# The relationship between crystal structure and compressibility of DHFR mutants

Katsuo KATAYANAGI*, Yasunori KAMIYA, Masaki KAWAMOTO,
Takehiro SATO, Eiji OHMAE, Kunihiko GEKKO
Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University,
Higashi-Hiroshima 739-8526, Japan

## Introduction

As well as its 3D structure, the flexibility of protein molecule plays an important role in its function. Although structure-function relationships of enzymes have been widely investigated, it seems difficult to predict precisely the effects of amino acid replacements on the stability and function. Thus, we are now studying the structure-flexibility-function relationships of *Escherichia coli* dihydrofolate reductase (DHFR), which has four flexible loops. The flexibility of the whole molecule can be characterized by measuring the compressibility. From this criterion, DHFR is classified as "soft" protein and is suitable for the present study.

## Results and Discussion

As we have already reported, we, at first, focused on the loop around the site 67 for the study of flexibility. This site was selected because position 67, being 29.5 Å away from the active site Asp27, is most flexible in a loop (residue 64-72) as revealed by large B-factor of the wild-type structure. We could find out the clear and linear correlation between the total volume of cavity from X-ray structure and compressibility.

Next, we focused on site 121, which is 19 Å away from active site. In both case of 67 and 121 site mutants, the large difference of distribution of the cavities between the wild-type and DHFR mutants was observed. The fact that these changes in cavities distributed all over the molecule suggests that the mutations at this position, being located in the surface loop, affect the structural dynamics and function of this enzyme by the long-distance effects as found by the double mutation experiments.

Comparing to loops at sites 67 and 121, the loop around site 145 is quite different. According to the results of double mutant study, the "long range interaction" was not observed between the sites of 67 and 145. Thus, we crystallized the DHFR mutants of A145X. The following table indicates all the mutants, which we have collected during current and previous proposal number.

G67 → A, V, L, S, T, D, C
G121 → A, D, F, Y, H, F, C
A145 → L, G, S, R, H, I

The data of compressibility experiments were already collected for these mutants. The crystals were obtained as a folate binary complex by vapour diffusion method at 4°C. The diffraction data, up to 2.1 Å resolution, were collected using beam line BL18B and BL 6B at Photon Factory. The crystal belonged to the space group $P6_1$ with lattice parameters a=93.0Å c=74.4Å. The initial phase was determined by the molecular replacement method. Then the crystallographic refinement was carried out using a program X-PLOR. As for the site 145 mutants, the structural analysis is now in progress. Then, we are planning to compare the distribution of the cavities between the wild-type and DHFR mutants, using the probe radius of 0.8 Å.

The crystal of DHFR mutants were not so stable during capillary mounting or cryo-mounting. The large number of crystals (about 10crystals) had to be tried to obtain a good diffraction dataset. This may be due to the fact that DHFR belongs the soft protein. The factor of "long distance interaction" will be identified through the viewpoint of detail 3D structure combined with double mutant analysis and the result of compressibility.

## References

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* kkata@sci.hiroshima-u.ac.jp