The Structure determination of S-ovalbumin, the stabilized form of ovalbumin by alkaline treatment

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Ovalbumin, a major component of egg white proteins, is grouped into a family of serine proteinase inhibitors (SERPIN), which controls serine proteinases involved in diverse physiological reactions, because of the close similarity in the primary and tertiary structures. SERPIN undergo a unique conformational change upon exertion of inhibition; after receiving the proteolytic cleavage at P1-P1' site, the reactive center loop is inserted into the central β-sheetA, thereby driving the trapped proteinase to the far opposite site. But ovalbumin is non-inhibitory and lacks the loop insertion mechanism due to the side chain of the P14 site, which works as a hinge for the loop-insertion, is Thr in most inhibitory serpins, but in ovalbumin it is a bulkier Arg339. We could recently proved this hypothrsis by the X-ray structural analysis of an ovalbumin mutant R339T in which Arg339 is replaced by Thr residue.

One of the most interesting phenomena is the presence of S-ovalbumin which is a stable form derived by alkaline treatment of ovalbumin. S-ovalbumin seems to be a conformer of ovalbumin caused by a small chemical composition change. Though several hypotheses have been presented to explain this molecular conversion, the structure of S-ovalbumin has not been determined. It should be clear that the loop insertion process is involed in the formation of S-ovalbumin from the open loop form of ovalbumin.

In the present beam time, we have examined the diffraction of the crystal of S-ovalbumin in order to determine the fine structure of S-ovalbumin. S-ovalbumin was prepared by incubation of ovalbumin with 0.1 M glycine/NaOH buffer for one week at pH 9.9 and 30°C. S-ovalbumin was then purified by ion exchange column chromatography. It was crystallized by hanging-drop vapor diffusion method using ammonium sulfate as a precipitant. The resultant crystals belong to a space group of P1 with cell dimensions of a = 62.441, b = 70.493, c = 82.419 Å, α =87.341, β = 71.735 and γ = 75.849°. Four molecules were estimated in an asymmetric unit. The diffraction data was collected with λ = 0.9 Å and a Mar Research CCD detector adjusted the detector-crystal distance of 154 mm under a cold nitrogen gas stream at BL41XU beamline. The crystals were freezed in liquid nitrogen after a bleaf incubation with cryosolvent containing the mother liqid and 15% glycerol. Total of 360 images were collected with the exposure time of 5 s/frame and the phi rotation angle of 1°/frame. The collected images were processed with a program of HKL2000. Total of 380,829 reflections containing 109,050 unique data were collected with 91.1% completeness and R_{merge} of 0.074 up to 1.8 Å resolution.

We are now trying the structure determination of S-ovalbumin by molecular replacement method by using the coordinate of ovalbumin with a program pakages of CNS-SOLVE and CCP4.

X-ray Crystallographic Study of the Vesicular Assemblies of Membrane Proteins

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Some membrane proteins are known to form uniformly-sized vesicular assemblies when they are co-crystallized with native lipids. One example is bacteriorhodopsin, a light-driven proton pump found in H. *salinarium*. Another example is the light-harvesting chlorophyll protein complex (LHC-II) from pea thylakoid membranes. In the case of bacteriorhodopsin, spherical vesicles of a diameter of ~50 nm are formed in a pre-crystallization solution containing the detergent octylthioglucoside and, above a critical concentration of precipitant, the vesicles are packed into an optically isotropic crystal. In the case of LHC-II, vesicular assemblies of a diameter of 27 nm are formed in a pre-crystallization solution containing nonylglucoside and, at a high precipitant concentration, the vesicles are packed into an optically isotropic crystal. The vesicular assemblies of these membrane proteins are not always stable. In fact, slight alteration of the crystallization condition induces fusion of the bacteriorhodopsin vesicles, yielding a birefringent hexagonal crystal. This crystal has been shown to be composed of stacked membranes, in each of which the trimeric protein-lipid complexes are arranged in a honeycomb lattice. Similarly, LHC-II can be crystallized into a birefringent hexagonal cryatal which diffracts X-rays up to 3 Å resolution. For better understanding of the crystallization mechanism, it is important to determine the detailed structure of the vesicular assemblies.

In this study, we investigated an octahedral crystal of bacteriorhodopsin that was produced by close packing of the vesicular assemblies at low temperature. X-ray diffraction data show that this crystal belongs to the space group *F23* with cell dimensions a= b= c= 747 Å. Since the Bragg diffraction spots were observable only at a small scattering-angle region (< 45 Å), it was difficult to carry out a conventional crystallographic analysis. Instead, we investigated the dependence of the diffraction intensity on the reflection angle. When the intensity is plotted against the scattering angle, a periodic variation is seen. Such a variation would be expected for a crystal that is composed of hollow spheres. But the whole part of the diffraction data was not fully explained by any simple spherical-shell model. Since the bacteriorhodopsin vesicle contains native lipids whose hydrocarbon chains have a lower electron density than that of the bulk solvent, it is rationale to assume that the amplitude of the structure factor at a small scattering angle is given by the following equation

$$\langle|F(s)|\rangle = (\rho_{lip}-\rho_{sol})\,FT(M_{lip})\exp(-B_{lip}s^2) + (\rho_{pro}-\rho_{sol})\,FT(M_{pro})\exp(-B_{pro}s^2)$$

where ρis the average electron density of the lipid, the protein or the bulk solvent region, and FT(M) is the fourier transform of the mask of the lipid or the protein region; the exponential represents a dumping factor reflecting fluctuation or irregularity of the shape of each mask. The result shows that the bacteriorhodopsin vesicle has a diameter of 460 Å at the outer surface. This is much smaller than the value expected for tight packing of the vesicular assembly, suggesting that the C-terminal tail of the protein extrudes largely from the outer surface of the vesicle.

The same procedure was used to analyze diffraction data of the octahedral crystal of LHC-II that belongs to *F23* with cell dimensions a = b = c = 360.6 Å. The result suggests that the LHC vesicle is composed of a high electron-density domain with a thickness of 45 Å and a low electron-density shell with a thickness of 28 Å.