presence of glycine. It implies the usefulness of this\e chemical chaperone in reduction of diabetic complications such as cataract.

Keywords: Catalase, Chemical Chaperone, Glycation, Glycine.

Abstract No.289

Thermodynamic Aspects of Disulfide Bridge Introduction and Bioluminescence Color Shift in Firefly Luciferase

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Relationship between stability, color shift and position of flexible loop in firefly luciferase is investigated. Bioluminescence reaction, which uses luciferin, Mg2+-ATP and molecular oxygen to yield an electronically excited oxyluciferin, is carried out by the luciferase and emit visible light. Multi-color bioluminescence is developed using introduction of single/double disulfide bridges in firefly luciferase through structural stabilization and displacement of functional loops. The bioluminescence color of firefly luciferases is determined by the luciferase structure and assay conditions. Single and double disulfide bridges are introduced into firefly luciferase to make separate mutant enzymes. By introduction of disulfide bridges using site-directed mutagenesis in Photinus pyralis luciferase the color of emitted light was changed to red or retained. Multicolor bioluminescence is accompanied with displacement of critical loops in red-emitter luciferases without significant changes in green emitter mutants. Thermodynamic analysis revealed that among mutants, L306C-L309C mutant with a single disulphide bridge shows a remarkable stability against urea denaturation and also considerable increase in kinetic stability with changes in emission spectra towards red.

Keywords: Bioluminescence, Disulfide Bridges, Luciferase, Red-Emitter, Site-Directed Mutagenesis.

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Biotechnological Applications of Peroxidases

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Peroxidase enzymes that may find ubiquitously in animals, plants and microorganisms, are able to oxidize a wide range of reductants in the expense of peroxides as acceptors[1,2]. Most part of our knowledge on the structure–function relationships and catalytic mechanisms of peroxidases is indebted on horseradish peroxidase.In classical peroxidases, ferric protoporphyrin IX is the prosthetic group and imidazole the fifth iron ligand. The characteristic activity of peroxidases is two electron oxidation, which proceeds through formation of compounds I and II. Compound I is reduced back to the ferric resting state either by a two sequential one-electron transfer processes from peroxidase to substrates or by two-electron oxidation process associated with the ferryl oxygen transfer to substrates[3]. The radicals produced in the reaction generally evolve nonenzymatically to nonradical products by pathways characteristic of each substrate (coupling, dismutation etc.).Chloroperoxidase (CPO) is unique among the peroxidases because it contains a cysteinic thiolate as the fifth axial ligand of the heme instead of the imidazole ligand. For this reason, many of its spectroscopic and chemical properties are similar to those of cytochrome P-450. CPO is unusually versatile: it catalyzes not only the reactions typical of peroxidases but also those of catalases and monooxygenases, and it is also almost unique in catalyzing halogenation reactions in the presence of halide ions and H2O2. Peroxidase-catalyzed reactions exist in four categories[4]:1. Oxidative dehydrogenation 2.Oxidative halogenations 3. H2O2 dismutation 4. Oxygen-transfer reactions: Benzylic/allylic hydroxylation, Alcohol oxidation, Epoxidation, Indole oxidation, Heteroatom oxidation (Sulfur oxidation, Nitrogen oxidation)Present work reviews and discusses recent biotechnological developments and applications of peroxidase family including homogeneous, heterogeneous, artificial peroxidases and miniperoxidases.

Keywords: Peroxidase, Chloroperoxidase, Application, Reaction.
