently long after the exposure The absence of topa quinone, absorbing at 480 nm, was confirmed by single-crystal microspectrometry before crystals were subjected to X-ray diffraction measurements. Diffraction data of these crystals at cryogenic temperature were collected on CCD cameras installed in the BL44B2 and BL44XU stations at SPring8, Japan. The structures of these crystals were evaluated carefully by using several electron density maps and refined by using a program XPLOR. The structures of three district intermediates were determined at atomic resolution. The molecular mechanism of the topa quinone biogenesis will be discussed on the basis of these X-ray snapshots.

Keywords: TOPAQUINONE BIOGENESIS, COPPER AMINE OXIDASE, FREEZE-TRAPPED INTERMEDIATE