Stevens, T. H., Martin, C. T., Wang, H., Brudvig, G. W., Scholes, C. P., & Chan, S. I. (1982) *J. Biol. Chem. 257*, 12106–12113.
Sutin, N., Weaver, M. J., & Yee, E. L. (1980) *Inorg. Chem. 19*, 1098–1100.
Taniguchi, V. T., Sailasuta-Scott, N., Anson, F. C., & Gray, H. B. (1980) *Pure Appl. Chem. 52*, 2275–2281.
Taniguchi, V. T., Ellis, W. R., Jr., Cammarata, V., Webb, J., Anson, F. C., & Gray, H. B. (1982) *Adv. Chem. Ser. No. 201*, 51–68.
Urry, D. W., Wainio, W. W., & Grebner, D. (1972) *Biochem. Biophys. Res. Commun. 27*, 625–631.
Vanderkooi, J., & Erecińska, M. (1974) *Arch. Biochem. Biophys. 162*, 385–391.
van Gelder, B. F. (1966) *Biochim. Biophys. Acta 118*, 36–46.
Vanneste, W. H., & Vanneste, M.-T. (1965) *Biochem. Biophys. Res. Commun. 19*, 182–186.
Wainio, W. W. (1983) *Biol. Rev. 58*, 131–156.
Wang, H., Blair, D. F., Ellis, W. R., Jr., Gray, H. B., & Chan, S. I. (1985) *Biochemistry* (following paper in this issue).
Wikström, M. K. F., Harmon, H. J., Ingledew, W. J., & Chance, B. (1976) *FEBS Lett. 65*, 259–277.
Wilkström, M., Krab, K., & Saraste, M. (1981a) *Cytochrome Oxidase: A Synthesis*, Academic Press, London.
Wikström, M., Krab, K., & Saraste, M. (1981b) *Annu. Rev. Biochem. 50*, 623–655.
Wilms, J., Veerman, E. C. J., Konig, B. W., Dekker, H. L., & van Gelder, B. F. (1981) *Biochim. Biophys. Acta 635*, 13–24.
Wilson, D. F., & Nelson, D. (1982) *Biochim. Biophys. Acta 680*, 233–241.

Temperature Dependence of the Reduction Potential of Cu_A in Carbon Monoxide Inhibited Cytochrome *c* Oxidase†

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ABSTRACT: The temperature dependence of the reduction potential of the Cu_A site in carbon monoxide inhibited cytochrome *c* oxidase has been measured with a spectroelectrochemical method adapted to the relatively weak near-infrared absorption of this copper ion. These measurements, together with parallel measurements on the 604-nm absorption due to Fe_a, indicate that an interaction between Cu_A and Fe_a causes the reduction potential for one of these sites to be decreased by approximately 40 mV upon reduction of the other. The temperature dependence of the Cu_A reduction potential indicates a relatively large and negative standard entropy of reduction of Cu_A ($\Delta S^{\circ\prime} = -48.7 \pm 2.3$ eu). Possible implications of the intersite redox interaction and the large standard entropy of reduction of the Cu_A site are discussed.

The Cu_A site in cytochrome *c* oxidase exhibits unique spectroscopic properties (Beinert et al., 1962; Peisach & Blumberg, 1974; Stevens et al., 1982), which have made it the subject of many chemical [Gelles & Chan (1985) and references cited therein] and spectroscopic (Stevens et al., 1982) investigations. Since the flow–flash kinetic studies of Gibson & Greenwood (1965), which demonstrated that this copper ion can undergo oxidoreduction on a time scale comparable to or faster than the overall oxidase turnover, it is generally accepted that Cu_A functions as a catalyst of electron transfer between cytochrome *c* and the dioxygen reduction site (Fe_{a_3} and Cu_B) of the enzyme. The unusual spectroscopic parameters of the site, which must reflect a structure different from those of other metalloprotein copper sites, have led Chan et al. (1979) to suggest that Cu_A is also important in the energy-transducing functions of cytochrome oxidase, specifically as the site of redox-coupled proton pumping.

The reduction potential of the Cu_A site has been measured by van Gelder et al. (1977) at a range of pH values and in different detergents. These potentiometric titrations were carried out by the addition of oxidizing and reducing agents to achieve the desired potentials while the level of oxidation of Cu_A was monitored by an absorption band at 830 nm that is associated with cupric Cu_A (Beinert et al., 1980). The extinction coefficient of this absorption is approximately 2000 M^{-1} cm^{-1}, which makes monitoring the Cu_A site by this method more difficult and less precise than monitoring the heme sites, which have much stronger absorptions. This circumstance probably accounts for the fact that few room-temperature measurements of the Cu_A reduction potential have been undertaken (Tiesjema et al., 1973; van Gelder et al., 1977; Schroedl & Hartzell, 1977a; Babcock et al. 1978), while measurements of the heme potentials have been made repeatedly (Wilson et al., 1972; Tiesjema et al., 1973, Schroedl & Hartzell, 1977a,b; Babcock et al., 1978; Kojima & Palmer, 1983).

A thorough understanding of the thermodynamics of reduction of Cu_A is essential because of the importance of Cu_A in the electron-transfer functions of cytochrome *c* oxidase and because this copper site may be unique among cuproproteins

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in fulfilling a role in energy transduction. We have therefore undertaken spectroelectrochemical measurements of the reduction potential of Cu_A in carbon monoxide inhibited cytochrome oxidase at different temperatures between 1 and 29 °C, using a protocol specifically designed to increase the precision of measurements of the 830-nm absorption. Our measurements on Cu_A, together with parallel measurements of the 604-nm absorption due to Fe_a, furnish evidence for an anticooperative interaction between Cu_A and Fe_a that causes the reduction potential for either one of these sites to be reduced by approximately 40 mV upon reduction of the other. The temperature dependence of the Cu_A reduction potential indicates that the standard entropy of reduction of this site is relatively large and negative compared to those of other metalloprotein copper sites. The thermodynamic parameters for the reduction of Cu_A deduced from the measurements are (all relative to the normal hydrogen electrode) $\Delta G^{\circ\prime}$ (25 °C) = −6.64 ± 0.08 kcal mol^{-1} (285 ± 3 mV vs. NHE),[1] $\Delta H^{\circ\prime}$ = −21.1 ± 0.7 kcal mol^{-1}, and $\Delta S^{\circ\prime}$ = −48.7 ± 2.3 eu.

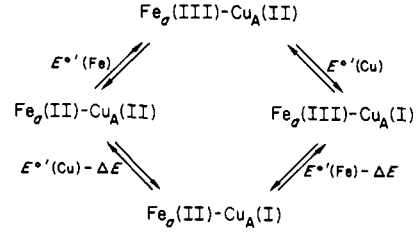
Materials and Methods

Enzyme. Beef heart cytochrome *c* oxidase was prepared as in Ellis et al. (1985). The enzyme concentration used in these experiments was typically 40 μM. The following redox mediators were added: hexaammineruthenium trichloride, 5 equiv; pentaammine(pyridine)ruthenium perchlorate, 5 equiv; (hydroxymethyl)ferrocene, 2.5 equiv; 1,1′-bis(hydroxymethyl)ferrocene, 2.5 equiv. Samples were degassed by 3 cycles of evacuation and flushing with purified argon. One atmosphere of carbon monoxide (Matheson, 99.9%) was then added. The samples were transferred to a glovebox to be loaded into the spectroelectrochemical cell.

Spectroelectrochemistry. Spectroelectrochemistry was carried out as described by Ellis et al. (1985) except that a 2-cm path-length sample cell was employed. The working electrode was a thin gold foil that lined the bottom and the sides of the cell. The sample was stirred by a small magnetic stirring bar. The cell configuration in the gas-tight shroud is such that after 3–4 h of equilibration it becomes an isothermal cell (Taniguchi et al., 1982); i.e., the sample solution and the reference electrode are thermostated at the same temperature. The enzyme was first fully reduced by equilibration at −200 mV vs. SCE until no further change occurred in the absorbance at 604 nm. Titration was then carried out by increasing the potential in 30-mV steps between −130 and 140 mV (vs. SCE) and rereduction in staggered 30-mV intervals. Equilibration at each potential was monitored by the absorbance at 604 nm and was considered complete when no further absorbance change was detectable during a period of 30 min. The equilibration times required (ca. 2 h for each potential) were significantly longer than those for thin-layer electrochemistry experiments, so a complete titration (both oxidative and reductive) required up to 48 h. The absorbance change accompanying oxidation was reproduced upon rereduction to within 2% in the experiments below 20 °C and to within 9% at 23 °C. At 29 °C, the difference was much greater (ca. 30%), probably owing to aggregation and/or denaturation of the enzyme.

Data Analysis. The redox state of Cu_A was monitored by the intensity of the absorption band centered at 830 nm. The bulk of available evidence indicates that this band is due almost entirely to Cu_A (Beinert et al., 1980; Blair et al., 1983). The intensity of the absorbance was quantitated as the area under the spectrum and above a straight line connecting the data at 740 and 900 nm. This area was taken as a measure of the concentration of oxidized Cu_A in calculating values of log (oxid/red) at each potential. The total absorbance change accompanying oxidation was used in calculating log (oxid/red) for the oxidative titration, and the total absorbance change accompanying rereduction was used in calculating log (oxid/red) for the rereductive titration, thus partially compensating for irreversibility in the absorbance changes. The redox state of Fe_a was monitored simultaneously by the absorbance change at 604 nm, which is due entirely to Fe_a in the carbon monoxide inhibited enzyme (Greenwood et al., 1974; Anderson et al. 1976).

Scheme I



The measured absorbance change for both Cu_A and Fe_a did not show the behavior expected of single-electron acceptors. However, the behaviors of both Fe_a and Cu_A were well accounted for by postulating an interaction between these sites that causes the reduction potential for one to be decreased by approximately 40 mV upon reduction of the other. The reaction scheme appropriate to this situation is shown in Scheme I. In Scheme I, $E^{\circ\prime}$(Fe) and $E^{\circ\prime}$(Cu) are the reduction potentials of the iron and copper sites when their respective interaction partners are oxidized, and ΔE is an interaction potential that measures the decrease in reduction potential of one site which accompanies the reduction of the other site.

The equilibrium equations that describe Scheme I were used in a nonlinear least-squares fitting program to calculate the best fit values for $E^{\circ\prime}$(Cu), $E^{\circ\prime}$(Fe), and ΔE. The optimal values of these parameters were calculated by using both the data for Cu_A at 830 nm and the data for Fe_a at 604 nm. The data were corrected for incomplete oxidation at the highest potentials employed (140 mV vs. SCE), as described by Ellis et al. (1985). Finally, the values of $E^{\circ\prime}$(Fe) estimated directly from the 604-nm data were used as fixed parameters in refitting the Cu_A data at each temperature, thus improving the determination of $E^{\circ\prime}$(Cu) and ΔE. The interaction potential ΔE deduced from the Cu_A data (39 ± 2 mV) was in agreement with that deduced from the Fe_a data (42 ± 6 mV).

The reduction potentials of Cu_A measured at various temperatures were fitted to a straight line to obtain an estimate of the standard entropy of reduction. The standard entropy was then corrected for the fact that the cell is isothermal. This correction, according to Ellis et al. (1985), is −15.3 eu. The reported error estimates in $\Delta H^{\circ\prime}$, $\Delta G^{\circ\prime}$, and $\Delta S^{\circ\prime}$ are derived from the standard errors of determination of the relevant parameters in this fit.

Results

Near-infrared absorbance difference spectra obtained during a spectroelectrochemical titration at 6 °C are shown in Figure 1. The spectra indicate that the titration is very nearly reversible except for minor base-line drift. The absorbance near 830 nm was measured as the area under the curves and above a straight line connecting the data points at 900 and 740 nm; this method compensates for changes in base-line slope and

[1] Abbreviations: NHE, normal hydrogen electrode; SCE, saturated calomel electrode.

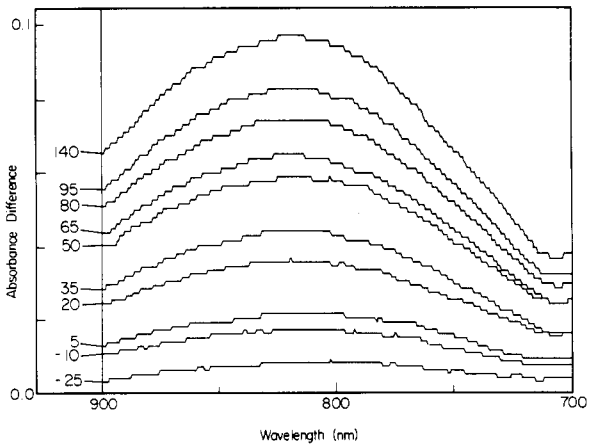


FIGURE 1: Near-infrared absorbance difference spectra obtained during a potentiostatic titration of carbon monoxide inhibited cytochrome *c* oxidase at 6 °C. The indicated potentials are relative to SCE; all spectra are referenced to the spectrum of the fully reduced enzyme obtained at a potential of −200 mV. The enzyme concentration was approximately 40 μM. The redox mediators employed do not make a significant contribution to the absorbance changes observed in this region of the spectrum.

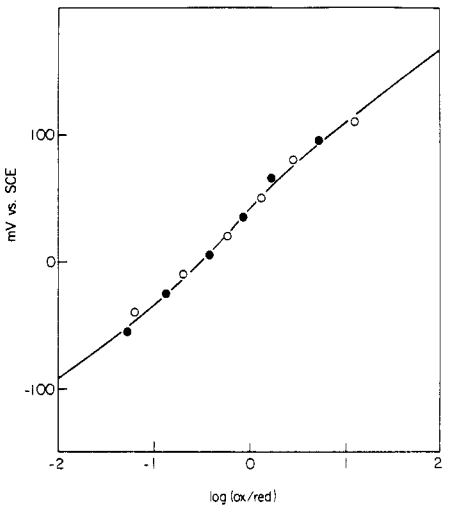


FIGURE 2: Nernst plot of the absorbance changes measured during the titration of Figure 1. The line through the data points is the computer-generated best fit appropriate to the interaction model described in the text. (Open circles) Oxidation; (filled circles) re-reduction.

offset. The difference spectrum obtained during the high-potential half of the titration (140 to 35 mV) was not significantly different from that obtained during the low-potential half of the titration (35 to −200 mV) (spectra not shown), indicating that the properties of the chromophore did not change during the course of the titration. A change in the shape of the spectrum would be expected, for example, if reduction of Fe_a (whose reduction potential is close to that of Cu_A under these conditions) caused a large change in the structure of the Cu_A site.

A Nernst plot of the absorbance differences measured in the same titration is shown in Figure 2. Under all conditions examined, the absorbance changes did not exhibit the behavior predicted by the Nernst equation for a single-electron acceptor. The same type of behavior was observed in the Fe_a chromophore at 604 nm, which was monitored simultaneously in these experiments. A Nernst plot with a slope different from that expected is a strong indication of cooperative behavior (Cornish-Bowden & Koshland, 1975). The behaviors of both Fe_a and Cu_A are well accounted for by postulating an interaction

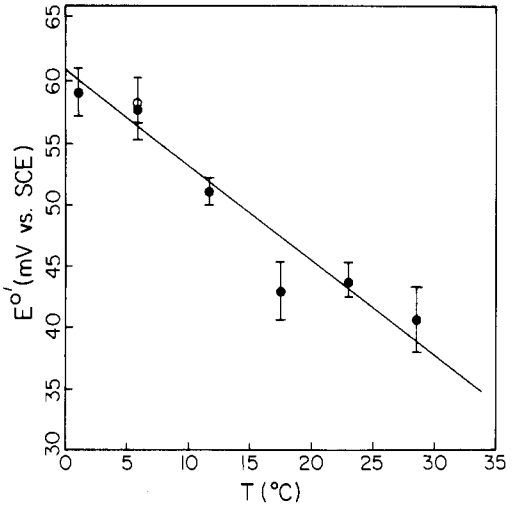


FIGURE 3: Temperature dependence of the reduction potential of Cu_A. The quantity plotted is $E°'$(Cu), the reduction potential of the Cu_A site when Fe_a is oxidized. The straight line through the data points is the computer-generated best fit and leads to the following estimates of the thermodynamic parameters for the reduction of Cu_A (after correction for the isothermal condition under which the experiments were performed): $\Delta G°'$(25 °C) = −6.64 ± 0.08 kcal mol^{-1}, $\Delta S°'$ = −48.7 ± 2.3 eu, and $\Delta H°'$ = −21.1 ± 0.7 kcal mol^{-1}.

between these sites that causes the reduction potential for one to be lowered by approximately 40 mV upon reduction of the other. The line through the data points in Figure 2 is the computer-generated best fit appropriate to this interaction model. (The interaction model and the fitting method employed are described in greater detail under Materials and Methods.) In such an interactive situation, the conventionally defined midpoint potential is no longer a useful measure of the intrinsic properties of the redox site(s) because it is sensitive not only to the intrinsic reduction potential of the site in question but to the potential of the redox site with which it interacts and to the magnitude of the interaction. For this reason, the potential of the Cu_A site when the Fe_a site is oxidized, designated $E°'$(Cu) and obtained from the best fits of the data to the interaction model, was selected as the relevant quantity.

The values of $E°'(Cu_A)$ measured at various temperatures between 1 and 29 °C are plotted in Figure 3. The thermodynamic quantities deduced from a straight-line fit to the data are (relative to the normal hydrogen electrode) $\Delta G°'$(25 °C) = −6.64 ± 0.08 kcal mol^{-1}, $\Delta H°'$ = −21.1 ± 0.7 kcal mol^{-1}, and $\Delta S°'$ = −48.7 ± 2.3 eu. The mean interaction potential deduced from the fits to the Cu_A data was 39 mV and was independent of temperature within the standard error of the determinations. The interaction potential inferred from the fits to the Fe_a data obtained during the same titrations was 42 mV. Given the uncertainty in these determinations, the interaction potentials obtained by the two methods are in satisfactory agreement.

Discussion

Thermodynamic Parameters. The standard entropy of reduction of the Cu_A site is compared with those of several other metalloprotein copper sites in Table I. All of the thermodynamic data available for comparison are for the type I or blue coppers [for a review of the properties of type I coppers, see Gray & Solomon (1981)]. These sites are known to function as efficient catalysts of electron-transfer reactions, but they are not involved in energy conservation or transduction. Compared to the blue coppers, the Cu_A site has a relatively large negative entropy of reduction, 17 eu more

Table I: Thermodynamic Parameters for the Reduction of Metalloprotein Copper Sites

protein	$\Delta G^{\circ\prime}$ (kcal mol^{-1})[a]	$\Delta S^{\circ\prime}$ (eu)	$\Delta H^{\circ\prime}$ (kcal mol^{-1})
azurin (*Pseudomonas aeruginosa*)[b,e]	−7.10	−31.7	−16.6
azurin (*Alcaligenes denitrificans*)[b,f]	−6.37	−23.2	−13.3
azurin (*Alcaligenes faecalis*)[b,g]	−6.22	−29.6	−15.0
plastocyanin (*Phaseolus vulgaris*)[b,e]	−8.30	−18.0	−13.7
stellacyanin (*Rhus vernicifera*)[b,e]	−4.41	−19.8	−10.3
laccase type 1 (*Polyporus versicolor*)[c,h]	−17.99	−13.9	−22.1
cytochrome *c* oxidase Cu_A (*Bos taurus* heart)[d]	−6.64	−48.7	−21.1

[a] At 25 °C. All thermodynamic parameters are relative to the normal hydrogen electrode. [b] Phosphate buffer, ionic strength, 0.1 M, pH 7.0. [c] In 0.2 M phosphate buffer, pH 5.4. [d] In 0.1 M phosphate buffer, pH 7.0. [e] Taniguchi et al., 1980. [f] E. M. Baker, W. R. Ellis, Jr., T. Loehr, and H. B. Gray, unpublished results. [g] W. R. Ellis, Jr., I. Pecht, and H. B. Gray, unpublished results. [h] Taniguchi et al., 1982b.

negative than that of *Pseudomonas aeruginosa* azurin, which is most nearly comparable. At the pH employed for the azurin study (Taniguchi et al., 1980) it is expected that reduction is partially linked to protonation of an ionizable group in the protein, probably His-35 (Corin et al., 1983; Canters et al., 1984), so that a component of the observed entropy of reduction may be due to protonation rather than to reduction per se. The reduction potential of Cu_A is weakly if at all pH dependent near pH 7 (van Gelder et al., 1977), so protolysis is not expected to contribute to its measured entropy of reduction.

The relatively large entropy change that occurs upon reduction of Cu_A may reflect a substantial protein conformational change, possibly related to a role of the copper site in energy transduction (i.e., proton pumping). Alternatively, it may be caused by the large overall negative charge of the oxidase or by a relatively buried disposition of the copper site, which shields it from interaction with the solvent, necessitating more extensive tightening of the protein structure upon reduction (Taniguchi et al., 1982a). The entropy of reduction of the Fe_a site is also relatively large and negative, but the reduction potential of this site is independent of ionic strength, suggesting that the overall protein charge is not very important in determining its reduction potential and reduction entropy. Further spectroscopic studies of the structure of the reduced Cu_A site are needed to test the suggestion that significant structural changes accompany oxidoreduction of this site.

It is not known whether Cu_A is reduced by cytochrome *c* directly or only via cytochrome *a* during turnover of the oxidase. The standard entropy change that accompanies electron transfer from cytochrome *c* [$\Delta S^{\circ\prime}(Fe_c) = -28.5$ eu; Taniguchi et al., 1982a] to Cu_A is approximately −20.2 eu, and that accompanying electron transfer from Fe_a to Cu_A is approximately 2.1 eu (Ellis et al., 1985) (assuming that the entropy of reduction of Cu_A is not greatly changed upon binding cytochrome *c* to the oxidase). The standard free-energy change accompanying electron transfer from cytochrome *c* to Cu_A at physiological temperature, predicted by extrapolation of the present measurements, is -0.40 ± 0.09 kcal mol^{-1}.

Interaction between Cu_A and Cytochrome a. In the CO-inhibited cytochrome oxidase used in this study, only two of the four metal sites in the enzyme undergo oxidoreduction, so long as very highly oxidizing potentials are not reached (Anderson et al., 1976; Wilson & Nelson, 1982). This system is well suited, because of its simplicity, to the study of interactions between these sites. We have found that multiple interactions occur among the sites in the uninhibited enzyme. Various alternatives to intramolecular site–site interactions may be proposed to explain non-Nernstian behavior, including electrostatic effects within a membrane-like aggregate (Walz, 1979) or intradimeric interactions (Wikström et al., 1981). It is therefore important to test a particular interaction model in every way possible and verify that the data are not systematically at variance with the predictions of the model. The data obtained in this study are consistent with the proposed Cu_A/Fe_a interaction model in two key respects: first, the interaction potential ΔE inferred from the data on the iron site is the same, within experimental error, as that inferred from the data on the copper site; and second, the potentials of the copper site that are inferred from the computer fits to the iron data, while poorly determined by the fits and thus subject to considerable variation from one experiment to another, are in agreement with the Cu_A potentials determined directly from the copper data.

The anticooperative interaction between Cu_A and Fe_a could take place via either an electrostatic mechanism or a conformational mechanism. The electron-spin relaxation of Cu_A in the CO-inhibited enzyme is made slower upon reduction of Fe_a (Brudvig et al., 1984), which suggests that these sites interact magnetically. However, magnetic dipolar broadening of the Cu_A resonance by the spin on Fe_a has not been detected. Assuming that the change in copper spin relaxation upon Fe_a reduction reflects only magnetic dipolar interaction between the sites, the EPR results imply an intersite distance between 13 and 26 Å. A 40-mV electrostatic interaction at this distance seems unlikely.

Close examination of the line shape of the Cu_A EPR resonance shows that one of its *g* values shifts slightly (by approximately 3 G at X-band) upon reduction of Fe_a (Brudvig et al., 1984). This indicates that Fe_a reduction induces a minor structural change at the Cu_A site. It is reasonable to associate this structural change with a change in the reduction potential of the copper: the EPR spectra of the blue copper protein azurin from two different species (*Alcaligenes faecalis* and *Pseudomonas aeruginosa*) are also only slightly different (unpublished observations), yet the reduction potentials of these proteins differ by 43 mV (Rosen et al., 1981). The anticooperative redox interaction between Fe_a and Cu_A is thus most plausibly explained by a mechanism in which these sites communicate via conformational change(s).

The proposed redox interaction is likely to have implications for the behavior of the oxidase during turnover. Because of this interaction, Cu_A and Fe_a will tend to accept only one electron between them, and it will be thermodynamically less likely for both sites to be reduced simultaneously. The reduction potential of Fe_a is substantially increased upon oxidation of the Fe_{a_3}/Cu_B site (Kojima & Palmer, 1983; Goodman, 1984), so the effective reduction potential of Fe_a will probably be significantly greater than that of Cu_A under turnover conditions (where the Fe_{a_3}/Cu_B site is mostly oxidized). If electron transfer between Cu_A and Fe_a is very rapid, so that these sites are at redox equilibrium with each other, this would mean that the Cu_A/Fe_a redox interaction will not be manifested as a splitting of their reduction potentials as is observed in the present experiments. The first electron into these sites would transfer rapidly to Fe_a and reside on Fe_a most of the time. The Cu_A site would not be substantially reduced until the transfer of a second electron into these sites; in this case, the pertinent reduction potential of the Cu_A site will be

the *lower* of the two measured here. However, the electron transfer between Cu_A and Fe_a is not necessarily as rapid as is sometimes suggested (Antalis & Palmer, 1982; Wilson et al., 1975). The available kinetic data may be interpreted as well if it is postulated instead that Cu_A can accept electrons *directly* from cytochrome *c*. The presence of two different functional cytochrome *c* binding sites on the oxidase (Ferguson-Miller et al., 1976; Wilms et al., 1981) and the proximity of Cu_A to residues that are involved in binding cytochrome *c* (Millett et al., 1982) are consistent with this proposal. If electron transfer between Fe_a and Cu_A is relatively slow, the Fe_a and Cu_A sites could each take on one of two different effective reduction potentials, depending upon the redox states of their respective interaction partners. Since Fe_a is mostly oxidized in the mitochondrial steady state, the *higher* of the two potentials measured here will most often be the pertinent potential for Cu_A.

Registry No. Cu, 7440-50-8; Fe, 7439-89-6.

REFERENCES

Anderson, J. L., Kuwana, T., & Hartzell, C. R. (1976) *Biochemistry 15*, 3847–3855.

Antalis, T. M., & Palmer, G. (1982) *J. Biol. Chem. 257*, 6194–6206.

Babcock, G. T., Vickery, L. E., & Palmer, G. (1978) *J. Biol. Chem. 253*, 2400–2411.

Beinert, H., Griffiths, D. E., Wharton, D. C., & Sands, R. H. (1962) *J. Biol. Chem. 237*, 2337–2346.

Beinert, H., Shaw, R. W., Hansen, R. E., & Hartzell, C. R. (1980) *Biochim. Biophys. Acta 591*, 458–470.

Blair, D. F., Martin, C. T., Gelles, J., Wang, H., Brudvig, G. W., Stevens, T. H., & Chan, S. I. (1983) *Chem. Scr. 21*, 43–53.

Brudvig, G. W., Blair, D. F., & Chan, S. I. (1984) *J. Biol. Chem. 259*, 11001–11009.

Canters, G. W., Hill, H. A. O., Kitchen, N. A., & Adman, E. T. (1984) *Eur. J. Biochem. 138*, 141–152.

Chan, S. I., Bocian, D. F., Brudvig, G. W., Morse, R. H., & Stevens, T. H. (1979) in *Cytochrome Oxidase* (King, T. E., Orii, Y., Chance, B., & Okunuki, D., Eds.) pp 177–188, Elsevier/North-Holland, Amsterdam.

Corin, A. F., Bersohn, R., & Cole, P. E. (1983) *Biochemistry 22*, 2032–2038.

Cornish-Bowden, A., & Koshland, D. E., Jr. (1975) *J. Mol. Biol. 95*, 202–212.

Ellis, W. R., Jr., Blair, D. F., Wang, H., Gray, H. B., & Chan, S. I. (1985) *Biochemistry* (preceding paper in this issue).

Ferguson-Miller, S., Brautigan, D. L., & Margoliash, E. (1976) *J. Biol. Chem. 251*, 1104–1115.

Gelles, J., & Chan, S. I. (1985) *Biochemistry 24*, 3963–3972.

Gibson, Q. H., & Greenwood, C. (1965) *J. Biol. Chem. 240*, 2694–2698.

Goodman, G. (1984) *J. Biol. Chem. 259*, 15094–15099.

Gray, H. B., & Solomon, E. I. (1981) in *Copper Proteins* (Spiro, T. G., Ed.) Chapter 1, Wiley-Interscience, New York.

Greenwood, C., Wilson, M. T., & Brunori, M. (1974) *Biochem. J. 137*, 205–215.

Hartzell, C. R., & Beinert, H. (1974) *Biochim. Biophys. Acta 369*, 318–338.

Kojima, N., & Palmer, G. (1983) *J. Biol. Chem. 258*, 14908–14913.

Millett, F., Darley-Usmar, V. M., & Capaldi, R. A. (1982) *Biochemistry 21*, 3857–3862.

Peisach, J., & Blumberg, W. E. (1974) *Arch. Biochem. Biophys. 165*, 691–708.

Rosen, P., Segal, M., & Pecht, I. (1981) *Eur. J. Biochem. 120*, 339–344.

Schroedl, N. A., & Hartzell, C. R. (1977a) *Biochemistry 16*, 4961–4965.

Schroedl, N. A., & Hartzell, C. R. (1977b) *Biochemistry 16*, 4966–4971.

Stevens, T. H., Martin, C. T., Wang, H., Brudvig, G. W., Scholes, C. P., & Chan, S. I. (1982) *J. Biol. Chem. 257*, 12106–12113.

Taniguchi, V. T., Sailasuta-Scott, N., Anson, F. C., & Gray, H. B. (1980) *Pure Appl. Chem. 52*, 2275–2281.

Taniguchi, V. T., Ellis, W. R., Jr., Cammarata, V., Webb, J., Anson, F. C., & Gray, H. B. (1982a) *Adv. Chem. Ser. No. 201*, 51–68.

Taniguchi, V. T., Malmström, B. G., Anson, F. C., & Gray, H. B. (1982b) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3387–3389.

Tiesjema, R. H., Muijsers, A. O., & van Gelder, B. F. (1973) *Biochim. Biophys. Acta 305*, 19–28.

van Gelder, B. F., van Rijn, J. L. M. L., Schilder, G. J. A., & Wilms, J. (1977) in *Structure and Function of Energy-Transducing Membranes* (van Dam, K., & van Gelder, B. F., Eds.) pp 61–68, Elsevier/North-Holland, Amsterdam.

Walz, D. (1979) *Biochim. Biophys. Acta 505*, 279–353.

Wikström, M., Krab, K., & Saraste, M. (1981) *Cytochrome Oxidase: A Synthesis*, Academic Press, London.

Wilms, J., Veerman, E. C. I., Konig, B. W., Dekker, H. L., & van Gelder, B. F. (1981) *Biochim. Biophys. Acta 635*, 13–24.

Wilson, D. F., & Nelson, D. (1982) *Biochim. Biophys. Acta 680*, 233–241.

Wilson, D. F., Lindsay, J. G., & Brocklehurst, E. S. (1972) *Biochim. Biophys. Acta 256*, 277–286.

Wilson, M. T., Greenwood, C., Brunori, M., & Antonini, E. (1975) *Biochem. J. 147*, 145–153.