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THE DYNAMICS AND OXIDATION STATE DEPENDENCE OF COMPLEX FORMATION BETWEEN CYTOCHROME C PEROXIDASE AND CYTOCHROME C

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Abstract The dynamic complex formed between two redox proteins, cytochrome c peroxidase (ccp) and cytochrome c (cytc), was characterized by energy transfer measurements. In Mgccp, magnesium porphyrin is substituted for heme to produce a fluorescent ccp donor; the heme in cytc acts as an energy acceptor. At low temperatures (77 K) Mgccp shows a simple decay when alone in solution but exhibits a complex behavior when bound to cytc. At room temperature the decay is simplified. This suggests a highly plastic binding domain in which conformational states of the cytc:ccp complex trapped at low temperature reequilibrate on the nanosecond time scale. The oxidation state of cytc also effects the nature of the complex. The reduced cytc shows a higher affinity for ccp and for three peroxidase mutants with surface mutations of Asp --> Lys at positions 37, 79 and 217 as demonstrated via the ionic strength dependent binding behavior of all four to a cytc affinity column. Higher affinity of CcP for reduced cytc over oxidized is consistent with the direction of electron transfer of the peroxidase reaction from the reduced to the oxidized forms of cytc and subsequent release of oxidized cytc product.

Keywords: Complex, cytochrome c, dynamics, oxidation state, cytochrome c peroxidase, fluorescence

INTRODUCTION

Cytochrome c (MW 13,000, pI 11) and cytochrome c peroxidase (MW 34,000, pI 5.4) are soluble heme containing yeast proteins. In vivo they participate in the reaction;

$$2H^+ + H_2O_2 + 2Fe(II)\ cytc \xrightarrow{ccp} 2H_2O + 2\ Fe(III)\ cytc$$

These proteins are a convenient model for biological electron transfer. Both proteins can be isolated in pure form and their high resolution crystal structures have been published. Computer modeling has provided a lock and key binding model in which complementary charged residues on cyt c and ccp play key roles in the complexation[1].

X-ray diffraction of cocrystalized ccp and cytc failed to give any information regarding the binding face of the couple due to disorder in the crystal[2]. Computer modeling only provides a static model for the complex. However, crystal structure does not always mirror protein surfaces in solution. Experiments have been performed to determine whether the binding is static or dynamic, and if the residues implicated in the model are important.

RELATIVE BINDING AFFINITY OF CCP AND ITS MUTANTS TO CYTOCHROME C MEASURED BY AFFINITY CHROMATOGRAPHY

Activated Thiol Sepharose 4B, a thiol functionalized agarose material, was attached to yeast Iso-1 Cytochrome C via cys 102, opposite the heme face (the suspected ccp binding domain) as described Azzi et al.[3] Either reduced or oxidized cytc could be covalently bound to the thiol functionalized agarose bead. The resultant affinity column is used to compare binding between mutants as a function of pH, ionic strength and oxidation state.

Pure ccp was introduced to the column, pre-equilibrated to low ionic strength. Ccp bound to the cytc and was then washed off with a phosphate gradient. Integrated chromatograms are shown in Figures 1a and b. A vertical line at any ionic strength gives relative binding affinities for the mutant peroxidases shown. Peroxidases bind more strongly to reduced cytc than oxidized cytc. Note the differences in scale between oxidized and reduced cytc columns. Binding affinities are K37<<K217<native<K79 for oxidized and K37<<K79<native<K217 for reduced cytc columns. Both experiments indicate that the change at position 37 disrupts binding to cytc.

These results agree with measurements of relative binding affinities obtained using steady state fluorescence[4] and are predictive of trends in electron transfer rates.

THE DYNAMICS OF CYTOCHROME C AND CYTOCHROME C PEROXIDASE COMPLEX[5]

The tight binding protein complex formed by two redox partners, cytc and ccp, has been characterized by dynamic energy transfer measurements. In Mgccp, magnesium(II) is substituted for iron(III) in the heme of ccp to produce a fluorescence energy donor, while iron(III) cytc acts as an energy acceptor. At low temperatures (77K), Mgccp shows a simple decay when free in solution but gives a complex decay

FIGURE 1A

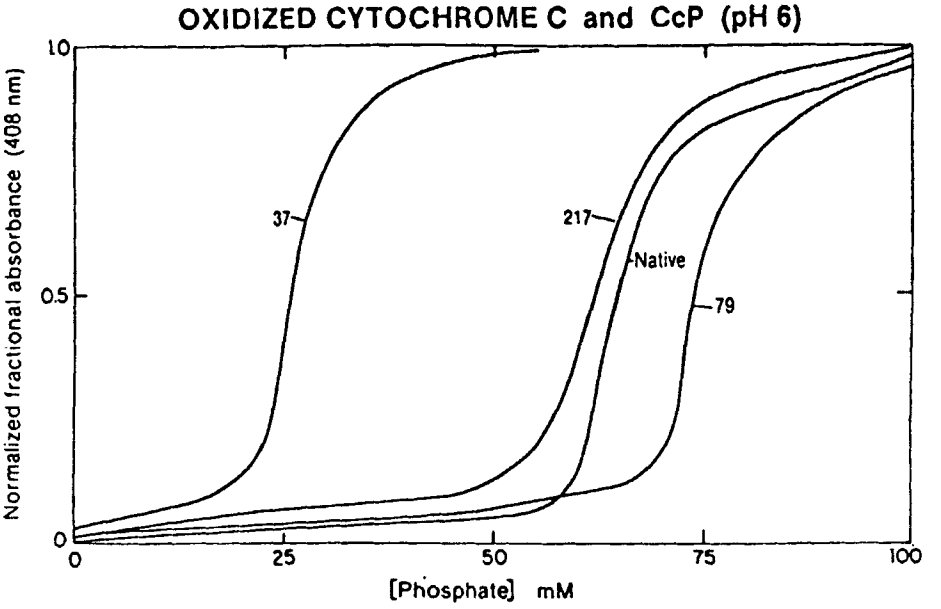
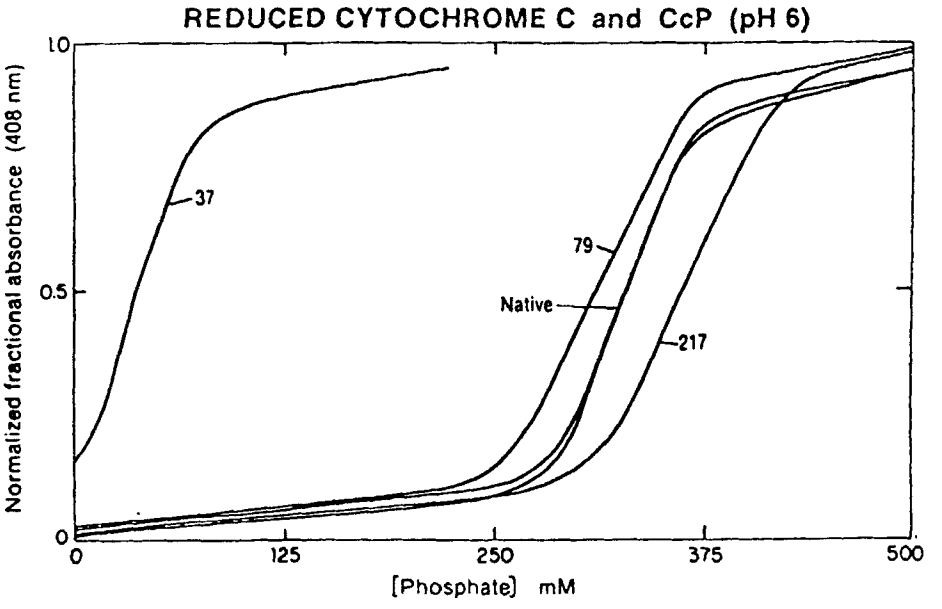


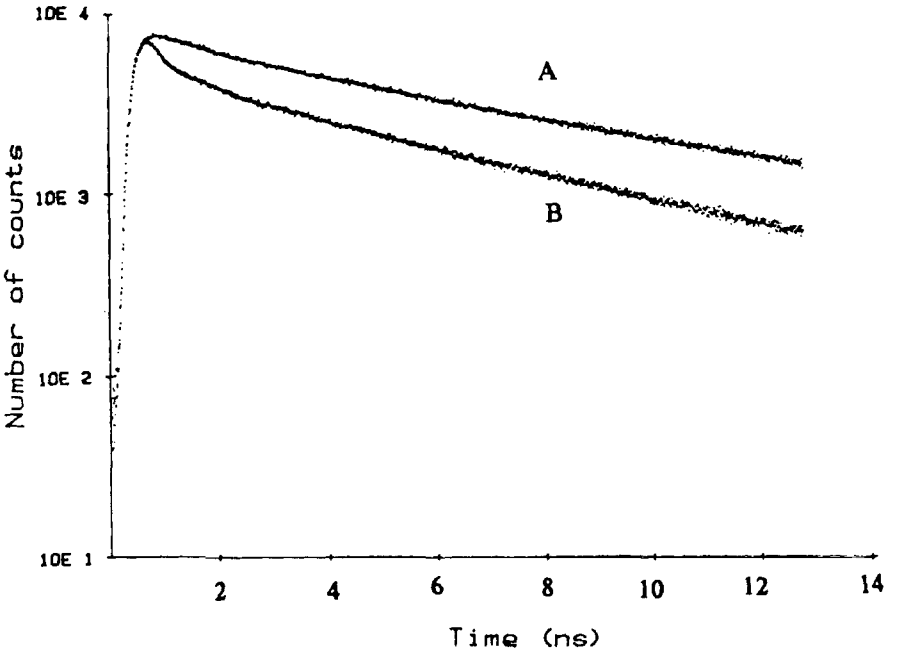
FIGURE 1B



when bound to the energy acceptor Fe(III) cytc. On warming to room temperature, the decay profile is considerably simplified. This change suggests that individual conformational states which are trapped at low temperature in the ccp:cytc complex reequilibrate on the nanosecond time scale via two-dimensional diffusion.

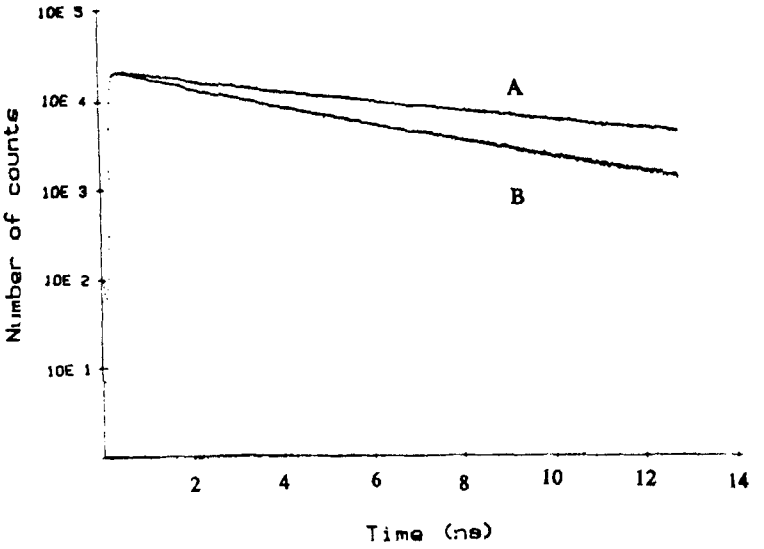
The method of single-photon-counting has been used in this dynamic study. SPC decay profiles of the Mgccp (A) and Mgccp:Fe(III) cytc complex (B) at 77K are shown in Figure 2. For the low temperature experiments, either Mgccp or the Mgccp/Fe(III) cytc complex was dissolved in a buffer (0.01 M phosphate pH 7.0) and cooled to 77K in an optical dewar. The excitation and emission wavelengths for Mgccp were 556nm and 600nm respectively.

figure 2.



SPC decay profiles of Mgccp (top) and Mgccp:Fe(III) cytc complex at 300K (bottom) are shown in figure 3. The decay profiles at both temperatures were fit with deconvolution using the Fortran program FDCC.

figure 3



The radial distribution describing the Mgccp: Fe(III) cytc complex at 77K extracted from energy transfer measurement. The 77K data for the energy transfer couple was fit using an exponential series program written by John Marohn. The data was fit using an exponential series of forty-five terms. Simple analytical formulas were derived which relate the recovered lifetime distributions to the corresponding distribution of donor to acceptor distances.

CONCLUSIONS

The complex formed by cytc and ccp is dynamic, with many rapidly equilibrating binding sites. Computer modeling describes a static complex is insufficient to explain the behavior of this complex. If the Poulos and Kraut model is describing the most commonly occupied or equilibrium binding site, altering described residues by mutagenesis should give roughly equivalent decreases in binding activity, this is clearly not the case. The aspartic acid residues are not of equal importance, and altering them

may assist complexation. Furthermore, these results are predictive of electron transfer trends between mutants.

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