03-Crystallography of Biological Macromolecules

PS-03.07.21 CRYSTAL STRUCTURES OF LYS49 PHOSPHOLIPASES A2 FROM *BOTHROPS ASPER* AND *BOTHROPS GODMANI* VENOMS by R.K.Arni[1]*, R.Ward[2] and J.M.Gutierrez[3], [1]Department of Physics, UNESP-IBILCE, Sao Jose do Rio Preto-SP, Brazil. [2]EMBL, Heidelberg, FRG. [3]Instituto Clodomiro Picado, Costa Rica.

Recent sequence analysis of phospholipases A2 has indicated the existence of a variant whose characteristic feature is the complete absence of catalytic activity (Maraganore et al., 1987, J.Biol.Chem., 259, 13839) as a result of the substitution of Asp49 by Lys49 in the calcium binding loop. These proteins have been shown to cause liposome leakage by a novel calcium independent process (Rufini et al., 1992, Biochem., 31,12424). Crystals of B.asper PLA are orthorhombic, space group P2(1)2(1)2(1), a=50.2, b=67.8 and c=88.0A, contain a dimer in the asymmetric unit and diffract to 2.8A. Crystals of B.godmani PLA are tetragonal, space group P4(1)2(1)2, a=b=60.6, c=84.7A, Contain a monomer in the asymmetric unit and diffract to 2.6A. The structures have been determined by molecular replacement and refined by simulated annealing (X-PLOR, Brunger, A.T., 1988, J. Mol. Biol., 203,803) and conventional least-squares methods. The results of the structure determinations and structural implications determinations and structural implications of the substitution of Asp49 by Lys49 will be presented.

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PS-03.07.22 X-RAY CRYSTALLOGRAPHIC ANALYSIS OF THE PHOTOSENSITIVE NITRILE HYDRATASE. J. Honda*, N. Kamiya, T. Nagamune, K. Kiribuchi, H. Sasabe, H. Iwasaki and I. Endo, The Institute of Physical and Chemical Research (RIKEN), Saitama 351-01, Japan.

The nitrile hydratase (NHase) hydrates various nitrile compounds to the corresponding amides (Asano, Y. *et al.*(1980). *Agric. Biol. Chem.* 44, 2251-2252). The NHase from bacteria *Rhodococcus* has active and inactive forms, and the inactive form is readily converted to active form by light irradiation (Nagamune, T. *et al.*(1990). *Biochem. Biophys. Res. Commun.* 168, 437-442). This photosensitive NHase consists of 2 subunits $\alpha\beta$ each with molecular weights of 22,787 (206 residues) and 23,428 (212 residues) respectively, and it has 2 iron atoms per molecule and a quinone-like cofactor (Nagamune, T. *et al.*(1991). *J. Mol. Biol.* 220, 221-222). In order to understand its enzymatic mechanism and ultimately the photoactivation mechanism, we have performed an X-ray crystallographic analysis of this NHase. The inactive form of NHase, which is a more stable form of the enzyme, was purified from *Rhodococcus*, and crystallized by vapor-diffusion method. The crystal belongs to orthorhombic system with space group $P2_12_12$, cell dimensions of a=117.4 Å, b=145.7 Å, c=52.1 Å and V_m value of 2.4Å^3/Da with 2 molecules per asymmetric unit as described previously (ibid.). The intensity data of the inactive NHase crystal was collected using Weissenberg camera and Imaging Plate (Sakabe, N. (1991). *Nuclear Instr. Methods Phys. Res.* A303, 448-463) at beamline 6A2 in the Photon Factory, KEK, Japan. The data of up to 2.2 Å resolution was collected from only one crystal with a total of 155,481 observed reflections and 33,167 independent reflections, processed using WEIS (Higashi, T. (1989). *J. Appl. Crystallogr.* 22, 9-18) which was 72% of the total reflections measurable at this resolution. The data gave an overall Rmerge ($\Sigma|I-\langle I\rangle|/\Sigma I$) of 6.8%. The rotation function in polar angles was calculated using POLARRFN (by W. Kabsch) which indicated a non-crystallographic 2-fold axis parallel to the a-b plane at ϕ=45°. This result is consistent with the image of transmission electron microscopy of NHase microcrystals which reveals the molecular packing of the NHase. A survey of heavy atom derivatives is currently in progress.

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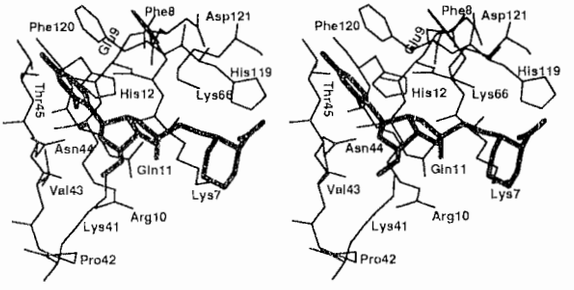
PS-03.07.23 MONOCHROMATIC X-RAY STRUCTURE ANALYSIS OF RIBONUCLEASE A AS A PRELIMINARY TO TIME-RESOLVED LAUE ANALYSIS. By T. Nonaka*, E. Tanno and Y. Mitsui, Department of BioEngineering, Nagaoka University of Technology, Kamitomioka, Nagaoka, Niigata, Japan.

We plan to study dynamics of reaction mechanism of bovine pancreatic ribonuclease A (EC 3.1.27.5), which cleaves single-stranded RNA, by time-resolved Laue method. To detect a substantial fraction of unique reflections in a single shot using a Laue camera developed by Dr. N. Watanabe of Photon Factory at Tsukuba, Japan, high symmetry crystal form is preferable. We have crystallized intact ribonuclease A (Sigma Type XII-A) by a vapor diffusion method to yield trigonal $P3_221$ ($a = b = 64.19$ Å, $c = 64.96$ Å, $\gamma = 120$ °) crystals. Monochromatic X-ray diffraction data were collected at 15 °C using a Rigaku R-axis IIc imaging plate detector, using one crystal. The data set with intensity $|F| \geq 1\ \sigma$ is 75.7 % complete to 1.7 Å resolution with an R_{merge} of 0.049. The coordinate set of a semisynthetic ribonuclease A (Martin, P. D. *et al.*, (1987). *J. Biol. Chem.* **262**, 15930-15938; Brookhaven Protein Data Bank code 1SRN) was used as a search model for rotation and translation search. The catalytic center of the current model is shown below with a proposed caged substrate (an *o*-nitrobenzyl derivative of cyclic cytidine 2',3'-monophosphate) superimposed.



PS-03.07.24 THE 3-D X-RAY STRUCTURES OF (1-3,1-4) -ß- GLUCANASE AND (1-3,1-3) -ß-GLUCANASE FROM BARLEY GRAIN TO 2.6A RESOLUTION. By J.N. Varghese*, T.P.J. Garrett, L. Chen[a] P.B.Hoj[a] and G.B. Fincher[a], Biomolecular Research Institute, Parkville, Victoria, Australia 3052,[a] La Trobe University, Bundoora, Victoria, Australia 3083.

The most important enzymes in the depolymerizatoin of the walls of the starchy endosperm in germinating barley are the (1-3,1-4) -ß-glucan 4-glucanohydrolases. These enzymes catalyse the hydrolysis of (1-4) -ß-glycosyl linkages in (1-3,1-4) -ß-glucans, only where the glycosyl residue is preceded by a (1-3) linked glycosyl residue. The (1-3,1-3) -ß-glucanase is also expressed at relative high levels in germinating barley, but the functional significance is unclear and could be related to its antifungal properties.

The 3-D structure of the (1-3,1-4) glucanase has been determined by M.I.R. methods to 2.6 A, using three heavy atom derivatives and anomalous dispersion. The 3-D structure of the (1-3,1-3) glucanase which has 50% sequence homology with the (1-3,1-4) glucanase, was solved by molecular replacement using a 3Å MIR electron density of the (1-3,1-4) enzyme, and subsequent non-crystallographic symmetry averaging (there are two independent images in the asymmetric unit.

