The Use of Microbeam X-Ray Diffraction for the Characterization of Crystal Structure of Rice Starch

Starch consists mainly of amylose and amylopectin. It occurs as water insoluble particles in the form of "starch granules" in plant tissue, and is generally classified into three types (A, B, and C) according to the wide-angle X-ray diffraction (WAXD) pattern given by their amylopectin crystalline structures. Cereal starch, including rice starch, had an A-type WAXD pattern, tuber starch a B-type, and bean and root starch a C-type (a mixture of A- and B-types) [1].

A genetic analysis using amylopectin mutants showed that a specific gene was important in determining the amylopectin structure and crystal pattern. The *amylose-extender* (*ae*) mutant, with a defect in the starch branching enzyme IIb function, accumulated starch which had a reduced ability to gelatinize due to a change in the fine structure of amylopectin with enriched long chains. The powder WAXD analysis of purified *ae* starch showed a B-type diffraction pattern, whereas wild-type rice starch showed an A-type. The *sugary1* mutant, with reduced isoamylase 1 activity, accumulated water-soluble polyglucans named as phytoglycogen. The phytoglycogen contained more short-glucan chains than wild type amylopectin, in contrast to the enriched longer glucan chains in *ae* amylopectin. In some *sugary1* lines, endosperm cells were clearly separated into a starch region and a phytoglycogen region as evidenced by iodine staining [2].

The localization of starch granules in the kernel is important, because it influences the gelatinization properties of rice. Powder WAXD analysis, which was typically used to characterize the crystalline structure of starch, tells us nothing about localization because the starch for this analysis is generally extracted and purified from tissue, destroying localization information and possibly damaging the starch granules. The aim of this study was to visualize starch localization and the starch crystalline structure by microbeam WAXD analysis.

We used mutant lines generated by treating of fertilized egg cells of japonica rice cv Kinmaze (wild type) with *N*-methyl-*N*-nitrosourea [3]. The use of double mutants that include a *waxy* (*wx*) defective gene ensures that the starch consists of essentially amylopectin, which makes up most of its crystal structure. The mature kernels from *ae*, *wx* double mutant (*wx/ae*) and *sugary1*, *wx* double mutant (*wx/sugary1*) were used as materials. The experiments were performed at beamline **BL40XU** with a high-flux beam ($\lambda = 0.083$ nm) of 5 μm in diameter. WAXD patterns were recorded using an image intensifier coupled to a cooled CCD camera [4]. 0.2 mm and 0.02 mm thick slices were cut from rice kernels using a cryostat, and the crystalline structure was scanned sequentially in constant intervals as shown by the arrows in Fig. 1.

Microbeam WAXD analysis visualized the starch localization and crystalline structure (Fig. 2). Amylose-free starches in the native rice kernels of the *wx* and *wx/ae* mutants showed A- and B-type WAXD patterns, respectively, with no difference between the outer and inner regions of the patterns. In contrast, the *wx/sugary1* mutant kernel showed an A-type diffraction pattern in the outer region and amorphous in the inner region. The higher intensity at small angles in the outer regions of *wx/sugary1* (scanning positions near 0 and near 1600) is likely due to scattering derived from density fluctuations in *sugary* amylopectin clusters. A chain-length distribution analysis of polyglucans in wild type and mutant kernels showed that *ae* amylopectin had more long chains and fewer short chains than the wild type and *waxy* amylopectin. In the *wx/sugary1* kernel, the phytoglycogen in the inner region had many more short chains than the amylopectin in the outer region had. These results indicate that the branch chain length in polyglucans is crucial in determining the starch crystalline structure [5].

Several lines of *ae* mutants with mutations at same locus but with varying phenotypes have been isolated previously. The starch from *ae* mutant line EM16 was a typical B-type detected by powder WAXD but that from EM129 was C-type. When microbeam WAXD analysis was applied to the EM129 kernel,

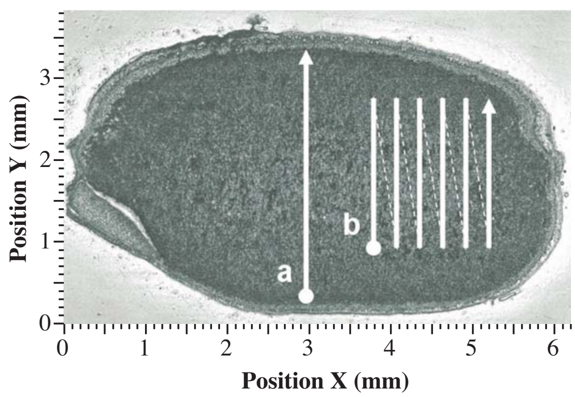

Fig. 1. A rice kernel slice of wild type under the microscope. WAXD profiles were scanned linearly **(a)** or two-dimensionally **(b)**.

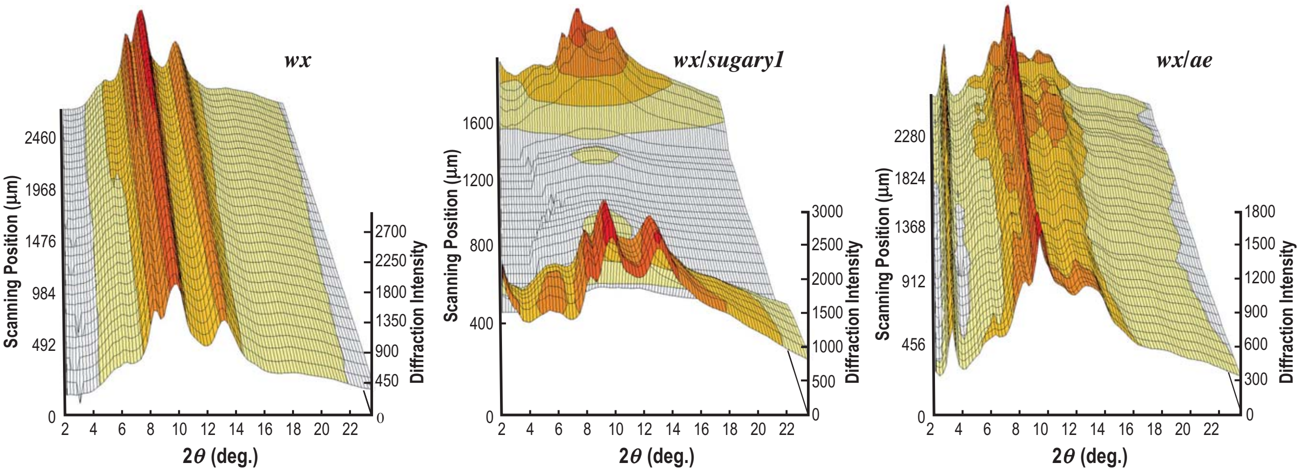

Fig. 2. Microbeam WAXD profiles of rice kernel starch granules. Diffraction patterns were recorded sequentially at intervals of 25 µm along the arrow **(a)** in Fig. 1.

C-type diffraction patterns scanned at different positions were classified to Ca- and Cb-type (Fig. 3). This result agreed with the idea that the C-type is the mixture of A- and B-type crystalline structures [1]. We did not observe any pattern in the regional distribution of Ca- and Cb-type starch. In conclusion, microbeam WAXD is a useful method to map the distribution of starch crystalline structures in the rice kernel.

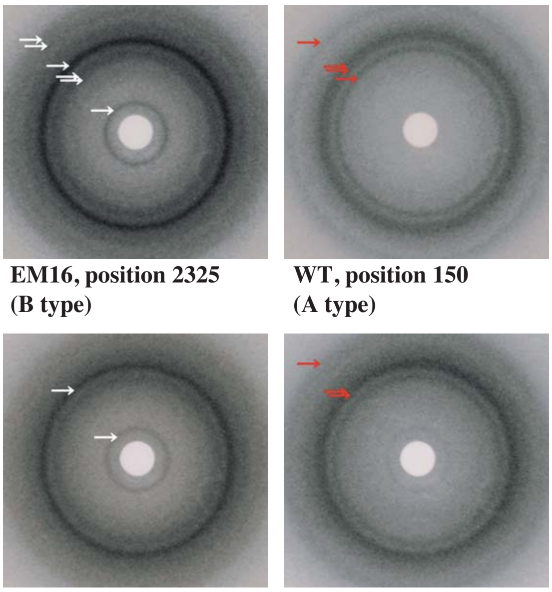

Fig. 3. Microbeam WAXD patterns detected using thin slices (20 µm). Diffraction patterns were recorded as shown in **(b)** of Fig. 1. White and red arrows show B- and A-type specific signals, respectively. C-type patterns from an *ae* kernel of EM129 were classified as Ca- and Cb-type.

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