1A3b Changes in the collective motion induced by site-directed mutation to the residue in flexible loop of dihydrofolate reductase revealed by NMR spin relaxation analyses

- An insight into the structural dynamics and function relationship -

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Protein structure contains intrinsic dynamics that is determined by overall structure or long-range interaction. Molecular dynamics simulation has shown that there are collective motions in protein structures, allowing the concerted movement of residues in distal positions. The collective motions, which are recognized as corresponding to the normal modes of protein structure, occur in time regions of μsec to msec. The structural motions in this time region should have functional significance, because most of the enzyme reactions occur in the same time scale. Although various experimental and theoretical researches have suggested the roles of the protein structural dynamics in biological functions, there is no clear evidence for that. To understand how the structure dynamics is engaged in exerting protein function, we have to establish the experimental and analytical approach to change the dynamics but keeping the structure unchanged. In considering the research background of the structural dynamics and function relationship, we have started to explore the effects of the site-directed mutation on the structural dynamics of *E. coli* dihydrofolate reductase (DHFR).

Our group has determined the kinetic parameters of a various types of mutant DHFRs, which have site-directed mutations to the three flexible loops (βC-βD, βF-βG and βG-βH). In the present study, we have focused on the βC-βD loop, especially Gly67 mutants. This loop is far distant from the active site, but some types of amino acid replacement to the loop caused the significant reduction in the enzyme activities. G67A mutant showed a small reduction in the turnover rate, k_{cat}, relative to the wild-type, while the reduction in k_{cat} was enhanced in G67V. In both mutants, K_m values were close to that of the wild-type. These functional changes induced by the site-directed mutation to this loop showed clear dependence on the replaced an amino acid residue. Through the nuclear spin relaxation analyses on amide ^{15}N by NMR, we found these functionally relevant mutations caused different effects on the motions of distal βF-βG loop, which should have an important role in reorganizing the active form of the Michaelis complex of DHFR.

We will present the details of the motional changes caused by the mutation to the βC-βD loop in relation to the function of DHFR.