Investigation on the local structural effects on chemical modification of cysteine residues on protein surface

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Adenylate kinase (Adk), a phosphoryl transfer enzyme for ATP/AMP and ADP, shows the conformational interconversion between OPEN and CLOSED forms. Using this characteristic, we previously constructed a photo-switching system of pyrene, where two pyrene molecules were conjugated onto cysteine residues of the mutant Adk (A55C/C77S/V169C).[1)] Furthermore, the reactivity of the thiol at Cys55 was found to be higher than that of the thiol at Cys169 although both cysteine residues are located on the protein surface. In general, the regioselective modification of cystine residues on the protein surface is difficult. Assuming that local structural effects of acidic amino acid residues (Asp54 and Glu170) located nearby these cysteine residues may provide the reactivity difference, we conducted the following molecular dynamics (MD) simulation and mutation studies. The MD simulation for the mutant Adk demonstrated that Glu170 tends to face to Cys169, whereas Asp54 took away from Cys55 to form a salt bridge with Lys50. Accordingly, we studied the reactivities of cysteine thiols for another mutant K50A/A55C/C77S/V169C because the removal of the salt bridge may bring about a similar situation for Asp54 and Glu170. In contrast to our expectation, the reactivity of the thiol at Cys55 was further enhanced on the mutation at Lys50. Lys/Ala50 and Asp54 are on a α-helix, whereas Cys55 is located on the edge of the α-helix. The Lys50Ala mutation causes the increase in the flexibility of the α-helix, and the structural perturbation is transmitted to Cys55. The finding indicates that the local flexibility near the chemical modification site influences the reactivities of cysteine thiols on the protein surface.[2)]

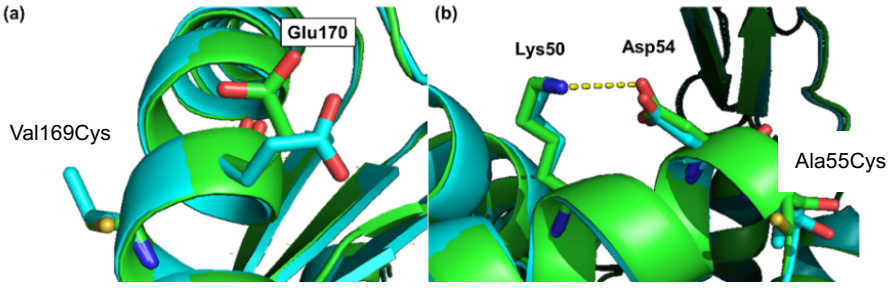

Figure 1. Local structures around Cys/Val169 (figure (a)) and Cys/Ala55 (figure (b)) obtained by MD simulation (blue backbone: the wild-type Adk; green backbone: mutant Adk (A55C/C77S/V169C)).

1) A. Fujii, S. Hirota, T. Matsuo, *Bioconjugate Chem.* **2013**, *24*, 1218. 2) T. Miyake, R. Tamaki, M. Asanuma, Y. Fukada, S. Hirota, T. Matsuo, *Bioconjugate Chem.* **2020**, *31*, 794.