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Modification of the heme active site to increase the peroxidase activity of thermophilic cytochrome P450: A rational approach

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ABSTRACT

The site specific mutants of the thermophilic P450 (P450 175A1 or CYP175A1) were designed to introduce residues that could act as acid–base catalysts near the active site to enhance the peroxidases activity. The Leu80 in the distal heme pocket of CYP175A1 was located at a position almost equivalent to the Glu183 that is involved in stabilization of the ferryl heme intermediate in chloroperoxidase (CPO). The Leu80 residue of CYP175A1 was mutated with histidine (L80H) and glutamine (L80Q) that could potentially form hydrogen bond with hydrogen peroxide and facilitate formation and stabilization of the putative redox intermediate of the peroxidase cycle. The mutants L80H and L80Q of CYP175A1 showed higher peroxidase activity compared to that of the wild type (WT) CYP175A1 enzyme at 25 °C. The activity constants (k_{cat}) for the L80H and L80Q mutants of CYP175A1 were higher than those of myoglobin and wild type cytochrome b562 at 25 °C. The optimum temperature for the peroxidase activity of the WT and mutants of CYP175A1 was ~70 °C. The rate of catalysis at temperatures above ~70 °C was higher for L80Q mutant of CYP175A1 compared to that of the well known natural peroxidase, horseradish peroxidase (HRP) that denatures at such high temperature. The peroxidase activities of the mutants of CYP175A1 were maximum at pH 9, unlike that of HRP which is at pH ~5. The results have been discussed in the light of understanding the structure–function relationship of the peroxidase properties of these thermostable heme proteins.

1. Introduction

Heme proteins play diverse roles in biological systems [1,2]. These proteins having the same heme prosthetic group are responsible for many different types of functions such as oxygen storage and transport, electron transfer, oxidation and oxygenation of different substrates etc. The functional diversity of the heme in different heme proteins has been attributed partly to the differences in the amino acid environment around the distal heme pocket [1]. It however, still remains a great challenge to understand the molecular basis of the specific function of the heme in a particular type of heme protein environment [3,4]. The most common strategy for the understanding of the structure–function relationship for an enzyme involves the substitution of amino acid residues near the active site by site-directed mutagenesis followed by investigations of the effect of the substitution on the function of that enzyme [3,5–11].

Peroxidases are heme-enzymes catalyzing the one electron oxidations that use hydrogen peroxide as the electron acceptor and have interesting biocatalytic properties with potential use in biosensing and immunoassays [12,13]. The peroxidase catalytic cycle generally involves the formation of the high valent reactive intermediates (Scheme 1 known as the compound I (oxo-ferryl porphyrin(P) π-cation radical, (P+•)Fe(IV) O, CpdI) and the compound II (oxo-ferryl porphyrin, (P)Fe (IV) O, CpdII) that are responsible for their catalytic activity [12,14,15]. The reducing substrate (AH) is generally converted into a radical ($A^{\bullet}$), which subsequently forms dimeric or polymeric products. These reactive intermediates are suggested to be generated by the heterolytic cleavage of the O–O bond of hydroperoxy ferric heme iron. The catalytic cycle and the peroxide shunt pathway of the heme monooxygenases (P450 enzymes) have also been proposed to involve the highly reactive intermediate (compound I) [14]. The X-ray crystal structure analyses of these heme containing enzymes such as chloroperoxidase (CPO, PDB: 1CPO.pdb), horseradish peroxidase (HRP, PDB: 1ATJ.pdb), cytochrome *c* peroxidase (CcP, PDB: 2CYP.pdb) and other peroxygenases (some P450s like $P450_{bs\beta}$) suggest the presence of certain key residues at the distal heme pocket, which enhance the generation of compound I species by acting as acid–base catalysts and by stabilizing it as well by forming hydrogen bond with the ferryl oxygen [4,16]. A histidine is one of such important amino acid residue around the distal heme pocket that is found to be highly conserved in many heme-peroxidases including HRP [3]. The His42 and Arg38 present near the distal side of the heme in HRP are reported to be responsible for the very high rate of compound I formation as well as for the high activity of the enzyme [17–20]. In contrast to HRP, the distal Glu183 in CPO is shown to be the crucial residue to act as the acid base catalyst [19,21,22]. The cytochrome P450,

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