MS5. Structure and function of enzymes

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MS5-P1 A leucine residue in the L2 loop is critical for caspases 3 and 7 active site formation

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Various apoptotic signals can activate caspases 3 and 7 by triggering the L2 loop cleavage of their proenzymes. These two enzymes have highly similar structures and functions, and serve as apoptotic executioners. The structures of caspase 7 and procaspase 7 differ significantly in the conformation of the loops constituting the active site, indicating that the enzyme undergoes a large structural change during activation. To define the role of the leucine residue on the L2 loop, which shows the largest movement during enzyme activation but has not yet been studied, Leu168 of caspase 3 and Leu191 of caspase 7 were mutated. Kinetic analysis indicated that the mutation of the leucine residues sometimes improved the Km but also greatly decreased the kcat, resulting in an overall decrease in enzyme activity. The tryptophan fluorescence change at excitation/emission = 280/350 nm upon L2-L2' loop cleavage was found to be higher in catalytically active mutants, including the corresponding wild-type caspase, than in the inactive mutants. The crystal structures of the caspase 3 mutants were solved and compared with that of wild-type. Significant alterations in the conformations of the L1 and L4 loops were found. These results indicate that the leucine residue on the L2 loop has an important role in maintaining the catalytic activity of caspases 3 and 7.

Keywords: Leucine, L2 loop, caspase-3, caspase-7

MS5-P2 Structure-based engineering of Methionyl tRNA Synthetase for expression of photoactivatable proteins with photomethionine in *E. coli.*

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Photo-methionine (pM; L-2-amino-5,5-azi-hexanoic acid) with a diazirine group has a structural similarity with methionine (Met). In this work, we tried *in vivo* expression of pM containing proteins in *E. coli*. Expressions of proteins cloned into the pET-28a vector in *E. coli* B834 Met auxotroph were failed in the minimal medium with pM, but successful in that with Met. Engineering of *E. coli* methionyl-tRNA synthetase (MRS) for three residues interacting with the Met sulfur moiety (PNAS, 106: 15282, 2009) was not effective for expression of photoactivatable proteins with pM incorporation. Here we report that a MRS variant with mutations of five residues (two additional residues plus three reported residues) successfully allow the expression of various photoactivatable proteins. The side group of Met is accessible to its binding site of the wild type MRS through a narrow cavity (J Mol Biol, 306: 446, 2001), and the additional residues are possibly acting as a gate of the cavity. In this aspect, the side chain of pM with a bulky diazirine group may not access to the binding site of MRSwt, and therefore widening of the gate by mutation is considered to enable protein biosynthesis in the presence of pM. Indeed, co-transformation of MRS5m was allowed expression of various proteins in the presence of pM. The photoactivatable proteins were found to induce photocrosslinking with the specifically interacting proteins upon UV irradiation. The engineered MRS5m will be also useful for incorporation of noncanonical amino acids with the similar side chain of pM.

Keywords: Methionyl tRNA synthetase, protein engineering, photomethionine, photoactivatable protein expression in E. coli