

Prospects for X-ray Crystal Structure Analysis of Selenoproteins with SPring-8 Synchrotron Radiation

Masaki Yamamoto,^a Takashi Kumasaka,^a Eiki Yamashita,^b Hideaki Moriyama,^c Mamoru Sato,^{*,d} and Tatzuo Ueki^c

^aThe Institute of Physical and Chemical Research (RIKEN), 1-1-1 Kouto, Mikazuki-cho, Sayo-gun, Hyogo 679-5148, Japan, ^bInstitute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871 Japan, ^cJapan Synchrotron Radiation Research Institute (JASRI), 1-1-1 Kouto, Mikazuki-cho, Sayo-gun, Hyogo 679-5198, Japan, and ^dGraduate School of Integrated Science, Yokohama City University, 22-2 Seto, Kanazawa-ku, Yokohama 236-0027, Japan

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The impact of synchrotron radiation as a new X-ray source with its polychromatic nature and associated high intensity and fine collimation has brought important advances in the field of macromolecular crystallography. It has extended structure determinations of proteins to higher resolution, allowed use of smaller crystals with larger unit cells. In particular, selenoprotein is a suitable material for X-ray crystal structure analysis with synchrotron radiation, since its polychromatic nature and anomalous diffraction from the selenium atom(s) in the protein allow the multiple-wavelength anomalous-diffraction (MAD) method to be used for phase determination. RIKEN beam line I (BL45XU), installed in the SPring-8 synchrotron radiation facility, has been designed and developed to optimize MAD data collection based on a 'trichromatic concept'. This concept facilitates simultaneous data collection by use of a 'trichromator', of three intensity data-sets at three different wavelengths from a single protein crystal, and thus results in the minimization of systematic errors in the measurement of anomalous diffraction by the MAD method. The X-ray crystallographic analysis of selenoprotein with SPring-8 synchrotron radiation, therefore, results in very fast data collection and high resolution structural analysis using a single protein crystal, which should lead to elucidation of the structure-function relationship of selenoprotein.

Key words — selenoprotein, synchrotron radiation, MAD method, anomalous diffraction, trichromatic concept

INTRODUCTION

Synchrotrons are devices that circulate electrically charged particles such as electrons and positrons at nearly the speed of light. When the orbit of the relativistic particle is changed in a magnetic field, the electrons or positrons are accelerated toward the center of the ring and, thus emit electromagnetic radiation, which is so called synchrotron radiation.¹⁾ The main advantage of synchrotron radiation for macromolecular crystallography is the high intensity of its fine collimated beam, which

reduces the exposure time of a weakly diffracting crystal and improves the signal-to-noise ratio. In the case of the SPring-8 synchrotron facility, the intensity is much brighter than for a conventional X-ray source. Synchrotron radiation also differs from the conventional laboratory source in the spectrum of X-rays. Its continuous spectrum with high intensity allows tunability of wavelength. Any suitable wavelength over the wide spectral range can be selected with a monochromator. This property is very useful for the multiple-wavelength anomalous diffraction (MAD) method²⁻⁴⁾ for phase determination in the crystal structure analysis of proteins.

The MAD method, however, requires anomalous scatterer(s), usually native-metal or heavy atoms, that give a significant signal of anomalous diffraction from a protein crystal at a particular wavelength. A conventional method to introduce heavy

*To whom correspondence should be addressed: Graduate School of Integrated Science, Yokohama City University, 22-2 Seto, Kanazawa-ku, Yokohama 236-0027, Japan. Tel.: +81-45-787-2306; Fax: +81-45-787-2306; E-mail: msato@yokohama-cu.ac.jp

atom(s) into non-metalloproteins is ‘soaking’ the protein crystal in a stock solution containing a heavy atom compound that soaks into the crystal.⁵ Recently, gene technology has produced a new method to prepare heavy atom derivatives of non-metalloproteins. The method whereby methionines in proteins are genetically replaced by selenomethionines is more effective as compared with the soaking method.^{6,7} This is based on the fact that selenium is an atom that gives significant anomalous diffraction in the wavelength range covered by the SPring-8 synchrotron radiation. We describe herein the RIKEN beam line I (BL45XU), installed in the SPring-8 synchrotron radiation facility, designed and developed to optimize MAD data collection, and describe the prospects for X-ray crystal structure analysis of selenoproteins.

Multiple-wavelength Anomalous Diffraction Method Using the Trichromatic Concept⁸⁾

The most critical problem in macromolecular crystallography is phase determination of X-rays diffracted from the crystal, the so-called ‘phase problem’. The multiple isomorphous replacement (MIR) method⁹⁾ is mostly used to solve the phase problem, and requires at least two kinds of heavy-atom derivative, which modify chemically their native crystal with heavy atom(s) and should be isomorphous with the native crystal. However, preparation of the derivatives is problematic and two heavy-atom derivatives are often hard to prepare. On the other hand, the MAD method solves the phase problem by using diffraction data collected from one crystal of metalloprotein or heavy atom derivative of non-metalloprotein, which is not necessarily isomorphous with its native crystal.

Data collection for structural analysis by the MAD method is made using X-ray radiation of two or more different wavelengths. The trichromatic concept, using a ‘trichromator’ has been devised to satisfy the requirement for data collection of a single protein crystal at three different wavelengths. The schematic drawing of RIKEN beam line I (BL45XU) using the trichromatic concept is shown in Fig. 1. This concept is advantageous in terms of reduced data-collection time without changing the crystal and device setting, which results in minimizing systematic errors such as those from absorption, detector characteristics and radiation damage.

The trichromator produces three different monochromated X-rays with wavelengths of λ_1 , λ_2 , and λ_3 . λ_1 is called ‘remote’ and set at a value remote

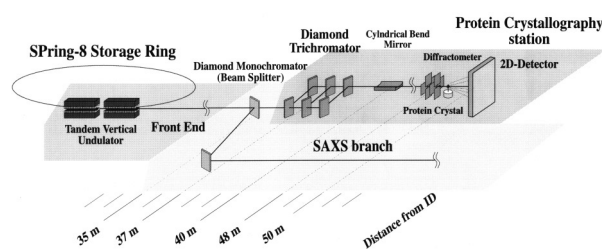


Fig. 1. The Schematic Drawing of RIKEN Beam Line I (BL45XU) Using the Trichromatic Concept

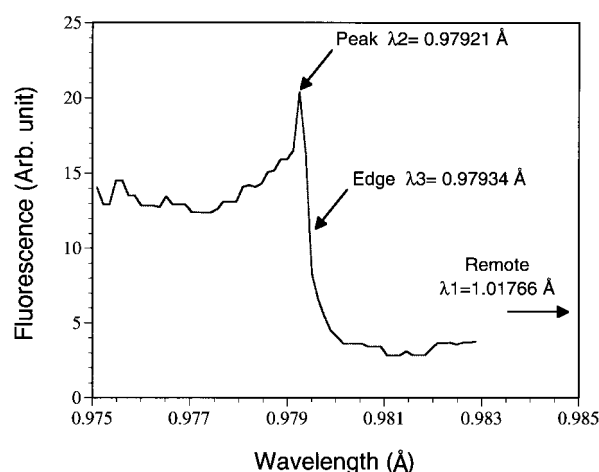


Fig. 2. X-Ray Fluorescence Spectrum of a Selenoprotein Crystal, in Which All the Methionine Residues Are Genetically Modified by Selenomethionines

The three wavelengths of X-rays that correspond to λ_1 (‘Remote’), λ_2 (‘Peak’) and λ_3 (‘Edge’) are indicated.

from an absorption edge of the heavy atom that shows significant anomalous diffraction. λ_2 , λ_3 are called ‘peak’ and ‘edge’, respectively and are set at values near and on the absorption edge to maximize the contribution of the anomalous diffraction effect. The absorption edge is determined from the X-ray fluorescence spectrum of the protein crystal by using a Si-PIN detector. Figure 2 shows the fluorescence spectrum of a selenoprotein crystal, in which all the methionine residues are genetically modified by selenomethionines. Once the three X-ray beams are tuned at three wavelengths for data collection, they are sequentially supplied without changing any experimental setting and the data collection is successively performed at each wavelength so as to minimize the background scattering arising from X-ray radiation of other wavelengths.

Configuration of Trichromator⁸⁾

The trichromator is composed of three pairs of transparent crystals arranged collinearly with fixed exits and introduces three monochromatic X-rays,

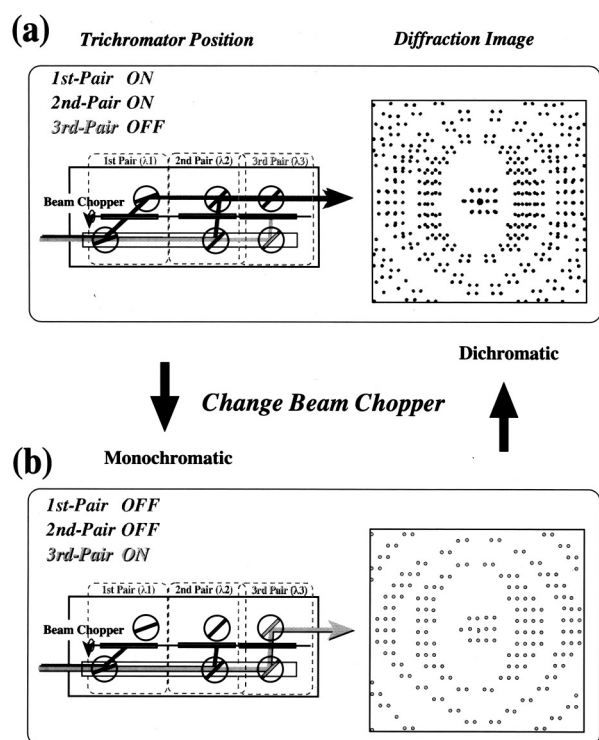


Fig. 3. Configurations of Trichrometer (Left) and Predicted X-Ray Diffraction Images (Right) of (a) Dichromatic Mode Using X-Rays with Two Wavelengths and (b) Monochromatic Mode Using X-Ray with One Wavelength

The trichromator is composed of three pairs of diamond crystals arranged collinearly with fixed exits. The three pairs (1st pair, 2nd pair, 3rd pair) of crystals produce three different monochromated X-rays with wavelengths of λ_1 , λ_2 and λ_3 , respectively. Three beam choppers are located between each pair of diamond crystals for dichromatic or monochromatic mode.

with different wavelengths, in an identical beam direction (Fig. 3). All of the transparent crystals are synthetic diamonds with a size of 7×7 mm², 0.3 mm or less in thickness and transmit about 70% of the incident beam. The reflection planes of the crystals are chosen to be (4 0 0) with Bragg geometry, and fundamental energy range is covered from 7.5 to 14 keV.

The diamond crystals located above and under the beam chopper are mounted on four- and three-axis goniometers, respectively. The four-axis goniometer consists of Bragg-angle rotation and fine-rotation stages, a translation stage along the primary-beam direction, a translation stage on the rotation stage, and crystal-tilting and fine-tilting stages. The three-axis goniometer has a Bragg-angle rotation stage with a high-resolution encoder.

In the case of protein crystals with smaller unit

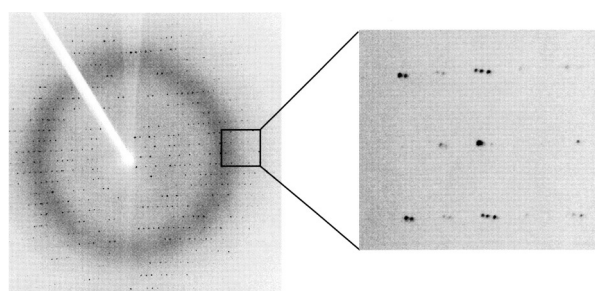


Fig. 4. X-Ray Diffraction Image of Hen Egg White Lysozyme Crystal, Taken at the RIKEN Beam Line I with Trichromatic Mode Using X-Rays with Three Wavelengths

cells, a high-efficiency mode is operated in the trichromator as shown in Fig. 3. In this mode, the beam chopper can select two or one wavelength(s) out of three to the experimental station and allows dichromatic ((a) in Fig. 3) or monochromatic mode ((b) in Fig. 3) for data collection. This operation mode dramatically reduces the time to collect the MAD data.

Observation of the X-ray Beam Emitted from the Trichromator

The validity of the trichromator was assessed by diffraction images of a hen egg white lysozyme crystal. As shown in Fig. 4, the trichromator successfully monochromated primary X-rays to those with three different wavelengths at the same time, giving three diffraction spots for each Bragg reflection on an X-ray detector (right in Fig. 4). These diffraction spots were displaced relative to one another according to incident X-rays with different wavelengths, indicating that the trichromator thus designed is well operated as designed and provides X-rays suitable for MAD analysis of protein crystals.

CONCLUSION

In conclusion, the 'trichromatic concept' is suited for crystallographic analysis of proteins, in particular selenoproteins. RIKEN beam line I based on this concept was developed to optimize MAD data collection and installed in a SPring-8 synchrotron radiation facility. It has been used for X-ray crystallographic analyses of many proteins and their crystal structures have been solved by the MAD method. Figure 5 shows a part of the electron density map of selenoprotein which was solved by the MAD method

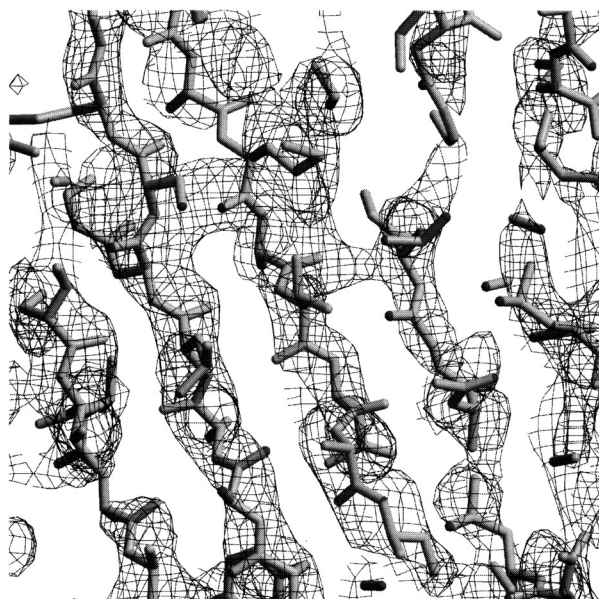


Fig. 5. A Part of the Electron Density Map of a Selenoprotein, in Which All the Methionine Residues Are Genetically Modified by Selenomethionines

using the RIKEN beam line I. The beam line should enhance the prospects for elucidation of the structure-function relationship of selenoprotein.

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