Converting cytochrome b_5 into cytochrome *c*-like protein

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Cytochrome b_5 (cyt b_5) is a well-studied *b*-type cytochrome with heme non-covalently bounded; its heme-holding ability depends mostly on the strong axial ligation provided by His63 and His39. Mutation studies on the axial ligands, aimed at creating new proteins with novel catalytic reactivity, have been limited due to the fact that such alteration led to substantial decrease of the heme-binding stability.[1] In order to study the possibility of formation of covalent linkage between heme and the protein matrix, we replaced residue Ser71 as well as Asn57 in the native cyt b_5 with cysteine by means of site-directed mutagenesis. Two major components, rb_5 N57C/S71C (red color) and gb_5 N57C/S71C (green color) were obtained. Formation of covalent linkages in rb_5 N57C/S71C was confirmed by acidified 2-butanone extraction test and also its ^{1}H NMR spectrum and electrospray mass spectrometry These studies coincide well with the crystal structure reported recently for cytochrome rC_{552} (PDB entry 1QYZ), which contains one extra oxygen atom since the 2-vinyl group of heme forms an unusual [heme-CO-CH_2-S-CH_2-C_α] linkage with cysteine residue.[2] This study demonstrates that (1) a cyt *c*-like cyt b_5 can be obtained by introducing cysteine residue close in space to the heme vinyl groups; (2) the cysteine-heme covalent linkage could be either in the form of thioether or [heme-CO-CH_2-S-CH_2-C_α] depending on their distance and spatial positioning; (3) the classic heme-binding peptide motif “CXXCH” is not essential for heme covalent attachment to protein matrix in cytochromes.

In addition, with heme covalently linked, the constructed cyt *c*-like cyt b_5 (rb_5 N57C/S71C) was tested for the peroxidase activity. The initial rates of the product formation catalyzed by unfolded rb_5 N57C/S71C and unfolded native cyt b_5 were found to be 3.5×10^5 $M\cdot S^{-1}$and 9.4×10^3 $M\cdot S^{-1}$, respectively. The considerably enhanced activity of unfolded rb_5 N57C/S71C could be attributed to an open crevice created for heme upon unfolding to facilitate the access of the peroxide substrate to the heme iron center.

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