Why Do We Want Synchrotron Radiation for Determining Protein Structures?

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Abstract

SR sources have largely contributed to the development of protein crystallography. SR has made it possible to determine the structure of biologically important proteins for which structure analyses would be difficult with the conventional sources. SR has made time-resolved crystallography a practical tool. This review will describe what we can do by using SR to protein crystallography.

1. Introduction

Proteins that are the largest group of macromolecules in the living cells play central roles in all life processes. To understand cellular processes, knowledge of the three-dimensional structure of proteins is vital. Two techniques are widely used for the structure determination of proteins at atomic details: X-ray diffraction and nuclear magnetic resonance (NMR). NMR method can be used only for small proteins with a molecular weight of less than about 20,000. On the other hand, X-ray diffraction method can give us the three-dimensional structure of large proteins, as well as small ones, at the precision of about 0.1-0.2 Å. In the present stage, therefore, the only general method available is X-ray diffraction method. However, since the structural models obtained by the X-ray diffraction method are based on interpretation of experimental data with errors, their models might have some errors. In a few cases, therefore, preliminary protein structures obtained by the X-ray method have been shown to be incorrect. As shown in later, these incorrect models have been corrected by subsequent refinement based on additional experimental data with higher accuracy and resolution obtained by use of synchrotron radiation.

Progress in research on the protein structure by X-ray has been slow, not only because of the difficulty of solving the phase problem, but also because of the difficulty of obtaining crystals that are large enough to apply crystallographic analyses. In recent decades, however, the X-ray method has made progress rapidly with the developments of genetic technology, computer graphics devices, X-ray detectors, computer software for structure analysis, and especially synchrotron radiation (SR). During the past 10 years, in fact, the structures of about 1500 proteins have been solved by the X-ray diffraction method, and an understanding of functions on the basis of these X-ray structures has greatly developed structural biology. Contemporaneously, new technologies, such as 'protein engineering' and 'structure based drug design', have been born.

SR has revolutionized a field of protein crystallography.

Contributions of SR source to protein structure determinations can be classified into some categories described below(1). Helliwell has given detail of this topic(2).

2. Crystals with small sizes or crystals with large cells

Weak scattering is a feature of the protein crystals. This comes mainly from the two reasons. The first is that protein crystals consist mainly of light atoms such as C, N and O. The second is the large size of their unit cells. In conventional X-ray sources, a crystal size of 0.3-0.5mm in all dimensions is regarded as optimal. Crystallization of proteins is a hit-and-miss work with no theory. Some proteins crystallize readily, but others do not at all. Moreover, even though a large crystal was obtained, it occurs often that the signal / noise ratio of diffraction data is too poor to analyze the crystal structure. Therefore, protein crystallographers have been forced to carry out structure analyses of proteins of which crystals could be obtained, rather than proteins with interesting biological properties.

High intensity and well-collimated beam of SR overcomes these problems and enables even the use of a crystal whose volume is as small as 2.0×10^4 μm^3. This will set protein crystallographers free from the tedious work of crystallization. Although small crystals tend to suffer radiation damage, this can be overcome by freezing them.

Virus and ribosome crystallography is one of the main uses of SR. The structure of various virus particles whose unit cells are very large has been unraveled using SR, and these have given us ideas or blueprints for designs of new vaccines or drugs.

3. Collection of high-resolution data

High-resolution data collection plays an important role for building correct and accurate models. SR gives usually higher resolution data than conventional sources. This may be due to the well-collimated geometry and high intensity of SR. Radiation damage of crystals degrades the high-resolution data. Most crystals show both time-dependent and dose-dependent radiation damages. With the use of SR the former can be overcome with the short exposure time, and the latter can be overcome with the selection of short wavelength. The largest group of publications related to protein structure analyses using the present SR sources deal with high-resolution data collection. Uses of the third generation sources by which higher resolution data will be obtained make probably the application of direct methods to proteins possible.

4. Rapid data collection

SR reduces the crystallographic data collection time.

Exposure times for a full data set are of the order of minutes to hours with the present monochromatic SR beam. The rapid data collection makes it possible to elucidate the structure / function relationship by structure comparisons among a series of mutants, within a reasonable period of time (see Table 1 and Fig. 1)(3,4,5,6).

Table 1 Diffraction measurements of glutathione synthetase

Source	AFC	PF
Wavelength(Å)	1.54.	1.04
Resolution(Å)	2.7	2.0
Number of crystals used	18	2
Time of data collection	1 month	3 hours
Number reflections observed	32,629	166,734
Merging R-factor(%)	3.3	5.3

AFC : 4-circle diffractometer with rotating anode Generater.
PF : Photon factory; SR source with Sakabe camera

In Laue geometry, exposure times are the order of milliseconds or less. Indeed, Laue diffraction patterns of lysozyme have been recorded with an exposure time of 120ps by using an undulator as source. Rapid data collection has opened up possibility of doing time-resolved crystallographic studies.

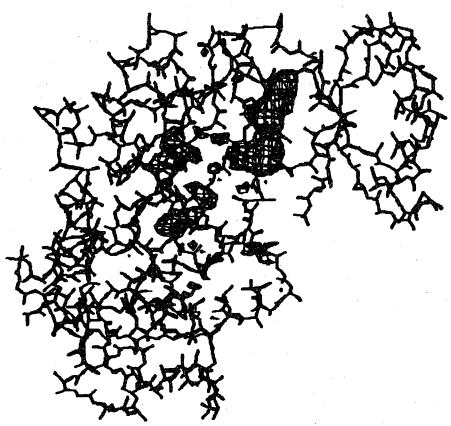

Fig. 1 Difference Fourier map superimposed on the main chain of glutathione synthetase (see also fig.2). Substrate binding sites were determined by the difference Fourier map calculated by using the data sets of the native and enzyme: substrate analogue complexes.

5. Optimized anomalous scattering

5.1 Distinction between metal ions with similar atomic numbers

SR differs from the conventional sources in its tunability. Any suitable wavelength in the spectral range can be selected with monochromater. For protein structure analyses, SR is usually turned to 1 Å or shorter wavelength. Tuning SR wavelength can optimize anomalous scattering of metal ions bound to protein molecules. This technique can be used to distinguish between similar metal ions. For example, the data set of SOD that contains Cu^{2+} and Zn^{2+} was obtained at wavelengths of 1.373 and 1.389 Å near the Cu K absorption edge (1.380 Å) and 1.275 and 1.299 Å near the Zn K absorption edge (1.287 Å). The Cu^{2+} and Zn^{2+} ions, which have only a one-electron difference, were successfully distinguished by comparing anomalous difference Fourier maps of these four different data(7).

5.2 Multiple wavelength anomalous dispersion method (MAD)

If a protein has anomalous scatters in its molecule, the difference in intensity between the Bijvoet pairs can profitably be used for the phase determination.

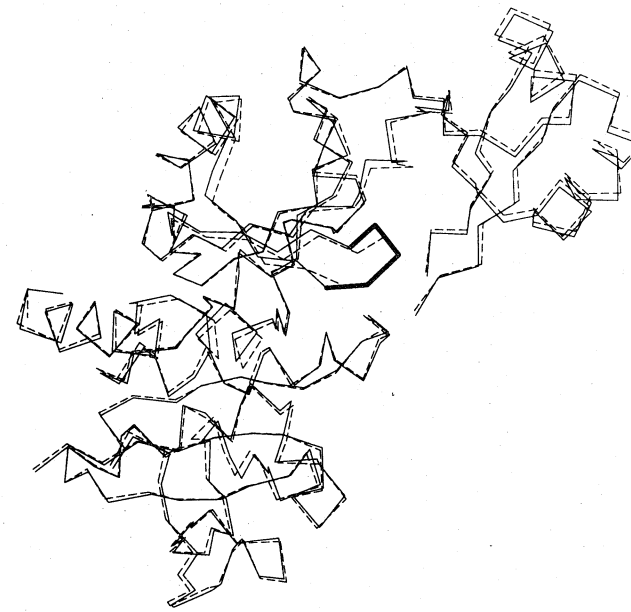

Fig. 2 Comparison of Cα backbone tracing of wild-type (dashed line) and loopless mutant of glutathione synthetase. Difference Fourir map shown in Fig. 1 suggested that the loop between Ile226 and Gly242 would play an important role to the specific function of this enzyme. In order to investigate the function of the loop, a loopless mutant was prepared by means of protein engineering techniques. The crystal structure of the loopless mutant was essentially identical with that of the wild-type enzyme, but the mutant lost its activity completely. Finally, it is confirmed that the loop in the enzyme stabilizes the intermadiate by preventing its decomposition by hydrolysis with water (4).

The principle of MAD method is rather old, but it became a technically feasible method by the advent of tunable SR sources. This method provides a more direct route to the protein structure determination.

5.3 Increase of phasing power:

The structure of a new protein is solved usually by means of multiple isomorphous replacement (MIR) that requires at least two different heavy-atom derivatives of the native protein. The anomalous scattering data of a heavy-atom derivative that was collected using appropriate wavelength works just like an additional heavy-atom derivative.

In *Serratia*-protease that contains Zn naturally, an useful heavy-atom derivative was only Sm. Diffraction data of native enzyme were collected with two wavelengths of 1.000 and 1.283 Å. The wavelength of 1.283 Å is shorter than that corresponding to the K absorption edge of Zn. The native data set collected at the wavelength of 1.000 Å were treated as the single "native" data set, while the native data set collected at the wavelength of 1.283 Å were treaded as different "Zn-derivative" data set. Diffraction data of the Sm-derivative were collected by two methods; with SR source(λ =1.283 Å) and with conventional source(Cu K α radiation, λ =1.541 Å). Thus, this enzyme structure was solved by single isomorphous replacement method supplemented with anomalous scattering effects from both the Zn in the native crystal and the Sm in the derivative crystal (8). This technique is important, because it reduces the number of heavy-atom derivatives in the multiple isomorphous replacement method.

6. Laue method and Time-resolved crystallography

The fully polychromatic SR beam with a smooth spectral profile, high intensity and small divergence is excellent source for Laue experiments. It is possible to measure diffraction data in an exposure time of milliseconds or less by an application of Laue method with SR.

A protein crystal usually has a large solvent region (30% to 80% in volume). Hence a reactant is accessible to the enzyme active site through solvent channels in the crystal. Therefore most of enzymes are still active even in their crystalline states. Hence it is possible to trace visually structure changes of protein, reactants and products during the reaction by applying time-resolved techniques to protein crystallography. However, this has not been achieved with monochromatic X-ray except for some special cases, because of the long time-scales needed for diffraction data collection compared with biochemical time-scales. On the other hand, Laue technique with SR dramatically reduced the diffraction data collection time and thus made time-resolved technique a practical experimental tool. However, in order to apply successfully this technique to protein crystallography, there are still some problems that must be overcome. For example, since for time-resolved structure determinations it is importance that a chemical reaction in the crystal is synchronized in all unit cells, one has to develop such a way that all protein molecules in the crystal start to react with the reactants simultaneously.

In future work, it might be possible to utilize the pulsed nature of the SR beam and to work on a picosecond time scale.

The importance of three-dimensional structure of proteins can not be overstated. SR sources have brought a revolution in the field of protein crystallography. The third-generation sources will lead to exciting more challenging works such as the structure determination of large macromolecular assemblies and more detailed time-resolved structure analyses.

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