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Structure of the Chitin-Protein Complexes in Insect Cuticle

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INTRODUCTION

Insect cuticle, where slender rod-like chitin filaments are embedded in a globular protein matrix, is an ideal fiber-reinforced composite. The composite structure realizes the extraordinary features of the material: tough and resilient but not brittle. In this study, therefore, we tried to analyze the structure of insect cuticle from the ovipositor of the ichneumon fly, in which chitin filaments were uniaxially oriented. For comparison, we also analyzed squid pen that is essentially a chitin-protein complex.

EXPERIMENT

Dried ovipositors of *Megarhyssa praecellens* and squid pens of *Todarodes pacificus* were analyzed without any treatment at the BL40B2 at the SPring-8, JASRI. They were X-rayed with the incident beam orthogonal to the long axis for 30-120 s with synchrotron radiation of wavelength 0.1 nm. The X-ray diffraction patterns were recorded on imaging plates (IP), and the IP to sample distance were calibrated using lead stearate (d = 4.97 nm).

RESULTS AND DISUSSION

Figure 1 shows small angle X-ray diffraction patterns of the ovipositor (A) and the squid pen (B).

In Figure 1A, three small angle reflections were observed on the equator at d = 6.44 nm, 3.73 nm, and 3.39 nm. This corresponds to hexagonal packing of chitin filaments in protein matrix with diameter a = 7.5 nm. However, only one broad small angle reflection was observed at d = 4.09 nm in Figure 1B. The arrangement of chitin filaments in the squid pen is less ordered.

Extra layer lines, which derived from protein were appeared with a spacing of 3.1 nm in Figure 1A; exactly three times of the classic chitin layer lines spacing of 1.03 nm. On the other hand, in Figure 1B, three small angle reflections d = 16.0 nm, 8.33 nm, and 5.11 nm were observed on the meridian. This result may suggest that squid pen protein has a periodicity about sixteen times of the chitin periodicity, 1.03 nm.

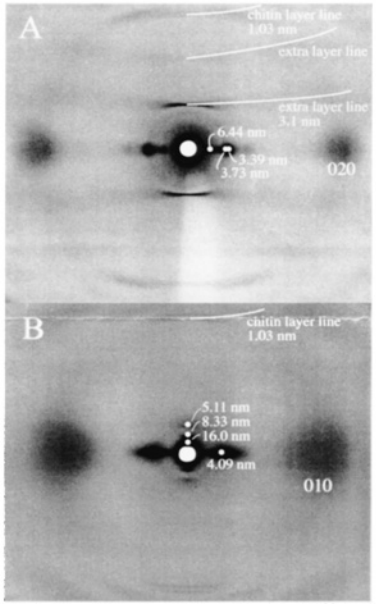

Figure 1 Small angle X-ray diffraction patterns of the ovipositor (A) and the squid pen (B).

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Crystallographic Studies on Yeast Homing Endonuclease VDE in Complex with Double-Stranded DNA Substrates

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Endonuclease VDE is a site-specific gene-homing endonuclease of *Saccharomyces cerevisiae*, which cleaves the non-palindromic sequence in the particular allele of the *VMA1* gene lacking the coding region for VDE itself. The nascent protein product of the *VMA1* gene undergoes folding-dependent protein splicing that excises out a 50-kDa intervening VDE segment from the polypeptide precursor and concomitantly joins N- and C-terminal regions into the 70-kDa subunit of the vacuolar H^+-ATPase. Using X-ray crystallographic methods, we recently elucidated three-dimensional structures of the splicing-precursor VDE and also of the generic VDE in complex with a 19-base-pair (bp) double-stranded DNA bearing a part of the *VMA1* sequence. For the purpose of further understanding of the structural mechanism of the specific recognition of the substrate DNA as well as the double-strand cleavages in which VDE requires Mg^{2+} ions, we carried out crystallographic studies on VDE in complex with pre-cleaved and half-cleaved substrates of double-stranded DNAs.

The VDE specimen was prepared in large quantities using an *E. coli* expression system. The specimen was precipitated from the cell lysate by ammonium sulfate and purified through steps of column chromatography. Purified DNA strands from solid-phase syntheses were annealed with corresponding complementary strands so as to form double strands. The prepared double-stranded DNAs were mixed with the VDE specimen, and resultant mixtures were subjected to crystallization trials using polyethylene glycol 3350 precipitants. The crystals of VDE complexes were obtained for 23-bp half-cleaved and 29-bp pre-cleaved DNAs in the absence of metal ions, and also for the 19-bp DNA in the presence of soaked Ca^{2+} ions.

Diffraction intensities were collected from these crystals at 100 K using synchrotron X-rays of the wavelength of 1.0000 Å and an ADSC Quantum 4D CCD detector equipped at the SPring-8 BL40B2 station. The crystals measured are isomorphous to each other, belonging to the $P2_1$ space group with estimated cell-parameters of a= 100 Å, b= 65 Å, c= 121 Å, and β= 104°. The data sets as processed with the HKL2000 suit were obtained for the crystals of the half-cleaved, pre-cleaved, and Ca^{2+}-soaked complexes at resolutions of 3.1 Å, 3.0 Å, and 2.8 Å, respectively.

Molecular replacement solutions were performed with the CNS program for a search model obtained from the 19-bp DNA complex. Through subsequent rigid-body and simulated-annealing refinements, the Ca^{2+}-soaked crystal was refined to an R factor of 21.6 %. Two Ca^{2+} ions substituted for Mg^{2+} ions were located between the phosphorus atoms of the scissile bonds and the side-chain atoms of the aspartic acid residues that are possibly involved in the enzymatic catalysis.

The crystal of the complex with the half-cleaved DNA is refined to the R factor of 24.5 %, and shows two nucleotides lying in an extension of the half-cleaved DNA. The structure refinement of the pre-cleaved complex is now in progress.