Development of the Program System for Protein Crystallography

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The Bio-Crystallography(MIR-OAS) beamline has been constructed for routine structure analyses of biological macromolecules[1]. Proteins, nucleic acids, and viruses wll be studies by crystallographers, biochemist, and virologist. For determining their crystal structures for themselves in couple with easily usable equipments, the development of convenient softwares is indispensable.

There are several methods to solve the crystal structure of macromolecules. The fundamental method is a multiple isomporphous replacement(MIR) method, where the prepared heavy atom derivatives supply the information of the phases of reflections from the native crystal[2]. The molecular replacement(MR) method becomes powerful in accordance with growing the data base of protein structures[3]. The method locates the similar structures with the rotational and translational operations in the unit cell. The utilization of multi wavelength anomalous dispersion(MAD) is promising for the synchrotron radiation[4]. Some wavelength gives a couple of real and imaginary parts of the anomalous dispersion effects, which depend on species of atoms and energy of X-rays. Unfortunately, this effect is too trivial to detect with the usual experiment. However, if you use the X-rays at the absorption edge of the atom and obtain quite accurate diffraction data from crystals, you will determine the protein structure with no additional experiments.

Any method have to be utilized in this beamline and the fundamental MIR method has been developed firstly. The heavy atom derivatives are prepared by soaking the crystal in the solution of the reagents. Even we works very carefully, it is not sure whether the heavy atoms are bound to the fixed position of every molecules in the unit cell. In this beamline, many crystals prepared but unknown whether it binds the heavy atoms are dedicated to experimens of the X-ray diffraction. From the X-ray experiments, a lot of intensity data sets are produced. For a moment, the hardware will produce a data set of one crystal within a hour. The software has to complete all calculations within this time. The first step of the analysis is to determine the scale factors and overall temperature factor among the data sets. This step is processed by the programs of start.e and restart.e. The input data is only a symbol of the space group, the cell parameters (a, b, c, α, β and γ) and molecular formula with the number of molecules in an asymmetric unit. The resolution, relative scale factorsand temperature factors are saved in the disk for the successive calculation. The distribution of the errors are listed in steps of resolution, $\sin\theta/\lambda$ and $F/\delta(F)$, respecitively. The second step is to know whether the heavy atoms are found in the unit cell. The vector verification method, that is, symmetrical minimum function[5], may be useful theoretically. In this calculation, the vectors between the positions related by the crystallographic symmetry are picked from the Patterson function. The minimum function follows the symmetrical minimum function which searches the vectors between a known position and the other on the Patterson function. Though these two programs have been coded with the double sorting algorithm, they takes a little longer computation time. For the grids of 70×70×80, about ten minutes by SUN-4/2 are necessary with the resolution of 20 to 4 Å, even though the Fourier transformation is carried with the FFT algorithm. The positions of the heavy atoms are refined by both Dickerson's[6] and Rossmann's methods[7]. The Rossmann's method may be useful for the present beamline because it works well even with a single derivative, though the Dickerson's method requests more than two derivatives for refinement. The difference Fourier synthesis is quite useful to determine the relative positions of the origins among the derivatives. Also, it should be used to pick minor sites of the heavy atoms after the refinement. Each map can be plotted on the SUN-4/2, and may be connected to the Turbo-Frodo(Bio-Graphcs CO.) through the t_mappage, and to the Phases through the t_fase.

All programs request the least input data, for example, what is the name of your crystal and name of derivative to be processed, whether the anomalous dispersion effects are included or not, so on. The size of a unit cell are infinite practically. The limit is the size of the two dimensional section of the map, which can be reset in the program with the Fortran's language, parameter. Though it takes much time for

the crystal of the larger cell, the calculation may run without troubles. It means that even the crystal of virus can be processed. A total package of the programs may be unified by the window system, which are now under development with the motif.

It should be emphasized that this system does not build the model but select the useful derivatives to solve the phases. When you leave the beamline, you bring several accurate data sets and their heavy atom's parameters which are enough to solve the structure. You can continue the determination of the structure with these data beside the station or your laboratory.

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