Y225F/A for Met-tRNA Synthetase reveals Importance of Hydrophobic Circumstance

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In *Thermus thermophilus* Met-tRNA synthetase, CP1 domain laps the entrance region of the hydrophobic pocket, on which Met binds. In order to investigate a possible role of this lapping in creation of hydrophobic circumstance around the enzymatic active center, *K*m values of substrates and *k*cat value for aminoacylation reaction for tRNA were comparatively measured for mutants Y225F and Y225A of Tyr225, which locates inside the ribbon portion of CP1 domain but out of the hydrophobic pocket for Met-binding. The crystal structures of these mutants determined were also closely compared with that of the wild type protein. Observed *K*m values for Met and tRNA are 27 and 4.7ìM for Y225F, 1300 and 11 ìM for Y225A and 30 and 4.5 ìM for wild type protein, respectively. On the other hand, observed *k*cat are 0.59 sec^{-1} for Y225F, 1.4 sec^{-1} for Y225A and 17 sec^{-1} for wild type, respectively. Remarked effect on the binding affinity for Met induced by Y225A may be due to the decreased hydrophobicity caused by mutation from Tyr to Ala, whereas little effect observed in Y225F reflects comparable hydrophobicity between Phe and Tyr. The decreased hydrophobicity may affect the conversion process of hydrated form of Met into un-hydrated form thereof, which process is a precedent step to Met loading into the hydrophobic pocket of the enzymatic center. In contrast, the considerably decreased kcat appears to suggest that the presence of the phenolic hydroxyl group of Tyr may play some determinant role in the coming-up step of the 3' terminal CCA of tRNA to the carbonyl group of Met.

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