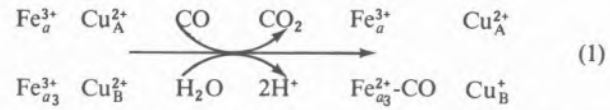
electron transfer-driven proton pumping, especially when a reasonable correlation of electron transfer rate and exothermicity is assumed; and 3) a consideration of the importance and possible mechanisms of electron gating suggests that copper sites in proteins are more attractive than heme sites as candidates for a proton pumping function. The ideas derived from the kinetic analysis of a proton pumping cycle have been used to construct a model for proton pumping by cytochrome *c* oxidase which emphasizes the role and possible mechanisms of electron gating. In this model, Cu_A is the site of proton pumping, and electron gating is effected by protonation-linked changes in the coordination environment of this copper ion. A consideration of the nature of the dioxygen reduction reaction demonstrates that *uncoupling* electron transfer from proton pumping may be desirable at particular steps in this reaction; a mechanism for this uncoupling which involves branched electron transfer pathways is suggested.

PS2.18 — TH

STEPHAN N. WITT
DAVID F. BLAIR
JOEL E. MORGAN
SUNNEY I. CHAN
Arthur Amos Noyes Laboratory
California Institute of Technology
Pasadena, California 91125
U.S.A.

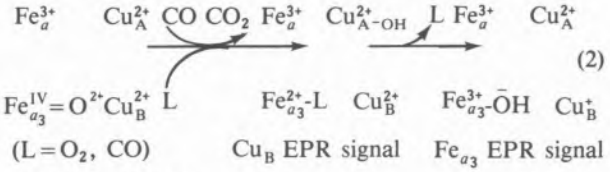
REACTIONS OF CYTOCHROME *c* OXIDASE WITH CARBON MONOXIDE

Cytochrome *c* oxidase oxidizes carbon monoxide to carbon dioxide via three distinct reactions. The «resting» oxidized form of cytochrome *c* oxidase is known to oxidize carbon monoxide very slowly to produce the mixed-valence CO compound ($t_{1/2} = 400$ min. at 277 K) [1,2]. We have observed that the «pulsed» form of cytochrome oxidase also oxidizes carbon monoxide to yield the mixed-valence CO compound. The latter reaction occurs much faster than with the resting enzyme ($t_{1/2} = 4$ min. at 277 K) [2]. Both reactions may be represented as follows:



(1)

In low temperature kinetic experiments using electron paramagnetic resonance (EPR) to follow the reduction of dioxygen by fully reduced cytochrome oxidase, we have recently trapped a highly reactive intermediate at the dioxygen reduction site in which dioxygen is reduced by three electrons. Upon incubation of samples containing this intermediate at 211 K and higher, we observed the formation, within 20 minutes, of two new EPR signals. One EPR signal is due to Cu_B and has been observed previously [3] under different conditions of sample preparation. The other EPR signal is attributable to low spin ferric cytochrome a_3. Both of the EPR signals are diagnostic of a partially reduced Fe_{a3}/Cu_B site. The EPR evidence thus indicates that the three electron-reduced dioxygen intermediate is being reduced by an electron donor other than the metal centers of the protein. Significantly, carbon monoxide is the only available reductant in the low temperature kinetic experiments [4]. Because CO provides two reducing equivalents, and since the Fe_{a3}/Cu_B site becomes only half reduced, the intermediate that oxidizes CO is by implication a ferryl cytochrome a_3/cupric Cu_B couple which is one oxidizing equivalent above the oxidized enzyme (equation (2)). The ferryl cytochrome a_3 intermediate is apparently highly reactive, exhibiting significant carbon monoxide oxygenase activity even at 211 K.



(2)

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PS2.19 — MO

ANN M. ENGLISH
TUN-CHEONG CHEUNG
JACK A. KORNBLATT
Enzymology Research Group
Chemistry and Biology Departments
Concordia University
1455 de Maisonneuve Blvd., West, Montreal H3G 1M8, Quebec
Canada

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 ($k = 1.7 \times 10^4$ s^{-1}) in the C^{III}:CCP^{II} complex ($\Delta E° = 0.46$ 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 l-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 ± 0.02 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:

$$C^{III}{:}CCP^{II} \xrightarrow{k_t} C^{II}{:}CCP^{III} \qquad \Delta E° = 0.46 \text{ V}$$

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-