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Electron Transfer between Cytochrome *c* and Cytochome *c* Peroxidase in Single Crystals

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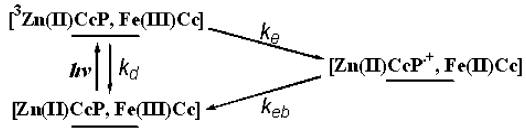
Studies of the natural redox partners cytochrome *c* (Cc) and cytochrome *c* peroxidase (CcP) have greatly advanced our understanding of interprotein electron transfer (ET) reactions.[1] By phototriggering redox chemistry from Zn porphyrins substituted at heme centers (Scheme 1), Hoffman and colleagues have elegantly shown that Cc/CcP ET reactions depend on solvent conditions, conformational gating, and multiple association sites of varied reactivity.[1a,b] Crystal structures reveal different interactions between CcP and the natural partner yeast isozyme-1 Cc (yCc) compared to CcP and the yCc homologue horse Cc (hCc).[2] While these structures likely define the general binding modes found in the 1:1 Cc/CcP solution complexes at low ionic strength, it is not clear whether the crystal structures represent ET active states.[1,3] To directly associate ET rates with defined structures, we have carried out ET measurements and structure determinations of Zn porphyrin-substituted CcP (ZnCcP) bound to either yCc or hCc in diffraction-quality single crystals. Our results show that quenching of CcP/ZnPorph[3] by Fe(III)Cc in the 1:1 solution complexes involves the protein associations defined by the crystal structures and that while relative forward ET rates (k_e) are likely conformationally gated, back rates (k_{eb}) are faster for yCc than for hCc because of better coupling.

Scheme 1



Crystals of yCc/ZnCcP (PDB code 1u74) and hCc/ZnCcP (PDB code 1u75) were grown from proteins and conditions[4] modified from those of the native protein complexes.[2] The respective crystals diffracted to 2.4 and 2.55 Å resolution (at 100 K), without requiring prior dehydration.[2] The resulting structures[4] are very similar to the room-temperature structures of the native protein complexes (2.3 and 2.8 Å resolution, PDB codes: 2PCC and 2PCB), although the improved resolution better defines the interface structure of the hCc/CcP complex (Figure 1). Notably, an unexpected phosphate anion coordinates to the CcP Zn porphyrin in both structures (Figure 1, inset), which may partly explain the dependence of ET reactivity on phosphate concentration.[1a,b] Similar to the native structures, hCc binds CcP in a rotated orientation compared to the native partner yCc (Figure 1). Consequently, the yCc/CcP complex has a shorter intermolecular metal-to-metal distance (26.4 Å) and porphyrin-edge-to-porphyrin-edge distance (19.1 Å) than the CcP/hCc complex (30.0 Å, 22.4 Å). In both crystal forms, all other Zn porphyrin-to-heme-iron interprotein distances generated by the crystal lattice exceed 35 Å, and thus they should not contribute appreciably to intermolecular ET reactions within the crystals.

Enhanced quenching of the ^{3}Zn porphyrin triplet excited state (^{3}ZnCcP) by Fe(III)Cc and Fe(II)Cc was measured in crystals by exciting ZnCcP with 550−580-nm laser pulses (8 ns) and monitoring (^{3}ZnCcP) by transient absorption at 460 nm.[4] The ^{3}ZnCcP decay rate (k_p) reflects both the intrinsic triplet-state decay, k_d, and decay processes resulting from the proximity of Fe(III)Cc (k_t): $k_p = k_d + k_t$.[8] In the absence of energy transfer, $k_t = k_e$. To obtain k_d, k_p was evaluated in crystals of Fe(II)yCc/ZnCcP, where quenching by ET is eliminated. Fe(II)yCc/ZnCcP crystals gave a monoexponential excited-state decay with a rate constant of $k_d = 88 \pm 3\ s^{-1}$ (Figure 2). ^{3}ZnCcP in complex with Fe(III)yCc decayed more quickly with a rate constant of $k_p = 322 \pm 21\ s^{-1}$ due to either ET with Fe(III)Cc[4] or an energy transfer process not operative with Fe(II)Cc (Figure

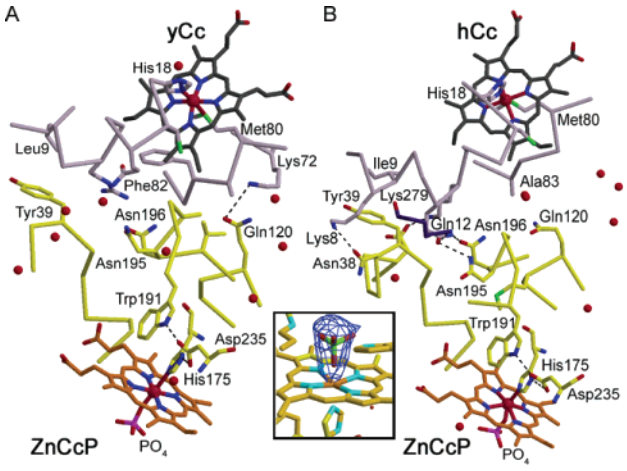


Figure 1. Interfaces of the yCc/ZnCcP (A) and hCc/ZnCcP (B) complexes bury a relatively small amount of solvent-accessible surface area that is atypically hydrophilic and has relatively low complementarity (1191 Å^2, 53% hydrophilic, Sc = 0.54[4] for yCc/ZnCcP and 1153 Å^2, 53% hydrophilic, Sc = 0.55[4] for hCc/ZnCcP). A Cc heme vinyl group directly contacts CcP only in the yCc/CcP complex, whereas a short (2.7 Å) intermolecular hydrogen bond (between hCc Gln12 and CcP Asn196) lies on the path directly between hemes only in the hCc/ZnCcP complex. Lys 279 (blue), from a symmetry-related molecule in the hCc/CcP crystal lattice, mediates a hydrogen-bonding network that couples hCc Gln12 to CcP Asn195. Inset: Omit electron density of phosphate bound to ZnCcP.

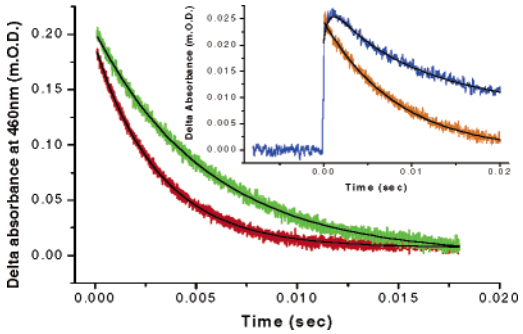


Figure 2. ^{3}ZnCcP decay kinetics monitored at 460 nm. (Normalized Δ-absorbance.) Fe(III)yCc/ZnCcP (red) and Fe(II)yCc/ZnCcP (green). Inset: Kinetics of hCc/ZnCcP ET. Kinetics for ZnCcP$^+$ formation and decay at 680 nm (blue) reflect rate of ^{3}ZnCcP decay at 460 nm (yellow) in crystals.

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2). The difference between k_p and k_d within a crystal of yCc/ZnCcP is 234 ± 24 s^{-1} at room temperature, in close agreement with the solution value of 266 ± 13 s^{-1} under low ionic strength conditions.[5a] In contrast, Fe(III)hCc quenches ZnCcP[3] less effectively ($k_p = 124 \pm 12$ s^{-1}; $k_t = 36 \pm 15$ s^{-1}), in good agreement with forward ET rates from previous solution studies ($k_e = k_t = 17 \pm 3$ s^{-1}).[5a,c]

Remarkably close agreement between crystal and solution quenching rates confirm that the crystal structures represent the associations of both yCc and hCc that determine solution reactivity under conditions of low ionic strength and low protein concentration. Thus, the ordered assemblies of the lattice govern quenching reactions in the crystal, despite high effective protein concentrations.

In solution, the charge-separated intermediates, Fe(II)hCc and ZnCcP$^+$, form with kinetics expected for quenching of ^{3}ZnCcP by ET to Fe(III)Cc.[5c] We established that ET to Fe(III)Cc quenches ^{3}ZnCcP in hCc/ZnCcP crystals by measuring formation of Zn(II)-CcP$^+$ at 680 nm. The fitted progress curve at 680 nm (Figure 2, inset) provides kinetic parameters that agree well with those derived from solution studies that monitored Fe(III)−heme reduction.[5c] Importantly, Zn(II)CcP$^+$ builds up and decays in crystals with kinetics that reflect the rate of ^{3}ZnCcP decay ($k_p = 154 \pm 20$ s^{-1}) and a back ET reaction (Scheme 1) that can be described by three components ($k_{eb}^1 = 1650 \pm 180$ s^{-1}, $k_{eb}^2 = 67 \pm 8$ s^{-1}, and $k_{eb}^3 = 10 \pm 3$ s^{-1}) of which the fraction (f) of the largest term dominates ($f^1 = 0.82$, $f^2 = 0.09$, and $f^3 = 0.09$).[4] In solution, a smaller k_{eb}^1 (757 ± 88 s^{-1}, $f^1 = 0.78$)[4] suggests that reduced motion in the lattice favors an especially ET active conformation.

We observe no formation of ZnCcP$^+$ in crystals of yCc/ZnCcP, consistent with solution studies of the 1:1 complex.[5c] Thus, either ^{3}ZnCcP quenching results from energy transfer (Förster mechanism) or k_{eb} exceeds k_e to the degree that no intermediate builds up. Two observations support ET quenching of ^{3}ZnCcP by Fe(III)Cc: (1) yCc/ZnCcP has closer and much better coupled redox centers than hCc/ZnCcP, yet hCc/ZnCcP undergoes ET and (2) yCc(FeII) does not quench ^{3}ZnCcP in the crystal. Spectral overlap considerations indicate that Förster energy transfer should be minimal and not distinguish Fe(II)Cc from Fe(III)Cc.[6] In cocrystals of Fe(III)Cc/ZnCc, Fe(III)Cc quenches ^{3}ZnCc by ET over a distance of 22 Å.[7c] Furthermore, Cc/ZnCcP complexes fixed in sol gels have ET activity that was interpreted as possibly resulting from the 1:1 complex.[5d] Nevertheless, in solution, most of the charge-separated intermediates generated by yCc/ZnCcP arise from yCc binding at a second site not accessed in the crystal.[1a,8c]

The correspondence between solution and crystal ET rates in this and other systems[7] indicates that crystallization does not affect potentials or reorganization energies of the protein redox centers. It follows that the proteins themselves provide the restrained motions necessary to stabilize charge-separated states and activate long-range ET.[8]

Differences in ET rates between yCc/CcP and hCc/CcP in crystals will primarily depend on the different associations within the respective complexes, because the two structures generate very similar environments for the redox centers. As others have found,[1a,2,9d] electron-tunneling calculations[9] of relative ET rate constants between yCc/ZnCcP and hCc/ZnCcP using either a distance-dependent exponential, bonding network model, or atom density model[9] predict faster tunneling rates for the yeast complex by many orders of magnitude.[4] Thus, the modest 5-fold faster rate we observe in the yCc/ZnCcP crystals for the forward ET rate constants (k_e) cannot be reconciled by application of straightforward electron tunneling models to the static crystal structures. In contrast, much faster back rates (k_{eb}) for yCc/ZnCcP agree with theory and predict the absence of an observable ET intermediate. Conformational gating at the interfaces could level the relative forward ET rates in the two complexes. Crystallographic refinement of TLS rigid body displacement parameters[4] for each protein in the two complexes indicates that peripheral regions of the Cc molecules show significant anisotropic displacements, but residues at the Cc/CcP interfaces are well-ordered (rms displacements $\ll 1.0$ Å). Furthermore, the crystal lattice prevents movement of hCc to the configuration of yCc in the yCc/CcP complex. Thus, if protein conformational fluctuations limit forward ET rates across the interfaces,[3c,d] these structural changes must be small. Once a productive conformation locks in, the relative back ET rates are apparently fast enough to be controlled by the coupling pathways and, hence, differentiate the yCc and hCc complexes.

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Supporting Information Available: Details of protein expression, purification, and crystallization. Details of crystal structure determination, rate calculations, and transient absorption experiments. Transient spectra of hCc/ZnCcP in solution. Solution quenching data of ZnCcP by yCc and hCc. This material is available free of charge via the Internet at http://pubs.acs.org.

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