STRUCTURE AND CHEMISTRY OF RIBONUCLEASES

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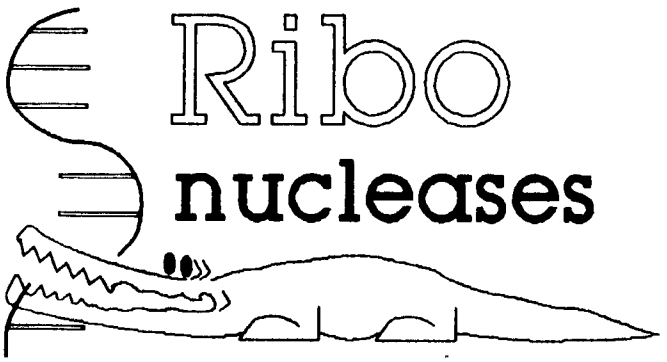

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NEW REFINEMENT PROGRAM FROG GIVES NEW POSSIBILITIES TO STUDY MACROMOLECULAR MODELS

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1. Program outline.

It is well known that automatical refinement is necessary for obtaining most important structural information. Presently, there are some widespread programs to refine atomic parameters but they often cannot solve problems which need rigid-group movement rather than those of individual atoms: here are whole molecule or domain position refinement etc. The full set of existing programs may allow one to core with most of the difficulties but to get a final model he should change programs during the work.

The FROG program refines models that can simultaneously consist both of :

- individual atoms and of
- groups of atoms, each moving as a rigid body during refinement.

Any possible stereochemical restraints may be applied for both individual atoms and rigid groups.

In addition to problems arising from subsequent replacement one program to another, all of these programs suffer from their own deffects. Most programs need large

refinement time and limit the size of the object.

! The FROG program has very low time cost owing to new algorithms (Lunin & Urzhumtsev, 1985).

! The only its essential restriction is that puts not less than two section of elestron density in program buffer array.

! No restrictions are placed on the space group and object size.

The more complicate are structural problems one deels with, the fine are effects which should be taken into account. The known programs can only minimize a once selected functional R which is quantitative representation of the user's requirements to a model (here some weight coefficients may be changed only).

! The FROG program fixes the general structure of R, rather than its concrete form. This latter is described by special input files, which allows it to be varied with no change in the FORTRAN text of the program. The functional R may include various requirements expressed in terms of atomic-blocked model parameter, or atomic parameters, or electron density distribution, or structure factors. One, in particular, can require that

! - modules of calculated structure factors correspond to the observed values;

! - phases of calculated structure factors fit their probability distributions (Lunin & Urzhumtsev,1985)

! - bond lengths, bond and dihedral angles, hirality volume etc. minimally deviate from given values; the same for planarity of some atomic groups;

! - non-bonded interaction energy including intermolecular interaction be minimal;

! - discrepancy in model symmetry be minimal;

! - model output from a given region of the unit cell be minimal.

The list may easily be extended. One, in particular, can introduce fitting of NMR-data or so-called "real space refinement criterion" or direct-method equations or so on.

The structure of molecular complexes takes special interest when mechanism of molecular aciton is investigated or protein engineering problems are solved. In the known programs special programmers efforts are needed to change the type of macromolecules to be refined.

The FROG program can simultaneously process macromolecules of different types; each of them can, as before, be devided into some rigid groups.

2. Application examples.

a) Refinement of τ-crystallin IIIb atomic model.

The three-dimensional structure of the protein τ-crystallin IIIb from calf lens is being investigated at the Protein Research Institute of the USSR Academy of Sciences together with the present authors (Chirgadze et al., 1986). Crystals of the protein belong to the $P2_12_12_1$ space group, a=58.7, b=69.5, and c=116.9 A. The asymmetric part of the unit cell contains 2 protein molecules, each with a molecular weight of 20KDa (173 aminoacid residues).

Refinement of the model consisting of individual atoms with stereochemical restraints against the first data set (with resolution 2.5A) produced an R-factor of 0.25 (Chirgadze et al.,1986).

At the second stage we used a new data set with a resolution up to 1.9A collected on the Daresbury synchrotrone. Changes in the conditions of data collection resulted in a modification of the molecular structure. Molecular package also was changed so that the unit cell parameters became a=57.4, b=69.2 and c=115.5A. This required a preli-

minary refinement of the model as a rigid body followed by refinement of flexible surface side chains with rigid internal structure, and only finally usual restraint refinement of individual atoms became possible.

A refinement cycle of τ-crystallin IIIb model at the resolution of 2.5 A (12000 reflections) took about 35 min of the CPU time on EC-1055M computer (300,000 op/sec).

b) Model "protein engineering" of fag T4 lysozyme.

The structure of the fragment 37-73 of phage T4 lysozyme (Weaver & Matthews,1987) resembles the "EF-hand" (Kretsinger & Nockolds,1973) of some proteins with the difference that it includes Gly51 instead of Asp51. Tufty & Kretsinger (1975) suppose that the reverse change Gly51 --> Asp51 allows the lysozyme segment 51-62 to take the form of Ca^{2+}-connecting loop of EF-proteins. A computing experiment has been executed to understand whether the fragment can hold "EF-structure" without stereochemical distorsions.

Initial model, phage T4 lysozyme complemented by a side chain of Asp on C_α-atom of Gly51, was gradually changed (Murzin & Vernoslova, 1988) by the FROG program to organize 51-62 residues into a conformation of 90-101 residues of carp parvalbumin.

The main part of the lysozyme model (residues 94-164) was fixed and some elements of the secondary structure (α-helixes 1-11, 40-46, 63-80, β-sheet 14-33) were moved as a rigid body. Loops and the rest of α-helix (residues 47-62) were modelled by individual atoms linked stereochemically. A refinement cycle of the model took 2 minutes on EC-1055M computer.

The final model satisfied all the stereochemical requirements, which suggests that target conformation is stereochemically possible.

c) Phase extension of pea lectin.

The structure of pea lectin is being analysed by Riskulov et al.(1985) at the Institute of Molecular Genetics of the USSR Academy of Sciences. Crystals of the protein belong to $P2_12_12_1$ space group, a = 51.0, b = 61.7 and c = 137.6 A. The asymmetric part of the unit cell contains one molecule with two similar subunits, their common weight is 52KDa (about 480 residues).

The method of multiple isomorphous replacement was applied to get an initial electron density map at a resolution of 3.0A. To refine and extend phases of structure factors, mixed atomic model (Lunin et al.,1985) was used.

This model consisted of partial model's atoms, interconnected by stereochemical restraints, and of independent dummy atoms which interacted with real atoms only. The total of atoms was about 4000.

At the final the phase set was extended from 3.0A to to 2.4 A, which improved the electron density map. This was used to increase the number of identified atoms from 40 to 70 per cent of the total.

d) Phase extension of carnation mottle virus.

The structure of carnation mottle virus (molecular weight about 7000 KDa, space group F23) is being investigated at the Institute of Crystallography of the USSR Academy of Sciences. The low-resolution structure of virus and approximate position of the molecule in the unit cell were determined by Morgunova et al.(1988).

The FROG program is utilizing to refine the model's positioning and, correspondingly, to extend the phase set.

A refinement cycle of virus model (more than 2 million atoms/unit cell) takes 7 hours for 10A refolution (6000 refl.) and 11 hours for 6A resoluiton (23,000 refl.) on EC-1045 computer with about 600,000 op/sec.

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