Electronic and vibrational spectroscopy of the cytochrome *c*:cytochrome *c* oxidase complexes from bovine and *Paracoccus denitrificans*

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Abstract

The 1:1 complex between horse heart cytochrome *c* and bovine cytochrome *c* oxidase, and between yeast cytochrome *c* and *Paracoccus denitrificans* cytochrome *c* oxidase have been studied by a combination of second derivative absorption, circular dichroism (CD), and resonance Raman spectroscopy. The second derivative absorption and CD spectra reveal changes in the electronic transitions of cytochrome *a* upon complex formation. These results could reflect changes in ground state heme structure or changes in the protein environment surrounding the chromophore that affect either the ground or excited electronic states. The resonance Raman spectrum, on the other hand, reflects the heme structure in the ground electronic state only and shows no significant difference between cytochrome *a* vibrations in the complex or free enzyme. The only major difference between the Raman spectra of the free enzyme and complex is a broadening of the cytochrome *a*₃ formyl band of the complex that is relieved upon complex dissociation at high ionic strength. These data suggest that the differences observed in the second derivative and CD spectra are the result of changes in the protein environment around cytochrome *a* that affect the electronic excited state. By analogy to other protein–chromophore systems, we suggest that the energy of the Soret π^* state of cytochrome *a* may be affected by (1) changes in the local dielectric, possibly brought about by movement of a charged amino acid side chain in proximity to the heme group, or (2) π – π interactions between the heme and aromatic amino acid residues.

Keywords: cytochrome *c*; cytochrome *c* oxidase; protein–protein interactions; spectroscopy

Protein–protein interactions play a critical role in biological signal and energy transducing systems. In respiratory electron transfer, for example, electrons are shuttled between the membrane-bound cytochrome *bc*₁ and cytochrome *aa*₃ (cytochrome *c* oxidase, or ferrocycytochrome-*c*:oxygen oxidoreductase, EC 1.9.3.1) components by transient formation of protein–protein complexes between these molecules and the soluble protein cytochrome *c* (Nicholls, 1982). In the case of the cytochrome *c*:cytochrome *c* oxidase complex, the interactions between the two proteins are largely electrostatic and are strong enough ($K_d \sim 10$ nM) that a 1:1 complex between the proteins can be isolated in vitro (Michel & Bosshard, 1984).

Several studies of the cytochrome *c*:cytochrome *c* oxidase complex suggest that conformational transitions in

both cytochrome *c* and the enzyme attend complex formation (Michel & Bosshard, 1984; Weber et al., 1987; Hildebrandt et al., 1990; Lynch et al., 1992). The structural details of these conformational transitions, however, are not well described. Hildebrandt and coworkers have used resonance Raman spectroscopy, with excitation under the cytochrome *c* Soret transition, to follow changes in heme group structure upon complex formation (Hildebrandt et al., 1990). These workers reported that the heme of cytochrome *c* undergoes a structural alteration in the complex that resembles the heme structure induced by low pH or polyanion binding to cytochrome *c* (Hildebrandt, 1991). Our group has studied the second derivative absorption spectrum of the complex between horse heart cytochrome *c* and bovine cytochrome *c* oxidase in the Soret region (Lynch et al., 1992). We have reported a significant change in the electronic transition of ferrocycytochrome *a* upon cytochrome *c* binding to cyanide-inhibited forms of the enzyme. The structural determinants of this change

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in the cytochrome *a* electronic spectrum are not fully understood at present.

To help elucidate the nature of the structural transitions that attend complex formation between these proteins, it may be informative to compare the enzyme from different species. Prokaryotic forms of cytochrome *c* oxidase are composed of far fewer protein subunits, and can serve as convenient models for the more structurally complex eukaryotic enzymes (Ludwig, 1987). Among these bacterial enzymes, the cytochrome *c* oxidase from *Paracoccus denitrificans* is particularly well suited as a model system (Ludwig, 1986). The *P. denitrificans* enzyme consists of three subunits that correspond to the three mitochondrially encoded subunits of the eukaryotic enzyme (subunits, I, II, and III). These three subunits show immunological cross-reactivity with antibodies raised against the corresponding subunits of the mammalian enzyme, as well as significant sequence homology. The three-subunit *P. denitrificans* enzyme contains all of the redox active metal cofactor and displays electron transfer and proton translocation activity similar to the mammalian enzyme (Ludwig, 1987).

In this study we compare the spectroscopic features of the 1:1 complexes between cytochrome *c* and the cytochrome *c* oxidase from two evolutionarily diverse species, bovine (*Bos taurus*) and *P. denitrificans*. We find that complex formation affects the electronic transitions of ferrocyanochrome *a* in both species; however, the electronic ground state vibrations of this chromophore are not significantly affected by cytochrome *c* binding.

Results

Second derivative absorption spectroscopy

We have previously shown that the second derivative absorption spectrum of ferrocyanochrome *a* is sensitive to ligand binding events at cytochrome *a*₃ in the purified bovine enzyme (Copeland, 1991; Sherman et al., 1991). We have further shown that ferrocyanochrome *c* binding affects the second derivative absorption spectrum of bovine cytochrome *c* oxidase in its cyanide-inhibited forms, but not in its unliganded or CO-inhibited forms (Lynch et al., 1992). Figure 1 compares the effects of ferrocyanochrome *c* addition to the cyanide-inhibited mixed valence forms of the bovine and *P. denitrificans* enzymes. In the absence of cytochrome *c*, the second derivative spectrum of this enzyme form displays two transitions associated with ferrocyanochrome *a*, at 442 and 450 nm, in both species. Cytochrome *c* binding collapses this two-band signature of ferrocyanochrome *a* into a single band at 443 nm for the bovine enzyme and 448 nm for the *P. denitrificans* enzyme (the differences in the second derivative spectra for these and other species will be more fully described in a subsequent report; Horvath et al., in prep.). For both species, this change in second derivative pattern is reversed

Fig. 1. Second derivative absorption spectra of the 1:1 complexes between ferrocyanochrome *c* and cyanide-inhibited mixed valence cytochrome *c* oxidase. **A:** Spectrum of the complex between horse cytochrome *c* and bovine cytochrome *c* oxidase at low ionic strength. **B:** Spectrum of the sample from **A** after addition of NaCl to a final concentration of 500 mM. **C:** Spectrum of the complex between yeast cytochrome *c* and *Paracoccus denitrificans* cytochrome *c* oxidase at low ionic strength. **D:** Spectrum of the sample from **C** after addition of NaCl to a final concentration of 500 mM. Each protein was present at a concentration of $\sim 8 \mu\text{M}$ in the buffer system described in the text.

when the ionic strength of the solution is increased with 500 mM NaCl. Similar results were obtained for the complexes prepared with the fully reduced cyanide-inhibited form of the enzyme for both species (data not shown). Thus the ferrocyanochrome *a* Soret transition of the cyanide-inhibited enzyme from both species is sensitive to complex formation with cytochrome *c*. As with the bovine enzyme, the second derivative spectra of the fully reduced unliganded or CO-inhibited forms of the *P. denitrificans* enzyme were not sensitive to cytochrome *c* binding.

Circular dichroism spectroscopy

Figures 2 and 3 illustrate the changes in Soret band circular dichroism (CD) that accompany disruption of the cytochrome *c* complexes by high ionic strength for the fully reduced and cyanide-inhibited mixed valence enzymes from bovine and *P. denitrificans*, respectively. The Soret CD band of cytochrome *c* oxidase occurs at 446 nm for the fully reduced bovine enzyme and at 448 nm for the fully reduced *P. denitrificans* enzyme. Binding of ferrocyanochrome *c* and subsequent disruption of the complex with NaCl has no effect on the wavelength maximum and little effect on the dichroic intensity of this band for either species. In contrast, the ca. 419-nm Soret transition of ferrocyanochrome *c* appears to split into two bands at 413 and 422 nm in the complex; this splitting is reversed upon disruption of the complex at 500 mM NaCl. These data are in good agreement with those reported earlier by Weber et al. (1987) for the bovine enzyme.

Fig. 2. CD spectra of the complexes between horse ferrocycytochrome *c* and bovine cytochrome *c* oxidase in its fully reduced (top) and cyanide-inhibited mixed valence (bottom) forms. For each panel the solid line represents the spectrum of the complex in low ionic strength buffer, and the dashed line represents the spectrum of the same sample after disruption of the complex with 500 mM NaCl. The high ionic strength spectra have been corrected for the dilution effects of the salt addition. Each protein was present at a concentration of $\sim 8 \mu\text{M}$ in the buffer system described in the text.

The Soret CD band of ferrocycytochrome *a* occurs at $\sim 445 \text{ nm}$ in the cyanide-inhibited mixed valence enzyme of both species. In this form of the enzyme, cytochrome *c* binding induces a 2–3-nm red shift of the ferrocycyto-

Fig. 3. CD spectra of the complexes between yeast ferrocycytochrome *c* and *Paracoccus denitrificans* cytochrome *c* oxidase in its fully reduced (top) and cyanide-inhibited mixed valence (bottom) forms. For each panel the solid line represents the spectrum of the complex in low ionic strength buffer, and the dashed line represents the spectrum of the same sample after disruption of the complex with 500 mM NaCl. The high ionic strength spectra have been corrected for the dilution effects of the salt addition. Each protein was present at a concentration of $\sim 8 \mu\text{M}$ in the buffer system described in the text.

chrome *a* band and a small increase in its dichroic intensity. In this enzyme form the ferricytochrome *a*₃ Soret band overlaps with the cytochrome *c* transition, making it difficult to evaluate changes in the composite band envelope. Once again, these complex-induced changes are reversed at high ionic strength, as seen in Figures 2 and 3.

Resonance Raman spectroscopy

Resonance Raman spectra of the cytochrome *c* complexes of bovine and *P. denitrificans* cytochrome *c* oxidase in their fully reduced and cyanide-inhibited mixed valence states were obtained with 441.6 nm excitation, in resonance with the ferroheme A absorption band of the enzyme. Figures 4 and 5 show the effects of salt-induced disruption of the complexes formed with the fully reduced and mixed valence bovine enzyme respectively. In Figures 6 and 7 we show the corresponding spectra for the complexes formed with the *P. denitrificans* enzyme. There are a number of differences between the spectra of the isolated enzymes from these two species that will be addressed in a separate communication (Lynch et al., in prep.). In terms of the effects of complexation with cytochrome *c*, however, neither enzyme shows any significant changes in its heme vibrational pattern between the intact and dissociated complex in either their fully reduced or mixed valence forms. The only reproducible changes that we have observed are a broadening and downshift ($\sim 1,665 \rightarrow 1,660 \text{ cm}^{-1}$) of the cytochrome *a*₃ formyl band in the intact complex, which is relieved when the complex is treated with 500 mM salt. This effect is highlighted in Figure 8 where we show the cytochrome *a*₃ formyl band on an expanded scale for the complex between horse heart cytochrome *c* and fully reduced bovine cytochrome *c* oxidase; a similar broadening of this band was also observed for the complex between yeast cytochrome *c* and *P. denitrificans* cytochrome *c* oxidase.

Discussion

The data presented here show clear evidence for changes in the electronic transitions of ferrocycytochrome *a* upon complex formation between cytochrome *c* and cytochrome *c* oxidase. In the second derivative, absorption spectrum changes are observed for cyanide-inhibited forms of the bovine enzyme but not for the unliganded or CO-inhibited states (Lynch et al., 1992). The same behavior is observed for the enzyme from *P. denitrificans*, further supporting the notion that this bacterial cytochrome *c* oxidase is a good functional model for the more complex mammalian enzyme. With respect to the Soret region CD, complex-induced differences in cytochrome *c* oxidase heme absorption are observed for the cyanide-inhibited mixed valence enzyme from both species. Thus, cytochrome *c* binding appears to affect the environment, or structure, of the cytochrome *a* cofactor of cytochrome

Fig. 4. Resonance Raman spectra of the 1:1 complex between horse ferocytochrome *c* and bovine cytochrome *c* oxidase in its fully reduced form. The bottom spectrum is that of the complex in low ionic strength buffer, and the top spectrum is the same sample after addition of NaCl to a final concentration of 500 mM. Each protein was present at a concentration of $\sim 30 \mu\text{M}$ in the buffer system described in the text. Each panel represents a different frequency region of the spectrum and is scaled arbitrarily to best illustrate the spectral features of that frequency region.

Fig. 5. Resonance Raman spectra of the 1:1 complex between horse ferocytochrome *c* and bovine cytochrome *c* oxidase in its cyanide-inhibited mixed valence form. The bottom spectrum is that of the complex in low ionic strength buffer, and the top spectrum is of the same sample after addition of NaCl to a final concentration of 500 mM. Each protein was present at a concentration of $\sim 30 \mu\text{M}$ in the buffer system described in the text. Each panel represents a different frequency region of the spectrum and is scaled arbitrarily to best illustrate the spectral features of that frequency region.

Fig. 6. Resonance Raman spectra of the 1:1 complex between yeast ferocytochrome *c* and *Paracoccus denitrificans* cytochrome *c* oxidase in its fully reduced form. The bottom spectrum is that of the complex in low ionic strength buffer, and the top spectrum is of the same sample after addition of NaCl to a final concentration of 500 mM. Each protein was present at a concentration of $\sim 30 \mu\text{M}$ in the buffer system described in the text. Each panel represents a different frequency region of the spectrum and is scaled arbitrarily to best illustrate the spectral features of that frequency region.

Fig. 7. Resonance Raman spectra of the 1:1 complex between yeast ferrocitochrome *c* and *Paracoccus denitrificans* cytochrome *c* oxidase in its cyanide-inhibited mixed valence form. The bottom spectrum is that of the complex in low ionic strength buffer, and the top spectrum is of the same sample after addition of NaCl to a final concentration of 500 mM. Each protein was present at a concentration of $\sim 30 \mu\text{M}$ in the buffer system described in the text. Each panel represents a different frequency region of the spectrum and is scaled arbitrarily to best illustrate the spectral features of that frequency region.

c oxidase. In contrast to these dramatic spectral differences, we observe very little change in the resonance Raman spectrum when cytochrome *c* is complexed to these enzymes. Previously, Hildebrandt and coworkers reported resonance Raman spectra of the cytochrome *c*:cytochrome *c* oxidase complex of the bovine enzyme using 407 nm excitation, in resonance with the Soret maximum for cytochrome *c* (Hildebrandt et al., 1990). Not surprisingly, these spectra were dominated by vibrational features of the cytochrome *c* heme group, although some cytochrome *c* oxidase vibrations could also be discerned. Most of the changes reported by Hildebrandt and coworkers were observed in vibrational bands associated with cytochrome *c*. However, these workers also reported relative intensity changes in some cytochrome *c* oxidase vibrational bands caused by cytochrome *c* binding. With the 441.6 nm excitation used in the present study, the vibrational pattern observed is entirely due to the heme A chromophores of cytochrome *c* oxidase. Under these

conditions we do not observe the same relative intensity changes reported by Hildebrandt and coworkers; however, in the absence of an internal intensity standard it is difficult to say whether there are any systematic changes in Raman intensity between the intact and disrupted complex spectra. The data from both groups agree, however, that no significant frequency shifts occur for the vibrational bands of cytochrome *c* oxidase. The only major change that we observe in the vibrational spectrum of the enzyme is a broadening of the $1,665\text{-cm}^{-1}$ band assigned to the formyl stretch of cytochrome a_3 . A similar broadening of the ferricytochrome a_3 formyl band, at $1,673\text{ cm}^{-1}$, was reported by Hildebrandt et al. for the complex between ferricytochrome *c* and fully oxidized bovine cytochrome *c* oxidase (Hildebrandt et al., 1990). This broadening, which is relieved upon disruption of the complex at high ionic strength, suggests that cytochrome *c* binding to the enzyme allows the formyl group of cytochrome a_3 to sample a larger range of configurations relative to the plane of the porphyrin macrocycle. This implies that cytochrome *c* binding effects a conformational change in the enzyme that is transmitted to the binuclear center and "opens up" the cytochrome a_3 binding pocket, thus eliminating steric or other protein-based restrictions to motion of the cytochrome a_3 formyl group.

At first glance it may seem anomalous that one could observe such large changes in the optical spectra but fail to see differences in the resonance Raman spectra of the same complexes. An explanation for these results can be gained from consideration of the physical principles underlying each spectroscopic method (Cantor & Schimmel, 1980). Thus both absorption and CD spectroscopy report the energetic difference between the electronic ground and excited states in the form of the wavelength (more correctly frequency) of maximum absorption, and the allowedness of the electronic transition in terms of the intensity of the absorption band. Under the conditions

Fig. 8. Resonance Raman spectra of the 1:1 complex between horse ferrocitochrome *c* and bovine cytochrome *c* oxidase in its fully reduced form. The spectra are shown on an expanded scale to highlight the cytochrome a_3 formyl stretching mode. The solid-line spectrum is that of the complex in low ionic strength buffer, and the dashed-line spectrum is of the same sample after addition of NaCl to a final concentration of 500 mM. Conditions were identical to those described for Figure 4.

used here, the resonance Raman spectrum reports the energetic differences between vibrational levels of the electronic ground state of the molecule, in the form of the frequencies of maximum Raman scattering (Spiro & Stein, 1977). For π - π^* transition, such as the Soret band of heme groups, changes in the surrounding solvent (or polypeptide in the case of a protein-embedded heme) that affect the energy of the π^* state are expected to affect the absorption and CD spectrum but are less likely to affect the frequencies of electronic ground state vibrational modes (Cantor & Schimmel, 1980). This phenomenon is well known, for example, for the π - π^* transitions of the aromatic amino acids of proteins. Red shifts of the π - π^* absorption bands of these residues are observed when the surrounding solvent polarity is increased by moving them from the hydrophobic interior of proteins into contact with aqueous solution during protein denaturation. Similar shifts are observed when comparing the UV spectra of the free amino acids in water and in 20% aqueous ethylene glycol (Herskovits & Laskowski, 1962). These solvent perturbations do not, however, significantly affect the frequencies of the Raman bands of these molecules when excited under either nonresonance or resonance conditions, although some effect on the Raman intensities has been noted (Copeland & Spiro, 1985; Hildebrandt et al., 1988). By analogy, the differences seen here in the optical spectra of cytochrome *a* upon complex formation with cytochrome *c*, and previously seen between the liganded and unliganded forms of the reduced enzyme (Sherman et al., 1991), could be due to changes in the polarity of the protein environment immediately surrounding the heme. Such polarity changes could be brought about, for example, by movement of a charged amino acid residue or a water molecule (Sassaroli et al., 1989) into proximity with cytochrome *a*.

Alternatively, Fowler et al. (1992) have recently shown that the red shifts associated with some photosynthetic pigments are due, in part, to π - π interactions between the chromophore and nearby tyrosine residues. When these tyrosines are replaced by site-directed mutagenesis, the lowest energy π - π^* transition of the chromophore is blue shifted by as much as 24 nm. In these systems excitonic effects due to pigment-pigment interaction also contribute to the observed red shifts (Fowler et al., 1992). In the case of cytochrome *c* oxidase the two heme A cofactors are thought to be too far apart (19 ± 8 Å) for this type of spectral interaction (Goodman & Leigh, 1987). However, π - π interactions between cytochrome *a* and aromatic amino acids could also play a role in producing the spectral perturbations observed in the electronic spectra reported here, while having minimal effect on the ground electronic state vibrations of cytochrome *a*.

In conclusion, the data presented here suggest that cytochrome *c* binding to the cytochrome *c* oxidase from either bovine heart or *P. denitrificans* does not affect the ground state structure of cytochrome *a*. Therefore, the

changes in cytochrome *a* electronic transitions, seen upon complex formation, most likely reflect changes in the protein environment immediately surrounding cytochrome *a* that affect the energy of the π^* state associated with the Soret absorption band of this chromophore. This interpretation implies that the 450-nm transition of ferrocycytochrome *a* that is seen in the second derivative absorption spectra of liganded forms of cytochrome *c* oxidase results from some specific interactions between the chromophore and the protein and is not an intrinsic property of low spin six coordinate heme A. This inference is supported by our findings that other ferrous heme A bis-histidine complexes, both in model compounds and other proteins, fail to display a 450-nm component in their second derivative spectra, and that partial denaturation of the CN-mixed valence enzyme by treatment with urea, guanidine hydrochloride, or heat result in a selective loss of the 450-nm band. These results will be presented in full detail in a subsequent communication (Horvath et al., in prep.).

Materials and methods

Bovine cardiac and *P. denitrificans* cytochrome *c* oxidase were purified and stored as previously described (Sherman et al., 1991). Horse heart cytochrome *c* was purchased from Sigma and further purified as described by Brautigan et al. (1978). Yeast cytochrome *c* was purified by the method of Sherman et al. (1968). Ferrocycytochrome *c* was prepared by addition of either sodium ascorbate or sodium dithionite to a ferricytochrome *c* solution until no further change in absorbance at 550 nm was observed. Residual reductant was then removed by gel filtration chromatography on a Bio-Rad P10 column (Lynch et al., 1992).

Fully reduced and cyanide-inhibited mixed valence cytochrome *c* oxidase was prepared in low ionic strength buffer (50 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid [HEPES], 0.5% dodecyl β -maltoside, pH 7.4) using sodium ascorbate and *N,N,N,N'*-tetramethyl-*p*-phenylene diamine (TMPD) as reductants as previously described (Ishibe et al., 1991). The 1:1 complexes with cytochrome *c* were prepared as described by Lynch et al. and disrupted at high ionic strength by the addition of NaCl to the protein solution to a final concentration of 500 mM (Lynch et al., 1992). For the bovine enzyme, horse heart cytochrome *c* was used in preparing the complex, and yeast cytochrome *c* was used to form the complex with the *P. denitrificans* enzyme.

Second derivative absorption and resonance Raman spectra were obtained as previously described (Ishibe et al., 1991). CD spectra were recorded with a Jasco J-600 CD spectrophotometer that was interfaced to an IBM personal computer for data acquisition and storage. Samples for CD spectroscopy were contained in the same anaerobic cuvettes as used for absorption measurements. CD spectra were acquired from 500 to 350 nm in 0.4-nm steps with an integration time of 1 s per step, and a spectral band-

pass of 0.2 nm. Each reported spectrum is the average of 10 such scans.

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References

- Brautigan, D.L., Ferguson-Miller, S., & Margoliash, E. (1978). Mitochondrial cytochrome *c*: Preparation and activity of native and chemically modified cytochromes *c*. *Methods Enzymol.* **53**, 128–164.
- Cantor, C.R. & Schimmel, P.R. (1980). *Biophysical Chemistry*, Part II, pp. 349–480. W.H. Freeman, San Francisco.
- Copeland, R.A. (1991). Conformational switching at cytochrome *a* during steady state turnover of cytochrome *c* oxidase. *Proc. Natl. Acad. Sci. USA* **88**, 7281–7283.
- Copeland, R.A. & Spiro, T.G. (1985). Ultraviolet resonance Raman spectra of cytochrome *c* conformational states. *Biochemistry* **24**, 4960–4968.
- Fowler, G.J.S., Visschers, R.W., Grief, G.G., van Grondelle, R., & Hunter, C.N. (1992). Genetically modified photosynthetic antenna complexes with blueshifted absorbance bands. *Nature* **355**, 848–850.
- Goodman, G. & Leigh, J.S., Jr. (1987). The distance between cytochrome *a* and *a*₃ in the azide compound of bovine-heart cytochrome oxidase. *Biochim. Biophys. Acta* **890**, 360–367.
- Herskovits, T.T. & Laskowski, M., Jr. (1962). Location of chromophoric residues in proteins by solvent perturbation. *J. Biol. Chem.* **237**, 2481–2492.
- Hildebrandt, P. (1991). Resonance Raman spectroscopic studies of cytochrome *c* at charged interfaces. *J. Mol. Struct.* **242**, 379–395.
- Hildebrandt, P.G., Copeland, R.A., Spiro, T.G., Otlewski, J., Laskowski, M., Jr., & Prendergast, F.G. (1988). Tyrosine hydrogen-bonding and environmental effects in proteins probed by ultraviolet resonance Raman spectroscopy. *Biochemistry* **27**, 5426–5432.
- Hildebrandt, P., Heimbürg, T., Marsh, D., & Powell, G.L. (1990). Conformational changes in cytochrome *c* and cytochrome oxidase upon complex formation: A resonance Raman study. *Biochemistry* **29**, 1661–1668.
- Ishibe, N., Lynch, S.R., & Copeland, R.A. (1991). The pH dependence of cytochrome *a* conformation in cytochrome *c* oxidase. *J. Biol. Chem.* **266**, 23916–23920.
- Ludwig, B. (1986). Cytochrome *c* oxidase from *Paracoccus denitrificans*. *Methods Enzymol.* **126**, 153–159.
- Ludwig, B. (1987). Cytochrome *c* oxidase in prokaryotes. *FEMS Microbiol. Rev.* **46**, 41–56.
- Lynch, S.R., Sherman, D., & Copeland, R.A. (1992). Cytochrome *c* binding affects the conformation of cytochrome *a* in cytochrome *c* oxidase. *J. Biol. Chem.* **267**, 298–302.
- Michel, B. & Bosshard, H.R. (1984). Spectroscopic analysis of the interaction between cytochrome *c* and cytochrome *c* oxidase. *J. Biol. Chem.* **259**, 10085–10091.
- Nicholls, D.G. (1982). *Bioenergetics, An Introduction to the Chemiosmotic Theory*. Academic Press, New York.
- Sassaroli, M., Ching, Y.-C., Dasgupta, S., & Rousseau, D.L. (1989). Cytochrome *c* oxidase: Evidence for interaction of water molecules with cytochrome *a*. *Biochemistry* **28**, 3128–3132.
- Sherman, D., Kotake, S., Ishibe, N., & Copeland, R.A. (1991). Resolution of the electronic transitions of cytochrome *c* oxidase: Evidence for two conformational states of ferrous cytochrome *a*. *Proc. Natl. Acad. Sci. USA* **88**, 4265–4269.
- Sherman, F., Stewart, J.W., Parker, J.H., Inhaber, E., Ship, N.A., Puterman, G.J., Gardisky, R.L., & Margoliash, E. (1968). The mutational alteration of the primary structure of yeast iso-1-cytochrome *c*. *J. Biol. Chem.* **243**, 5446–5456.
- Spiro, T.G. & Stein, P. (1977). Resonance effects in vibrational scattering from complex molecules. *Annu. Rev. Phys. Chem.* **28**, 501–521.
- Weber, C., Michel, B., & Bosshard, H.R. (1987). Spectroscopic analysis of the cytochrome *c* oxidase–cytochrome *c* complex. *Proc. Natl. Acad. Sci. USA* **84**, 6687–6691.