Introduction to single crystal X-ray analysis

I. What is X-ray crystallography?

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1. Introduction

All substances around us consist of atoms. The types of atoms and their three-dimensional arrangement define the structure of materials, therefore the nature of materials. Since the properties and functions of materials relate directly to its structure, there exist extensive researches for various materials such as semiconductor, electronic, food, pharmaceutical, or life science related materials.

However, we can't recognize the structure of materials at the atomic level because of the limited resolution mainly due to the wavelength, as long as we see objects with our eyes by using visible light. For example, we neither can distinguish a grain of table salt from that of sugar by their atomic level structures, nor can have a clear view of the turtle shell-shaped 6-membered rings (benzene rings) by just staring at the medicine for colds.

Recently, elucidation of molecular structures is becoming more common owing to the developments of various measurement techniques (See Table 1). Nuclear magnetic resonance (NMR), mass spectrometry (MS), and infrared spectroscopy (IR) are the typical examples. However, these spectroscopic techniques derive just a list of partial structures, and it is sometimes difficult to deduce the three-dimensional structure of a whole molecule. On the other hand, it is the molecular structure itself that is derived from the single crystal X-ray analysis (SCXRD). The single crystal analysis provides a unanimous conclusion that sometimes puts an end to arguments over molecular or crystal structures.

However, it is the fact that X-ray crystallography tends to be averted despite its efficiencies because it gives an impression to be difficult method requiring special knowledge. Through this series of articles, we would like to deliver an introductory course to the single crystal X-ray analysis to those who are not familiar with this technique. The course includes what X-ray crystallography is, what X-ray crystallography reveals, and how to solve the problems you will encounter in the future.

Table 1. Comparison between single crystal X-ray analysis and other methods.

	SCXRD	NMR	MS	IR
Nondestructive measurement	○	○	×	○
Sample form	Crystal	Liquid Solid	Solid Liquid Gas	Solid Liquid Gas
Measurable atomic types	All	Limited	All	All
Determination of configuration	○	○ (Indirect)	×	×
Determination of absolute structure	○	△ (Indirect)	×	×
Determination of crystal strcutre	○	×	×	×
Sample preparation	Difficult	Middle	Easy	Easy
Data interpretation	Direct	Indirect	Indirect	Indirect

SCXRD: single crystal X-ray analysis
NMR: nuclear magnetic resonance
MS: mass spectrometry
IR: infrared spectroscopy

2. History of X-ray crystallography

The history of crystallography starts with the discovery of X-rays in 1895 by Wilhelm Conrad Röntgen[1] whose name is used as a synonym of the X-ray photography in the medical field. In 1912, Max von Laue observed of the fist X-ray diffraction pattern from the copper sulfate crystal[2]. Next year, William Lawrence Bragg and his father William Henry Bragg discovered the Bragg's law[3] that makes it possible to calculate the positions of the atoms. In the 1910s, the structures of inorganic materials including sodium chloride, mineral and diamond were determined by the X-ray crystallography. The single crystal analysis was first applied to physics and chemistry.

In the 1920s, organic materials including hexamethylenetetramine were subjected to the study of X-ray crystallography. In the 1940s, biological materials, such as cholesterol, vitamin B12, and penicillin, were studied. The structure of insulin, a biological molecule, was finally solved by Dorothy Hodgkin in 1969 after 35 years of elaborating work[4]–[6].

In 1950, John C. Kendrew and Max F. Perutz solved the structure of heme-containing proteins, myoglobin[7] and hemoglobin[8], respectively. In 1953, James D. Watson and Francis Crick published the double helical structure of nucleic acids based on the X-ray diffraction patterns of DNA[9].

In the early 1980s, Ada Yonath started working on ribosome[10]. It is still flesh in our memory that the Nobel Prize in Chemistry in 2009 was awarded to Dr. Yonath for her contribution to the elucidation of the underlying mechanisms of protein synthesis along with Dr. Venkatraman Ramakrishnan and Dr. Thomas Steitz.

3. Why single crystal? Why X-ray?

By the way, why is a single crystal necessary and why are X-rays used for X-ray crystallography? For viewing the three-dimensional structure of molecules quantitatively, it is required to satisfy the following three

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conditions.

1) Types of atoms are distinguishable.
2) The position of the individual atom which is buried inside of the molecule can be determined even if the molecule is large like proteins.
3) The view of molecules from as many different directions as possible can be obtained. In addition, the relative positions among the molecules from different view can be determined precisely.

It is the X-ray crystallography that satisfies these conditions and currently it is the most popular analytical method to solve the three-dimensional structure of the molecule. The third condition is certainly satisfied by the nature of crystals.

Now, let's proceed to how to determine the molecular structure using X-rays. Talking of X-ray crystallography, you may have seen an image with black dots as shown in Fig. 1. These dots contain information to construct the molecular model of material you'd like to clarify its structure at the atomic level. The first step of X-ray crystallography is to collect several thousands to several tens of thousands of these dots. From the positions and intensities of these black dots, an atomic model can be determined.

The fundamentals of viewing the object using X-rays are similar to those of using a microscope for viewing the color and shape of the object. Let's think about using an optical microscope first. When parallel rays of light hit an object, only a shadow is projected to the screen. So you can't look the details of the object. At the same time as the shadow image is projected, most of the light is reflected. When we look at an object using an optical microscope, the objective lens gather and focus scattered light from the object. As a result, we can get the magnified and real image of the object.

By the way, visible light is an electromagnetic radiation with wavelength ranging from 400 to 700 nm. These wavelengths are visible to human eyes. However, submicroscopic objects requires shorter wavelength. Most molecules usually have dimensions in the range of a few to several dozens of Ås, and are far too small to be seen with the visible light. A typical wavelength used for crystallography is roughly 1 Å (0.1 nm). This is in the order of the covalent bonds and the radius of an atom. This is the reason why X-rays must be used for crystal structure determination.

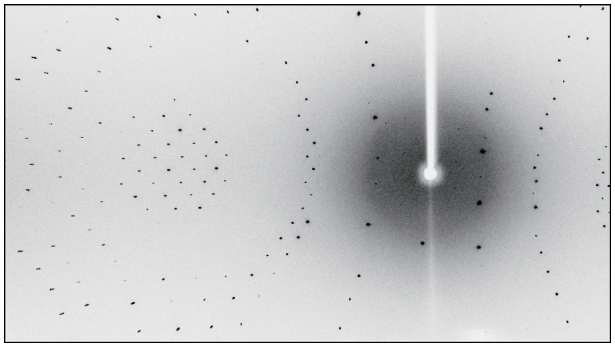

Fig. 1. X-ray diffraction pattern of single crystal.

Neutron radiation which is a kind of ionizing radiation can be used for viewing the object at the atomic level as well as X-rays. Although X-rays are scattered by the electrons of the atom, neutrons are scattered by the nuclei. For the determination of the positions of light atoms, chemical composition, or magnetic structure, neutron diffraction is superior to X-ray diffraction. However, there are limitations to use the neutron diffraction because of the weak diffraction intensities due to the extremely small scattering cross section. For example, it demands a considerably larger crystal than that of X-ray diffraction and it takes a long time for measurement. On the other hand, the ability of scattering X-rays is proportional to the atomic number (number of electrons), and thus X-ray diffraction is more suited to the structure determination of various materials from inorganic to organic compounds including proteins.

Let's get back to X-rays. There are no lenses to focus X-rays. However, one can substitute the calculation for the objective lens to reproduce a magnified image. (Strictly speaking, there is an optical element, Fresnel zone plate, which can focus X-rays and is widely used in the X-ray microscope.)

When parallel X-rays irradiate an object, only shadow image is projected on the screen. At the same time, most of the X-rays are reflected similar to visible light. It is called scattered X-rays. Scattered X-rays induce the phenomenon of coherent interference because X-rays can be thought as waves, and result in X-ray diffraction. If the object is a crystal, X-ray diffraction forms diffraction spots to the specific directions on the screen (black dots shown in Fig. 1). Each diffraction spot carries contribution from all atoms in the original object, and it is necessary to collect all diffraction spots to recreate the real image.

The information about the positions and the intensities of diffraction spots is recorded on the screen, so-called a "detector". Previously, the scintillation counter was used to measure the X-ray diffraction, and it could collect only one diffraction at a time. Recently, thanks to the development of two-dimensional detector, a large number of diffraction spots can be collected at a time, allowing it to reduce the measurement time substantially.

As shown in Fig. 2, based on the positions and the intensities of collected diffraction pattern, electron density distributions in crystals can be obtained after the calculation corresponding to the lenses in the microscope. X-ray crystallography assumes that atoms are present at the electron density maxima. Determination of positions of atoms, or atomic coordinates, results in a molecular model. The step of mathematical calculation might sounds difficult, but there is nothing to worry about performing this calculation because of the recent development of

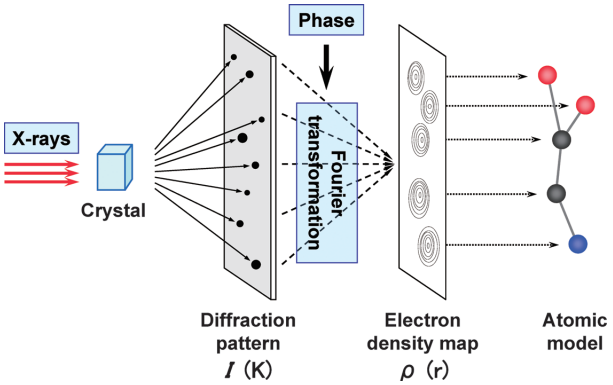

Fig. 2. Summary of technique.

software.

4. Methods

Three fundamental facts to understand the principles of X-ray crystallography are:

a) The X-ray scattering of materials is caused by the electron density ρ, and the scattering amplitude is the summation of scattering wave from an each electron. The scattering intensity is proportional to the square of the amplitude.
b) Electron density ρ within the crystal has a periodicity, which is virtually an infinite repetition in three independent directions.
c) Materials consist of atoms and the electron density of atoms stays similar even in different materials.

Among the equations derived from the facts listed above, the three most basic equations are the next three equations;

$$|F(K)|^2=I(K) \tag{1}$$

$$F(K)=\int_V \rho(r)\exp\{2\pi\imath(K\cdot r)\}dv_r \tag{2}$$

$$\rho(r)=\frac{1}{V}\int_{V_K} F(K)\exp\{-2\pi\imath(K\cdot r)\}dv_K \tag{3}$$

It is a natural feeling that you don't want to deal with difficult mathematical evaluation if possible. All you have to do is to keep the three equations above in your mind at least at the beginning of understanding X-ray crystallography.

The right-hand side of Eq. (1) can be obtained directly from the diffraction measurements. The $F(K)$ on the left-hand side is called structure factor, which is involved in the atomic positions directly. Eq. (2) shows that the structure factor can be calculated from the electron density ρ. On the other hand, Eq. (3) shows that the electron density within the crystal can be obtained from structure factors. It is important to know that the atomic positions are derived from this electron density.

However, Eq. (1) gives only the absolute value of $F(K)$ ($|F(K)|$), but the $F(K)$ is generally defined as a complex number; $F(K)=|F(K)|\exp\{i\varphi(K)\}$, where $\varphi(K)$ is phase, as represented in Eq (2). Electron density cannot be determined from Eq. (3) readily because of the loss of phase information in the diffraction experiment. This is so-called the "phase problem", and a large part of X-ray crystallography is devoted to finding correct phases.

Therefore, it is needed to determine the phases in some way. The direct method is almost uniquely employed to solve the phase problem for small molecule crystals. This methods estimate phases statistically from the amplitudes of the normalized structure factor. There are other methods for solving the phase problem in addition to the direct methods. One is the Patterson function which uses the square of the structure factors, that are intensities, as Fourier coefficients to eliminate phases, because the phase problem arises from using structure factors in the first place. This method is utilized in the heavy-atom methods which is applied to solving a strucutre containing heavy atoms and the Patterson search methods by rotating and then translating the Patterson function calculated from a known structure to match that from an observed intensities. Additionaly, the heavy atom isomorphous replacement method is used for protein crystals. With any one of these method, the key to the successful phasing is to collect accurate diffraction data to higher resolution (at higher diffraction angle).

5. Procedure

Now that you understand the principle, let's take a look at the flow diagram of single crystal X-ray analysis briefly (summarized in Fig. 3). Further details and technical descriptions in each step will be discussed in the successive articles.

5.1. Crystallization

The first and often most difficult step is to obtain an adequate crystal. In the crystallization, you have no choice but to proceed by taking the trial and error approach. It is independent from the development of the hardware and software. The truth is that obtaining only one well diffracting crystal assures good results.

Previously, a relatively large crystal was required for the diffraction measurement. However, small crystals with dimensions of 0.05 mm cube or smaller are becoming measurable by an in-house system. In addition, although it is said that a cube shaped crystal is preferable, yet a needle-like or a plate-like crystals can be used. The shape of the crystals is not critical in these days due to the development of robust absorption correction software.

5.2. Data collection

In the second step, diffraction data need to be collected on an X-ray diffractometer. The crystal is placed in the X-ray beam and the intensity of every

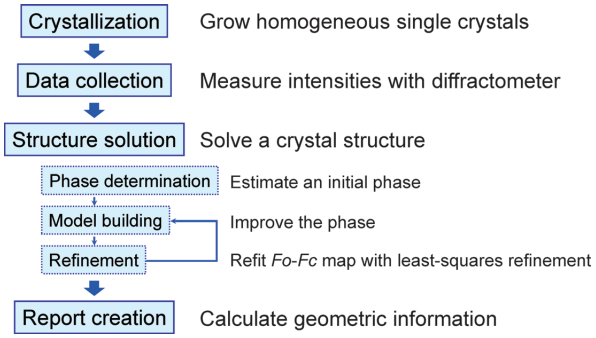

Fig. 3. Flow diagram of single crystal X-ray analysis.

diffraction spots is recorded by rotating the crystal. For the techniques of mounting crystals, please see the reference[11].

Once the crystal-like objects are acquired, try out a diffraction experiment. As is often the case with real experiments, diffractions are fuzzy or a crystal is twinned, even though the appearances of crystals are good. In contrast, it sometimes happens that excellent diffraction patterns can be observed from bad-looking crystals.

In the case of twinned crystals, it is possible to perform the data processing using advanced software[12]. However, crystals low in quality often leads to the struggle in the next step, solving the structure. If possible, such a problematic crystal should not be used for the data collection. You should take the trouble to change the crystal or may have to explore the crystallization conditions in some cases. 80% of the X-ray structure analysis may be considered to be finished if good crystals are obtained.

5.3. Solving a structure

After the data collection, the third step is to solve the structure. The information from the data collection is passed to the structural analysis program and the atomic level structure will be obtained finally. It is the most exciting moment that molecular structure appears on the computer screen.

People may think that the only intensity data is required for structure analysis. However, at least the chemical composition of your sample is necessary. In the cases where the constituent of the molecule is unknown, it is basically hard to conclude the crystal structure. So, the X-ray crystallography is the technique better at solving the crystal structure than at identifying the types of atoms.

As described in chapter 4, the elaborate and difficult calculations are performed in this step. Fortunately, however, software development provides the "black box" method, allowing us to perform the structure solution just like solving a puzzle.

The data are combined computationally with complementary chemical information to produce an electron density map. Then, atoms are fitted to the electron density map. After repetition of phase refinement and model fitting, the final refined atomic model can be obtained—now called a crystal structure. Nowadays there is a piece of software which automatically executes every step, and it takes only minutes or less before showing the molecular structure on the computer screen.

5.4. Creating a report

The information provided by the final model is atomic coordinates and temperature factors for each atom. With this information, one can easily calculate the bond distances and angles, and draw the molecular model and the Ortep diagram. Temperature factors, otherwise known as the thermal vibration parameters, describe the anisotropic vibrations of atoms.

International Union of Crystallography (IUCr) defines a file format called CIF for easy exchange of crystallographic information. Most program packages have a capability to output the final model in the CIF format.

Finally, the model may be submitted to a public database prior to publication—such as ICSD (Inorganic Crystal Structure Database), CSD (Cambridge Structural Database), or PDB (Protein Data Bank). The PDB is open to the public.

6. What reveals?

What does the single crystal X-ray analysis reveal? As well as determining the molecular structure, single crystal X-ray analysis reveals the chirality of a molecule.

The primary information is the molecular structure. Diamond, graphite and fullerene are the molecules which equally consist of carbon atoms alone, but their molecular structures are completely different. Having the same constituent atoms doesn't always mean to carry out the same function. And thus, investigating how the differences in the molecular structure effect on the materials with specific functionality helps us to design the new materials with desired functions. Furthermore, in the drug discovery, elucidating the three-dimensional structure can greatly contribute to the molecular design and refinement of the leads.

Next is the chirality. There are molecules which can't be superposed on its mirror image. It is called chirality. Human hands are the most familiar example of chirality. The molecule having a chirality is called a chiral molecule. The physical and chemical properties of two molecules in different handedness are almost the same, but the biological effects are sometimes quite different. Thalidomide is one the most famous cases caused by the chiral molecule. One of the two chiral molecules has teratogenicity. You will find the importance distinguishing the chirality.

Third is the molecular arrangement. A crystal is a solid material formed by the arrangement of constituent atoms or molecules in a periodic repetition extending in all directions. Even if the constituent molecule is the same, it sometimes happens that the molecular arrangements of resulting crystal vary depending on

the crystallization conditions. It is called packing polymorphism. Interestingly, the solid state properties such as melting point and the solubility alter because of the difference in the molecular arrangement. For example, the differences in the molecular packing of drugs results in differences in solubility, and leading to different medicinal effects. It is crucial to know the molecular arrangement in material design. In fact, there are many researches—such as modulated structure by altering the arrangement of anthracene molecules within the crystal[13], absorption mechanisms of gases going into the void space in the crystal arising from the specific combination of molecules[14], and nonlinear optical materials which modulate incident wavelength[15].

7. Concluding remarks

Single crystal X-ray analysis uses the phenomena of interference and diffraction of X-rays which arise from scattering by the electrons in the atoms. Furthermore, the electron density ρ is calculated from the diffraction intensity I, and the average three-dimensional arrangement of molecules or atoms in the crystal is revealed.

In the old days, you might have needed to have much knowledge and techniques on the crystallization, data collection and solving the structure including the fundamentals in crystallography for carrying out the X-ray crystallography by yourself. However, times have changed. The X-ray crystallography has become a freely usable technique for anyone with a little knowledge, because of the astonishing progress in both hardware and software.

The atomic world is beautiful. Once you could look into such a wonderful world by your own efforts, you will definitely become fascinated with this world. We wish we could invite you all into our world through this series of articles.

References

(1) W. C. Röntgen: *Nature*, **53** (1896), 274–276.
(2) W. Friedrich, P. Knipping and M. von Laue: *Sitzber. Math.-Phys. Klasse Bayer. Akad. Wiss. München*, (1912), 303.
(3) W. L. Bragg: *Proc. Cambridge Phil. Soc.*, **17** (1913), 43–57.
(4) D. C. Hodgkin: *Adv. Sci.*, **6** (1949), 85–89.
(5) C. Brink, D. C. Hodgkin, J. Lindsey, L. Pickworth, J. R. Robertson and J. G. White: *Nature*, **174** (1954), 1169–1171.
(6) M. J. Adams, T. L. Blundell, E. J. Dodson, G. G. Dodson, M. Vijayan, E. N. Baker, M. M. Harding, D. C. Hodgkin, B. Rimmer and S. Sheat: *Nature*, **224** (1969), 491–495.
(7) J. C. Kendrew, G. Bodo, H. M. Dintzis, R. G. Parrish, H. Wyckoff and D. C. Phillips: *Nature*, **181** (1958), 662–666.
(8) M. F. Perutz, M. G. Rossmann, A. F. Cullis, H. Muirhead, G. Will and A. C. T. North: *Nature*, **185** (1960), 416–422.
(9) J. D. Watson and F. H. C. Crick: *Nature*, **171** (1953), 737–738.
(10) A. Yamano: *The Rigaku Journal*, **27** (2011), No. 2, 1–5.
(11) M. Maeyama: *The Rigaku Journal (Japanese version)*, **38** (2007), 16–19.
(12) H. Sato and A. Yamano: *The Rigaku Journal (English version)*, **27** (2011), No. 2, 22–23.
(13) Y. Mizobe, N. Tohnai, M. Miyata and Y. Hasegawa: *Chem. Commun.*, (2005), 1839–1841.
(14) T. K. Maji, R. Matsuda and S. Kitagawa: *Nature Mater.*, **6** (2007), 142–148.
(15) K. Yamamoto, S. Iwai, S. Boyko, A. Kashiwazaki, F. Hiramatsu, C. Okabe, N. Nishi and K. Yakushi: *J. Phys. Soc. Jpn.*, **77** (2008), 074–709 (6 pages).