CO recombination as a probe of the Fe/Cu binuclear centre of terminal protomotive oxidases

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Cytochrome c oxidase and cytochrome bo are structurally and functionally homologous. Subunit I of each enzyme has a binuclear centre, comprising a high spin haem and a copper (Cyg), and a low spin haem. Recent models of subunit I [1,2] have assigned H419 (E. coli numbering) as the ligand to the high spin haem and H284, H333 and H334 as ligands to Cyg. The most widely conserved region of subunit I is that centred around H284, which is located in putative transmembrane helix VI [1,2]. We have determined the rate of carbon monoxide (CO) recombination in strains of both Saccharomyces cerevisiae [3] and Escherichia coli which carry mutations in this region of subunit I of cytochrome c oxidase and cytochrome bo, respectively.

Carbon monoxide, which binds to the high spin haem, can be photolysed with a quantum yield of almost one. The CO recombination following flash photolysis is monophasic and slow (7 x 10^-4 M^-1 s^-1 at room temperature, 1 atm CO) compared with many other haemoproteins (10^8 M^-1 s^-1). This rate constant is the same in whole cells, in membrane preparations or in the purified enzyme, and presumably reflects either the protein environment of the enzyme or the structure of the binuclear centre.

Figure 1 shows axial and vertical projections of helix VI of subunit I, on which are indicated the positions of the mutations we have examined. In some positions, we have employed several mutants. Mutations of positions V287 or Y288, close to H284, resulted in a decreased rate of CO recombination, but mutation of P285 caused an increase (Fig. 2). These effects are not likely to arise from a loss of Cyg, as it has been shown that mutants of H284 itself need not loose the metal [4]. Furthermore, the rate of CO recombination is much increased in mutants of H333 and H334, the other two Cyg ligands [5,6].

These results suggest that Y288 and V287 are close to the path of CO diffusion into the binuclear centre, so that mutations cause a change of either the conformation or environment of Cyg. The similarity of the effects of mutation of H284 and Y288 are consistent with the recent suggestion that Y288 might act as a Cyg ligand [2]. Replacement of P285, which is likely to cause a bend in the helix in the wildtype enzyme [1], may have caused a structural change in helix VI, resulting in a less occluded binuclear centre and an increase in the rate of CO recombination.

Mutations in this region of subunit I have consistent effects on the rate of CO recombination (Fig. 2) with either cytochrome bo or cytochrome c oxidase. The consistency of the results obtained confirm that the binuclear centres of the two enzymes have strong structural homology. Furthermore, they provide a basis for an analysis of the environment of Cyg and the binuclear centre.

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Figure 1. Projections of helix VI of cytochrome bo on which are indicated those residues which have been mutated in E. coli (C) or in S. cerevisiae (O). The angle about H284 (C) is shown in on the axial projection and no attempt has been made to indicate the effect of P285 on the helical structure.

Figure 2. Effect of mutations of cytochrome bo (O) or cytochrome oxidase (C) on the rate of CO recombination. The rates, relative to those of the wildtype enzyme (70 ± 3 s^-1 [3] and 50 ± 3 s^-1 [6] for cytochrome c oxidase (C) and cytochrome bo (O), respectively) are plotted as a function of the angle (θ) relative to H284 (θ = 0°). The number of mutations examined are indicated at each point.