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ELECTRON TRANSFER BETWEEN FERROCYTOCHROME *c* PEROXIDASE (CCP^{II}) AND FERRICYTOCHROME *c* (C^{III}): IONIC STRENGTH EFFECTS

CCP (EC 1.11.1.5) catalyzes the peroxidation of C^{II} [1]. The proteins form a tight complex [1,2], and electron transfer within the complex is believed to be rapid because of the large turnover number of CCP [1]. A recent study [3] also reported fast electron transfer ($k = 1.7 \times 10^4 \text{ s}^{-1}$) in the C^{III}:CCP^{II} complex ($\Delta E^\circ = 0.46 \text{ V}$) [4] following photoinduced electron transfer from C^{II} to CCP^{III}. However, when we attempted to corroborate this rate by a more direct measurement, we obtained a value of 0.2 s^{-1} [5], which is surprisingly slow. Since the crystal structures of both proteins and a computer model of the complex have been published [6,7], this is an ideal model to examine the structural factors important in controlling protein-protein electron transfer; thus, we decided to further probe the redox reactivity of the complex.

The stability of the C:CCP complex is reported to decrease significantly at high ionic strength [2]. Therefore, we expected a changeover from unimolecular to bimolecular electron transfer on increasing the salt concentration. We report here our findings on the ionic strength dependence of electron transfer from CCP^{II} to C^{III}.

EXPERIMENTAL

CCP was isolated by the method of NELSON *et al.* [8] and C was obtained from Sigma (type VI).

The reactions were carried out at room temperature as follows: CCP (3.3 μM) in 5–200 mM phosphate, pH 7.0, containing 0.008% acetophenone and 2% isopropyl alcohol, was sealed in a 1-cm cuvette and degassed. CCP^{II} was formed in situ by UV-irradiation [9] and 50–200 μl of C^{III} were added. Absorbance changes at 440 and 421 nm were followed using a rapid response spectrophotometer (HP Model 8451A; response time 0.1 s). The former wavelength monitors the decrease in CCP^{II} and the latter, an isosbestic point in the CCP spectrum, monitors the reduction of C^{III}.

RESULTS AND DISCUSSION

In 10 mM phosphate the decay at 440 nm and the growth at 421 nm are both exponential, and give rise to identical rate constants as we reported previously [5]. Initial C/CCP ratios of 0.5:1, 1:1, 2:1, and 3:1 were used and the observed rate constants fall within $0.22 \pm 0.02 \text{ s}^{-1}$. Essentially identical results were obtained at 5 mM phosphate. However, when the phosphate concentration was increased to 15 mM, the observed trace of absorbance vs. time indicated that a second, slower process was occurring. At 25 mM phosphate, the fraction of electron transfer occurring via the slow phase had increased at the expense of the fast phase, and at 50 mM phosphate only the slow phase was apparent. Increasing the phosphate concentration up to 200 mM phosphate caused no further changes in the observed kinetics. The absorbance changes occurring on the slow time scale were also found to be strictly first order and the measured rate constants are again independent of the C/CCP concentration within ratios of 0.5:1–3:1. These results are consistent with the scheme:



where k_t is 0.22 and 0.02 s^{-1} at low and high salt, respectively.

For the reaction to be unimolecular, complex formation between the reactants must be extensive. Since K_D for C^{III}:CCP^{III} is micromolar at low ionic strength [1,2], it is reasonable to suppose that K_D for the reactants is equally small. However, at high ionic strength, extrapolated values of K_D (obtained from absorbance changes on complexa-

tion [2]) are greater than millimolar which should give rise to bimolecular kinetics. This raises the possibility of a second C binding site on CCP which is further removed from the peroxidase heme and therefore does not give rise to spectral perturbations. Alternatively, the slow process could be a rate-limiting conformation change of the peroxidase followed by bimolecular electron transfer.

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PS2.20 — TU

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CYANIDE-INHIBITION STUDIES ON CYTOCHROME *c* OXIDASE USING RAPID-FREEZE/EPR

It has been known for a long time that cyanide inhibition of partially reduced cytochrome *c* oxidase occurs with a higher rate than with the oxidized or reduced enzyme. This partial reduction has been suggested to cause a conformational change, leading to a transition to a more active enzyme form and thus rendering its cytochrome a_3 site more available to cyanide binding. An enzyme model with only 2 conformational states, *resting* and the more active *pulsed* form, was found to be inadequate and the model had to be extended to include an *open* and a *closed* conformational state of both the pulsed and the resting form. A partial reduction of the 4 redox centers in the oxidase (cytochrome *a* and a_3 , Cu_A and Cu_B) was proposed to be the trigger mechanism for the transition from closed to open conformation, enabling fast cyanide binding.

To investigate the number of electrons necessary to trigger this conformational change, a rapid freeze/EPR investigation was performed. The data shows that the majority of the enzyme molecules in both resting and pulsed state are rapidly inhibited by cyanide when cytochrome *a* and Cu_A have been reduced and this prevents entry of more

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