Medical & healthcare, Drug development

Structures and reaction mechanisms of posttranslational modification enzymes targeting biosynthesis of novel functional peptides

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Abstract

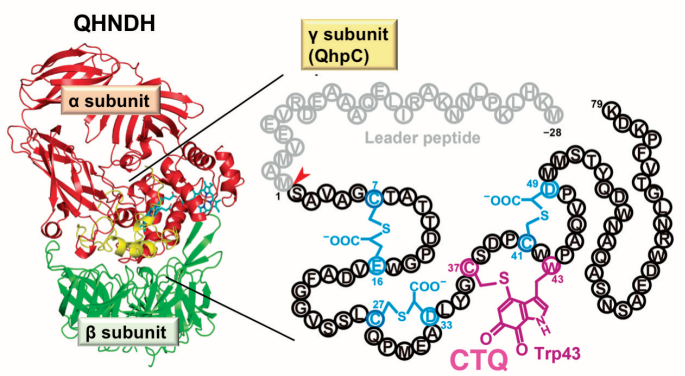
Specific chemical modification of proteins and peptides newly translated from genes, posttranslational modification, is essential for their functions and regulation. We focus the posttranslational modifications of enzyme proteins including generation of intermolecular crosslinks and quinone cofactor. Our aim is to apply these modification enzymes for constructing a new platform for drug development and novel functional peptides.

Background & Results

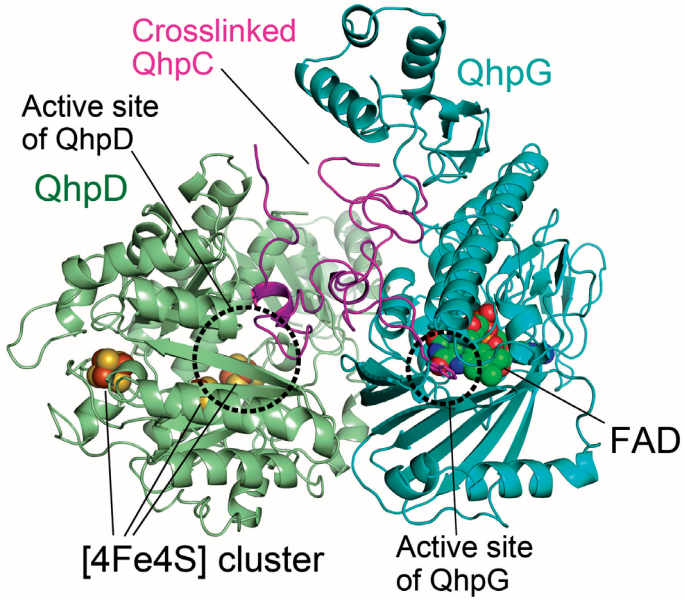
Quinohemoprotein amine dehydrogenase, derived from many Gram-negative bacteria, catalyzes oxidative deamination of various aliphatic primary amines for assimilation. The smallest γ-subunit (QhpC) in three subunits α β γ of QHNDH has two unique posttranslational modifications: three thioether crosslinks and a covalently bound quinone cofactor, cysteine tryptophylquinone (CTQ). The complex modification suggests the multi-step mechanism involved in several modification enzymes. Based on our previous studies, QhpC is translated as a precursor form including a 28-residue leader peptide at N terminal and forms a ternary complex with QhpD and QhpG. A radical *S*-adenosyl-L-methionine (SAM) enzyme QhpD forms three thioether crosslinks in QhpC and sequentially FAD-dependent oxygenase QhpG di-hydroxylates a CTQ precursor, Trp residue of QhpC, in the complex. Followed with removal of the leader peptide with a serine proteinase QhpE, the modified QhpC is transported to periplasm where CTQ is finally generated. As for QhpG, our group recently determined its X-ray crystal structure and clarified details of the reaction mechanism and the interaction with the substrate QhpC. QhpG is found be an atypical single-component oxygenase, catalyzing dihydroxylation of an unmodified Trp residue in the protein substrate. We are able to reconstitute these multi-step modification reactions without the last step in vitro. QhpD reaction can generate a multi-loop or single cyclic peptide, and QhpG is applicable to generate a reactive site such as dihydroxyl Trp and a tryptophyl quinone. It is expected that these enzymes are applicable as tools to construct novel functional peptides.

Significance of the research and Future perspective

Recently, middle-size biomolecules including macro cyclic peptides with various functions are paid to attention as a new drug framework because they have high affinity toward a target molecule and membrane permeability such as low molecular weight compounds. Peptide modification enzymes shown in the present work can be used for tools to develop new drugs or functional molecules on the cyclic peptides. Enzyme-based synthesis is possible under ambient temperature and pressure, and attains low environmental loading, agreeing with a concept of green chemistry.



X-ray crystal structure of quinohemoprotein amine dehydrogenase and multiple posttranslational modifications of QhpC.



Presumed model for ternary complex formed among radical SAM enzyme QhpD, FAD-dependent oxygenase QhpG, and substrate peptide QhpC.

Patent	
Treatise	Oozeki, Toshinori; Nakai, Tadashi; Okajima, Toshihide et al. Functional and structural characterization of a flavoprotein monooxygenase essential for biogenesis of tryptophylquinone cofactor. Nature Communications. 2021; 12(1): 933. doi: 10.1038/s41467-021-21200-9 Nakai, Tadashi; Tanizawa, Katsuyuki; Okajima, Toshihide et al. The radical S -adenosyl- L-methionine enzyme QhpD catalyzes sequential formation of intra-protein sulfur-to-methylene carbon thioether bonds. J. Biol. Chem. 2015; 290(17): 11144-11166. doi: 10.1074/jbc.M115.638320
URL	https://resou.osaka-u.ac.jp/ja/research/2021/20210210_2
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