Interaction of Cytochrome \(bd\) with Carbon Monoxide at Low and Room Temperatures

EVIDENCE THAT ONLY A SMALL FRACTION OF HEME \(b_{595}\) REACTS WITH CO*

Received for publication, December 21, 2000, and in revised form, March 27, 2001
Published, JBC Papers in Press, March 29, 2001, DOI 10.1074/jbc.M011542200

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Azotobacter vinelandii is an obligately aerobic bacterium in which aerotolerant dinitrogen fixation requires cytochrome \(bd\). This oxidase comprises two polypeptide subunits and three hemes, but no copper, and has been studied extensively. However, there remain apparently conflicting reports on the reactivity of the high spin heme \(b_{595}\) with ligands. Using purified cytochrome \(bd\), we show that absorption changes induced by CO photodissociation from the fully reduced cytochrome \(bd\) at low temperatures demonstrate binding of the ligand with heme \(b_{595}\). However, the magnitude of these changes corresponds to the reaction with CO of only about 5% of the heme. CO binding with a minor fraction of heme \(b_{595}\) is also revealed at room temperature by time-resolved studies of CO recombination. The data resolve the apparent discrepancies between conclusions drawn from room and low temperature spectroscopic studies of the CO reaction with cytochrome \(bd\). The results are consistent with the proposal that hemes \(b_{595}\) and \(d\) form a diheme oxygen-reducing center with a binding capacity for a single exogenous ligand molecule that partitions between the hemes \(d\) and \(b_{595}\) in accordance with their intrinsic affinities for the ligand. In this model, the affinity of heme \(b_{595}\) for CO is about 20-fold lower than that of heme \(d\).

Cytochrome \(bd\) is a terminal oxidase present in the respiratory chains of many bacteria (reviewed in Ref. 1, see an evolutionary tree in Ref. 2). The enzyme catalyzes reduction of molecular oxygen to water by ubiquinol or menaquinol as natural electron donors (3–5) but shows no sequence homology to the heme-copper quinol oxidases. Also, in contrast to these oxidases, cytochrome \(bd\) does not contain copper and, although generating \(\Delta\Phi\) (6–9), does not pump protons (10).

Cytochrome \(bd\)-type oxidases purified from Azotobacter vinelandii or Escherichia coli consist of two subunits and carry three iron-porphyrin groups: low spin heme \(b_{558}\), high spin heme \(b_{595}\), and a chlorin-type high spin iron-porphyrin group (heme \(d\)). All three redox centers are proposed to be located near the periplasmic side of the membrane (2). Heme \(b_{558}\) is directly involved in ubiquinol oxidation (11, 12). Heme \(d\) binds \(O_2\) and is involved in the trapping and reduction of oxygen (3). The specific role of heme \(b_{595}\) is still a matter of debate. One obvious possible role is the transference of electrons from heme \(b_{595}\) to heme \(d\) (13–15). However, because heme \(b_{595}\) is high spin, it is tempting to consider its involvement in dioxygen reduction; it was proposed that hemes \(b_{595}\) and \(d\) might form a binuclear dioxygen reduction center analogous to the heme-copper oxygen-reducing site in the cytochrome aa3- or bo3-type oxidases (16–19). In such a case, one might expect heme \(b_{595}\) to react with other exogenous ligands such as CO or NO as is typical of most high spin hemoproteins. Surprisingly, despite a long history of such studies, this essential and apparently simple question has not been answered by the apparently conflicting data. The intricate line shape of the absorption changes induced by CO binding with the fully reduced cytochrome \(bd\) in the Soret band as observed in conventional room temperature studies has long been considered to indicate CO binding with both hemes \(d\) and \(b_{595}\) (e.g. see Refs. 20–22 and references therein). However, the spectral features attributed to CO binding with \(b_{595}\) could be caused by a small bandshift of unligated heme \(b_{595}\) induced by CO interaction with the nearby heme \(d\) (23). Moreover, magnetic circular dichroism spectroscopy has shown that only a small fraction of heme \(b_{595}\), if any, in the E. coli cytochrome \(bd\) binds CO or NO at room temperature. On the other hand, low temperature photodissociation studies carried out by Poole and co-workers (24–26) on cytochrome \(bd\) from different bacteria have revealed consistently a simple pattern of photoinduced spectral changes assigned to the photodissociation of the cytochrome \(b_{595}\).CO complex. Recent femtosecond photobleaching studies have identified the Soret band of ferrous heme \(b_{595}\) at about 440 nm and support this interpretation (23). CO binding with about 15% of heme \(b_{595}\) at cryogenic temperatures was observed with the aid of Fourier transform infrared spectroscopy in the membrane-bound cytochrome \(bd\) from E. coli, but there was no binding in the isolated enzyme (18).

Conceivably, the enzyme may behave differently at cryogenic and room temperatures. On the other hand, the results obtained by different techniques and at different temperatures were also often done with different preparations and under different conditions, which complicates direct comparison of the data in the literature.

We considered it worthwhile to address this problem specif-
ically and compare the spectral changes associated with CO binding and photodissociation at room and low temperatures in the same preparation of purified cytochrome bd from A. vinelandii. The results confirm that CO photodissociation from heme $b_{595}$ is the sole major process revealed spectrophotometrically at temperatures around $-100^\circ$C. However, the fraction of heme $b_{595}$ that shows CO photodissociation at low temperature corresponds to only about 5% of the enzyme. These observations resolve the apparent contradiction between the room and low temperature experiments and corroborate the model of cytochrome bd interaction with exogenous ligands assuming negative cooperativity between hemes $b_{595}$ and d (22, 27).

**EXPERIMENTAL PROCEDURES**

Cytochrome bd was isolated from A. vinelandii strain MR8 overproducing cytochrome bd (28) essentially as reported (21). The cytochrome bd concentration was determined from the dithionite-reduced minus air-oxidized difference absorption spectra using a $\Delta A$ (628–605) value of 12 $\text{mM}^{-1} \text{cm}^{-1}$ (21). E. coli cytochrome bd was isolated from the membranes of strain GO105/pTK1 (29) as described (30), but the final hydroxyapatite chromatography step was omitted.

Low temperature experiments were performed using a dual-wave-length scanning spectrophotometer with low temperature facilities and photolysis arrangements as described (31–33). A typical experiment was performed as follows. Purified cytochrome bd in a buffer containing 100 $\text{mM}$ potassium phosphate, 0.5 $\text{mM}$ EDTA, 0.02% sodium $\text{D}$-dodecyl-$\text{D}$-maltoside, and 30% (v/v) ethylene glycol, pH 7.2, was reduced with a small amount of solid sodium dithionite for 15 min or by 10 $\text{mM}$ ascorbate/50 $\mu$M $N,N,N',N'$-tetramethyl-1,4-phenylenediamine for 25 min in a 2-mm path-length cuvette (total volume, 1 ml) and then bubbled with CO for 2 min at room temperature. The cuvette was transferred into a dark nitrogen atmosphere or an ethanol/dry-ice bath at $-78^\circ$C and frozen rapidly. After equilibration in the sample compartment of the spectrophotometer, a baseline was recorded and stored in the memory. The sample was subsequently photolysed for 3 min with the focused beam from a 150-watt tungsten lamp, and the difference absorption spectra (versus the stored baseline) were recorded. A reduced CO-unlabeled sample was prepared and frozen in a parallel manner to provide a baseline for the low temperature measurements of the CO-binding-induced absorption changes and compared with the photodissociation-induced spectral changes.

Laser flash-induced CO photodissociation/recombination time-resolved measurements at room temperature were done as described (34). Deconvolution of the kinetic curves of CO recombination with cytochrome bd at room temperature was done with the software package GIM (subroutine “discrete”) developed by A. L. Drachev in the A. N. Belozersky Institute of Physico-Chemical Biology (Moscow).

**RESULTS**

**Room Temperature Static Spectra**—Fig. 1 compares absorption changes induced by CO in the dithionite-reduced cytochrome bd from A. vinelandii (spectrum a) and E. coli (spectrum b) at room temperature. This side-by-side comparison of the two enzymes is important because some of the essential CO binding studies were performed with cytochrome bd purified from E. coli, whereas others were done with the A. vinelandii enzyme. Therefore, for generalization of the conclusions it is desirable to ensure that in the same study, the two enzymes show the same CO-induced spectral changes.

The two difference spectra are very similar and agree well with the published data (21, 22, 35). In the visible region, a typical red shift of the absorption band of ferrous heme d at $\sim 630$ $\text{nm}$ (presumably, the $Q_x$ transition) is observed, giving rise to a symmetric first derivative-shaped curve with a maximum at 632 $\text{nm}$ and a broad minimum centered around 432 $\text{nm}$ (decrease in free heme d) with an inflection point around 420 $\text{nm}$ between them. This major effect overlaps with a sharp first derivative-shaped feature with a maximum at 436 $\text{nm}$ and a minimum at 444 $\text{nm}$ dominated by perturbation of the ferrous heme $b_{595}$.$^{(25, 26)}$ As discussed in Ref. 22, room temperature absorption spectra provide no clear evidence for direct CO binding with heme $b_{595}$.$^{(25, 26)}$

**Low Temperature CO Photodissociation**—A difference absorption spectrum (CO-reduced minus reduced) recorded at $-100^\circ$C is shown in Fig. 2a. Changes in the 500–700 nm range are virtually identical to those at room temperature (Fig. 1a) and reflect mainly CO binding with heme d. In the Soret band, the changes recorded at low temperature are slightly different in line shape and showed some variability between the samples. Very similar results were obtained with the samples reduced by ascorbate + $N,N,N',N'$-tetramethyl-1,4-phenylenediamine instead of dithionite (data not shown).

**Photoinduced Absorption Changes**—Illumination of the frozen CO-ligated cytochrome bd with white light for 3 min brings about absorption changes indicative of a red shift of the Soret band (Fig. 2, spectrum b). The line shape (a maximum around 438 $\text{nm}$ and a minimum at about 420 $\text{nm}$ ) is in good agreement with earlier data (25, 26) and is typical of CO photodissociation from a high spin heme b (36). No photoinduced changes of heme d can be discerned in the far red region under these conditions (cf. also Refs. 24–26) because of geminate recombination of CO with the heme d, which is fast enough even at $-100^\circ$C (3). Much lower temperatures (4–5 K) are required to observe the photolysis and subsequent recombinant of CO with heme d (37). This property makes the low temperature photodissociation studies fairly selective for cytochrome bd, whereas at room temperature the changes from heme d in the Soret region overlap those of heme $b_{595}$.

At a 25-fold higher concentration of the enzyme, the matching absorption changes in the visible region can be resolved clearly (Fig. 3); the difference spectrum of the photoinduced changes shows peaks at 595 and 556 $\text{nm}$ and a trough at 571 $\text{nm}$, diagnostic of CO dissociation from a high spin rather than low spin heme b. Together, the data show that CO photodissociation from high spin heme $b_{595}$ is the sole effect revealed by static spectra in cytochrome bd at $-100^\circ$C.
Concentration was 20 mM. Conditions were as described in the Fig. 2 legend, but the cytochrome changes in the visible region of the absorption spectrum. Con

difference absorption spectrum (1 mM CO minus reduced); b, photodissociation spectrum (expanded 4-fold). The sample cell contained 0.8 μM cytochrome bd from A. vinelandii reduced with a few grains of solid dithionite. Other conditions were as described in the Fig. 1 legend for exception that 30% (v/v) ethylene glycol was used instead of 10% (v/v) glycerol.

The photoinduced CO photodissociation from heme bd as revealed by changes in the visible region of the absorption spectrum. Conditions were as described in the Fig. 2b legend, but the cytochrome bd concentration was 20 μM. The temperature was –100 °C.

Quantitation of the Absorption Changes—The photoinduced absorption changes at low temperatures cannot be quantitated directly with the use of known extinction coefficient values for the chromophores because of the well known effect of intensification of the absorption bands at cryogenic temperatures (typically ~10-fold) (38, 39). This phenomenon is mainly caused by an increase in the effective optical pathway because of the multiple reflection of light within the frozen sample, with a minor contribution from band narrowing. The extent of band intensification depends critically on the buffer/solvent system used, the exact procedure for freezing the sample, and other details that render comparison of the different series of experiment samples problematic (38, 39).

However, when the experiment is repeated under the same conditions, fairly reproducible results can be obtained. Therefore, to calibrate the absorption changes induced by photodissociation of CO at cryogenic temperatures, we compared their magnitude with that of the well resolved changes induced in the same samples by CO binding with heme d and recorded at the same low temperatures. In each experiment, a set of three low temperature spectra was recorded. First, a spectrum of a control sample reduced with dithionite was taken. Second, a spectrum of a similar sample that was treated with CO at room temperature in the dark before freezing was recorded (reduced + CO state). Third, after taking the low temperature spectrum of the reduced + CO state, the frozen sample was illuminated, and a spectrum of the photodissociated state was taken (reduced + COlight). Each of the low temperature spectra was recorded versus a frozen sample of buffer alone to compensate for light scattering. Subsequently, low temperature difference spectra were constructed for CO photodissociation at low temperature (reduced + COlight minus reduced + CO) compared with low temperature recordings of a sample prepared by CO binding at room temperature (reduced + CO minus reduced). In this way, the magnitude of the heme b595 response induced by low temperature photodissociation of CO was evaluated by comparison with the well separated absorption changes of heme d in the same frozen sample induced by CO binding.

Eight such experiments were performed, and the results are summarized in Table I. The normalized observed photoinduced absorption changes of heme b595 are almost two orders of magnitude smaller than a typical extinction coefficient of ~215 mM⁻¹ cm⁻¹ for a difference spectrum induced by CO binding with reduced high spin heme b proteins (36) and correspond to photodissociation of ~4.5% of heme b595 at ~70 °C and even less (~3%) at ~100 °C. The actual values may be slightly higher because of somewhat lower intensification of the Soret band changes relative to the α-range. In our samples, the a/γ intensification ratio was about 1.4. The corresponding values for the percentage of b595-CO photodissociation are given in Table I in brackets and give an upper limit for the effect (~4 and 6% of the heme at ~100 °C and ~70 °C, respectively).

CO Recombination—Independent evidence for CO binding with a small fraction of heme b595 in the same preparation of the enzyme is provided by the time-resolved studies of CO recombination with the reduced cytochrome bd after laser flash-induced photolysis of the ligand complex at room temperature (Fig. 4). The absorption changes induced by CO photodissociation were fully reversible in the dark, and their decay revealed several phases in general agreement with the data of Jüinemann et al. (40). The rapid phase is well separated kinetically and is characterized by τ of 18 ± 3 μs at ~1 mM CO (second order rate constant, k2 = 5.5 × 10⁸ M⁻¹ s⁻¹). A difference spectrum of this phase is shown in Fig. 4 by spectrum a, which is typical of CO binding with heme d (including a spectral perturbation of ferrous heme b595 induced by CO binding with heme d (23)). The slower part of recombination included 2–3 phases with τ values in the range of 0.2–2.3 ms, but the difference spectra of the phases were rather similar and indicated CO binding with a b-type heme in agreement with the findings in Ref. 40. Heterogeneity of the slow part of the response could reflect CO recombination with both b595 and low spin heme b558. In any case, the overall magnitude of the slow phase of absorption changes allows us to evaluate the upper limit of the CO-reactive fraction of heme b595.

An overall difference spectrum for the slow phases is given in Fig. 4 by spectrum b, which reveals a maximum near 440 nm and a minimum around 420 nm, similar to the photoinduced absorption changes at low temperatures. Its amplitude (Δε 420–440 = 4–6 mM⁻¹ cm⁻¹) allows an estimate of the upper limit for b595 reactivity toward CO and corresponds to 2–3% of this redox center. This value may be even less if part of the slow phase of CO recombination reflects binding of the ligand with the low spin heme b558 (40).

**DISCUSSION**

This work resolves the long standing controversy as to whether high spin heme b595 in cytochrome bd reacts with exogenous ligands like CO. Although the absorption changes
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Quantitation of the absorption changes induced by CO photodissociation from heme b595 at cryogenic temperatures

| Conditions | R-CO minus R ( binding of CO with heme d | R-CObd minus R-CO apparent ∆A (440–420) | ∆A (440–420)/ ∆A (640–622) | ∆A (440–420)/ ∆A (640–622) | Molar absorption | Fraction of b595 |
|------------|------------------------------------------|------------------------------------------|----------------------------|----------------------------|----------------|----------------|
| −100 °C    | 134 ± 7                                  | 61 ± 0.4                                 | 1.0                        | 1.0                        | 6.1 ± 0.4      | 2.8 ± 0.2      |
| −70 °C     | 188 ± 12                                 | 95 ± 2                                   | 1.0                        | 1.0                        | 9.5 ± 2        | 4.4 ± 1.0      |

a Assuming ∆A of 18 mm−1 cm−1 for CO binding with heme d at room temperature (21).

b Assuming ∆A of 215 mm−1 cm−1 for high spin b hemoprotein (36).

© Corrected for lower intensification in the Soret relative to the visible part of the spectrum.

induced by illumination of the CO complex of cytochrome bd at low temperatures pointed consistently to CO photodissociation from heme b595 (3, 24–26, 41), room temperature magnetic circular dichroism measurements allowing resolution of individual signals of ferrous heme b595 in the visible have shown that the heme does not respond significantly to either CO or NO (22). The present work shows that 1) there is CO binding with ferrous b595 as evidenced by both low temperature photodissociation of the bd-CO complex and time-resolved studies of CO recombination at room temperature, but 2) the binding involves only a small fraction of the heme, some 2–5%, that could well go unnoticed in magnetic circular dichroism studies.

Two alternative explanations for our finding can be considered. First, the results may provide support for the model of exogenous ligand binding with cytochrome bd suggested in Refs. 22 and 27. This model (A) implies that the two high spin hemes d and b595 are located very close to each other and form a dheme binuclear center with a capacity for only one molecule of exogenous ligand like CO. This molecule distributes between the two heme iron within the binuclear center in accordance with their intrinsic affinities for the ligand, heme d having a higher affinity (cf. Fig. 9 in Ref. 22). Within the framework of model A, our work indicates that the Kd of heme d for CO is about 20–30-fold lower that that of b595, so that about 95% of the ligand bound to cytochrome bd reposes on heme d (i.e. the ligand molecule trapped in the dheme binuclear center of the oxidase spends about 95% of time at heme d and ~5% at heme b595).

Model B proposes that the cytochrome bd population may be heterogeneous so that in ~95% of the enzyme the ligand can bind to heme d only, whereas in the remaining ~5% the ligand reacts with heme b595 (whether heme d in this 5% of the enzyme can also bind CO is difficult to deduce).

Model B may be more consistent with the recombination data that show heme b595-associated changes to be slower than those of heme d. Indeed, if there were free rapid (say, nanosecond) equilibration of CO between hemes d and b595 within the same heme-binding pocket, one might expect synchronous development of the absorption changes of the two hemes induced by CO recombination.

However, the above alternatives are not necessarily mutually exclusive and rather may pose a question as to what the time constant for equilibration between the states is.

if the characteristic time is short relative to an observation period in a particular type of experiment, model A applies. If the equilibration is slow, model A will transform to model B. The process may be slow if limited, for instance, by an exchange of the endogenous ligands in the coordination sphere of heme d (42). It must be emphasized that the above discussion refers to cytochromes bd from A. vinelandii and E. coli, showing much the same CO reactivity (e.g. Fig. 1). It is interesting that in cytochrome bd from Bacillus stearothermophilus, the major part of heme b595 binds CO at room temperature as can be inferred from the difference spectra in Fig. 1B of Ref. 43.

Acknowledgments — We thank R. Gennis for the strain of E. coli OG105/pTK1 and M. Johnson (Sheffield) for growing the A. vinelandii cells and excellent technical assistance. We also thank Dr. O. Gopta and A. Zsap for help in laser flash-photolysis experiments.

REFERENCES

1. Jünemann, S. (1997) Biochim. Biophys. Acta 1321, 107–127
2. Osborne, J. P., and Gennis, R. B. (1999) Biochim. Biophys. Acta 1410, 32–50
3. Poole, R. K. (1988) in Bacterial Energy Transduction (Anthony, C., ed.) pp. 231–291, Academic Press, London
4. Anraku, Y., and Gennis, R. B. (1987) Trends Biochem. Sci. 12, 262–266
5. Trumpower, B. L., and Gennis, R. B. (1994) Annu. Rev. Biochem. 63, 675–716
6. Kita, K., Konishi, K., and Anraku, Y. (1984) J. Biol. Chem. 259, 3375–3381
7. Miller, M. J., and Gennis, R. B. (1985) J. Biol. Chem. 260, 14003–14008
8. Roland, J. M., Gennis, R. B. (1984) Biochemistry 23, 445–453
9. Bertsova, Y. V., Bogachev, A. V., and Skulachev, V. P. (1979) FEBS Lett. 141, 369–372
10. Puustinen, A., Finel, M., Haltia, T., Gennis, R. B., and Wikström, M. (1991) J. Gen. Microbiol. 137, 3247–3255
11. Krasnoselskaya, I., Arutjunjan, A. M., Smirnova, I., Gennis, R., and Kostantinov, A. A. (1993) FEBS Lett. 327, 279–283
12. Dueweke, T. J., and Gennis, R. B. (1991) Biochemistry 30, 3936–3942
13. Green, G. N., Lawrence, R. M., and Gennis, R. B. (1986) Biochemistry 25, 2309–2314
14. Dueweke, T. J., and Gennis, R. B. (1991) Biochemistry 30, 3404–3406
15. Poole, R. K., and Williams, H. D. (1987) FEBS Lett. 217, 49–52
16. Hata-Tanaka, A., Matsuzara, K., Itoh, S., and Anraku, Y. (1987) Biochim. Biophys. Acta 893, 289–295
17. Kobayashi, K., Tagawa, S., and Mogi, T. (1999) Biochemistry 38, 5913–5917
18. Rothery, R. A., Houston, A. M., and Inglewed, W. J. (1987) J. Gen. Microbiol. 133, 3247–3255
19. Kushnirskaya, I., Arutjunjan, A. M., Smirnova, I., Gennis, R., and Kostantinov, A. A. (1993) FEBS Lett. 327, 279–283
20. Hill, J. J., Allen, J. O., and Gennis, R. B. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5863–5867
21. Tsukuba, M., Horii, H., Mogi, T., and Anraku, Y. (1995) J. Biol. Chem. 270, 20563–20569
22. Poole, R. K. (1994) Antonie Van Leeuwenhoek 65, 289–310

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21. Junemann, S., and Wrigglesworth, J. M. (1995) *J. Biol. Chem.* **270**, 16213–16220
22. Borisov, V., Arutyunyan, A. M., Osborne, J. P., Gennis, R. B., and Konstantinov, A. A. (1999) *Biochemistry* **38**, 740–750
23. Vos, M. V., Borisov, V. B., Liebl, U., Martin, J.-L., and Konstantinov, A. A. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 1554–1559
24. Poole, R. K., Scott, R. I., and Chance, B. (1981) *J. Gen. Microbiol.* **125**, 431–439
25. D'mello, R., Palmer, S., Hill, S., and Poole, R. K. (1994) *FEMS Microbiol. Lett.* **121**, 115–120
26. D'mello, R., Hill, S., and Poole, R. K. (1996) *Microbiology* **142**, 755–763
27. Borisov, V. B., Gennis, R. B., and Konstantinov, A. A. (1995) *Biochemistry* **60**, 231–239
28. Kelly, M. J. S., Poole, R. K., Yates, M. G., and Kennedy, C. (1990) *J. Bacteriol.* **172**, 6010–6019
29. Kaysser, T. M., Ghaim, J. B., Georgiou, C., and Gennis, R. B. (1995) *Biochemistry* **34**, 13491–13501
30. Miller, M. J., and Gennis, R. B. (1986) *Methods Enzymol.* **126**, 87–94
31. Poole, R. K., Salmon, I., and Chance, B. (1994) *Microbiology* **140**, 1027–1034
32. Chance, B., Graham, N., and Legallais, V. (1975) *Anal. Biochem.* **67**, 552–579
33. Kalnenieks, U., Galinina, N., Bringer-Meyer, S., and Poole, R. K. (1998) *FEMS Microbiol. Lett.* **168**, 91–97
34. Azarkina, N., Siletsky, S., Borisov, V., von Wachenfeldt, C., Hederstedt, L., and Konstantinov, A. A. (1999) *J. Biol. Chem.* **274**, 32810–32817
35. Lorence, R. M., Koland, J. G., and Gennis, R. B. (1986) *Biochemistry* **25**, 2314–2321
36. Wood, P. M. (1984) *Biochim. Biophys. Acta* **768**, 293–317
37. Poole, R. K., Sivaram, A., Salmon, I., and Chance, B. (1982) *FEBS Lett.* **141**, 237–241
38. Wilson, D. P. (1967) *Arch. Biochem. Biophys.* **121**, 757–768
39. Vincent, J.-C., Kumar, C., and Chance, B. (1982) *Anal. Biochem.* **126**, 86–93
40. Junemann, S., Rich, P. R., and Wrigglesworth, J. M. (1995) *Biochem. Soc. Trans.* **23**, 157
41. Poole, R. K., Salmon, I., and Chance, B. (1983) *J. Gen. Microbiol.* **129**, 1345–1355
42. Azarkina, N., Borisov, V., and Konstantinov, A. A. (1997) *FEBS Lett.* **416**, 171–174
43. Sakamoto, J., Koga, E., Mizuta, T., Sato, C., Noguchi, S., and Sone, N. (1999) *Biochim. Biophys. Acta* **1411**, 147–158
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\textit{J. Biol. Chem.} 2001, 276:22095-22099.
doi: 10.1074/jbc.M011542200 originally published online March 29, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M011542200

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