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Review

Where is 'outside' in cytochrome *c* oxidase and how and when do protons get there?

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Abstract

Cytochrome *c* oxidase moves both electrons and protons in its dual role as a terminal electron acceptor and a contributor to the proton motive force which drives the formation of ATP. Although the sequence of electron transfer events is well-defined, the correlated mechanism and routes by which protons are translocated across the membrane are not. A recent model [Michel, Proc. Natl. Acad. Sci. USA 95 (1998) 12819] offers a detailed molecular description of when and how protons are translocated through the protein to the outside, which contrasts with previous models in several respects. This article reviews the behavior of site-directed mutants of *Rhodobacter sphaeroides* cytochrome *c* oxidase in the context of these different models. Studies of the internally located lysine 362 on the K channel and aspartate 132 on the D channel, indicate that D132, but not K362, is connected to the exterior region. Analysis of the externally located arginine pair, 481 and 482, and the Mg/Mn ligands, histidine 411 and aspartate 412, which are part of the hydrogen-bonded network that includes the heme propionates, indicates that alterations in this region do not strongly compromise proton pumping, but do influence the pH dependence of overall activity and the control of activity by the pH gradient. The results are suggestive of a region of 'sequestered' protons: beyond a major energetic gate, but selectively responsive to the external environment.

Keywords: Cytochrome *c* oxidase; Magnesium; Proton; Arginine pair; Respiratory control

1. Introduction

In cytochrome *c* oxidase, four substrate protons ($4H_S^+$) are required for the reduction of O_2 to form $2H_2O$ at the active site. In addition, for every electron that is transferred from cytochrome *c* to cytochrome *c* oxidase, one proton is pumped through the protein giving four translocated protons ($4H_T^+$; $H_T^+/e^- = 1$) to add to the electrochemical gradient across the respiratory membrane for the synthesis of ATP.

Recent studies have attempted to determine how the oxygen chemistry is energetically coupled to the pumping of protons. Some insights have come from mutational studies [2–6] and the X-ray structures of cytochrome *c* oxidase [7–10] revealing at least two pathways that are common to bacterial and mammalian oxidases, designated as the D and K channels due to an aspartate (D132) and a lysine (K362) which are key residues in these proton channels. The aspartate (D132) appears to be connected via a hydrogen-bonded network involving H_2O and other residues, to a glutamate (E286) that is positioned between the two hemes (heme *a* and a_3). A recent model [1] suggests that protons supplied by the D

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channel are required during the reductive phase of the oxidase reaction to compensate for electron transfer from Cu_A at the external surface in subunit II, to heme *a* buried 19 Å into subunit I. Transfer of the proton from D132 to E286 and then to the vicinity of the heme a_3 ring D propionate is envisioned, en route to the heme *a* propionate or vicinity (site A) to stabilize the reduction of heme *a*. This involves a conformational event that repositions the glutamate. (There is some evidence for such a conformational change from IR and computer modelling studies [11–14].) Transfer back to the heme a_3 propionate (site B) would occur immediately to stabilize the first electron at Cu_B. Subsequently, a proton taken up through the K channel is proposed to allow heme a_3 reduction and O_2 binding, and to cause the repulsion of a proton from site B, resulting in a pumped proton. Further electron input is also neutralized by uptake of protons from the D pathway to sites A and B, which are ultimately pumped. The oxygen chemistry proceeds using protons from the D or K pathways, driving the release from A and B sites. This is a structurally explicit extension of the concept that neutralization of electron transfer to hemes in a region of low dielectric must be satisfied by protonation close to the heme [15] and that this uptake is part of the proton pump mechanism.

Here we consider whether our studies of various mutants in the D, K and possible exit channel in the region of the heme propionates are consistent with the Michel model or other mechanisms that have been proposed, such as the histidine shuttle [16] or the indirect coupling model [10].

Aside from the involvement of different proton paths, one major difference between current models is the extent to which the neutralization of electron input is invoked as contributing to the energy of proton pumping. Another area of controversy relates to the order of uptake of chemical versus pumped protons, and which channels are involved. The Michel model raises a further issue: where is 'outside', the physical location where protons have free access to the external solvent, in the oxidase structure?

Table 1
Comparison of activities of K362M and wild-type after reconstitution into lipids vesicles

Conditions	Steady-state O_2 consumption (e^-/s/aa_3)		Cyt. *c* oxidation (e^-/s/aa_3)	
	W.T.	K362M	W.T.	K362M
Coupled	96.0	0.17	26.4	0.0115
With valinomycin	100	0.18	132	0.0133
Uncoupled	1380	0.20	243	0.0243
RCR[a]	7.55	1.22	9.21	2.11

Measurements were made either in a Gilson oxygraph with 0.016 nmol W.T. and 1.6 nmol K362M COVs (steady-state) or in a stopped-flow Olis-rsm spectrophotometer (cyt. *c* oxidation as in Fig. 2).

[a]RCR = respiratory control ratio = uncoupled rate/coupled rate.

2. What is the role of the K channel and its key lysine residue?

Originally the K channel (Fig. 1) was designated as the substrate proton channel. It lies directly below the active site and the lysine, K362, and threonines above it, T359, T352, could connect via waters directly to a tyrosine, Y288, that is covalently bound to a histidine ligand of Cu_B [10,17]. This tyrosine is proposed to donate a substrate proton and an electron [18] in the formation of the chemical intermediate P (alias compound C). Mutation of K362 results in extremely low activity ($< 0.05\%$ of wild-type) and heme a_3 becomes very difficult to reduce, whereas heme *a* and CuB are readily reduced [6,19–22]. It has been postulated that the lysine could be merely required as a proton trap for neutralization of charge at heme a_3 [19]. However, since T359 and T352 mutants have similar properties to the lysine mutants [6] except with higher residual activity, this suggests that a conducting pathway is involved.

Our studies show that the residual activity of the K362M mutant increases when the membrane potential is dissipated, both in steady-state measurements of oxygen consumption (Table 1) and in pre-steady-state (8 turnovers) stopped-flow measurements of cytochrome *c* oxidation (Fig. 2, Table 1). This observation indicates that impaired proton uptake in the K pathway cannot be overcome by protons supplied from the exterior, as seen in the D132 mutants [4,23,24]. Thus the K pathway does not appear to lead to an externally accessible site, supporting its proposed role as a substrate proton channel.

Substantial increase in K362M activity is achieved

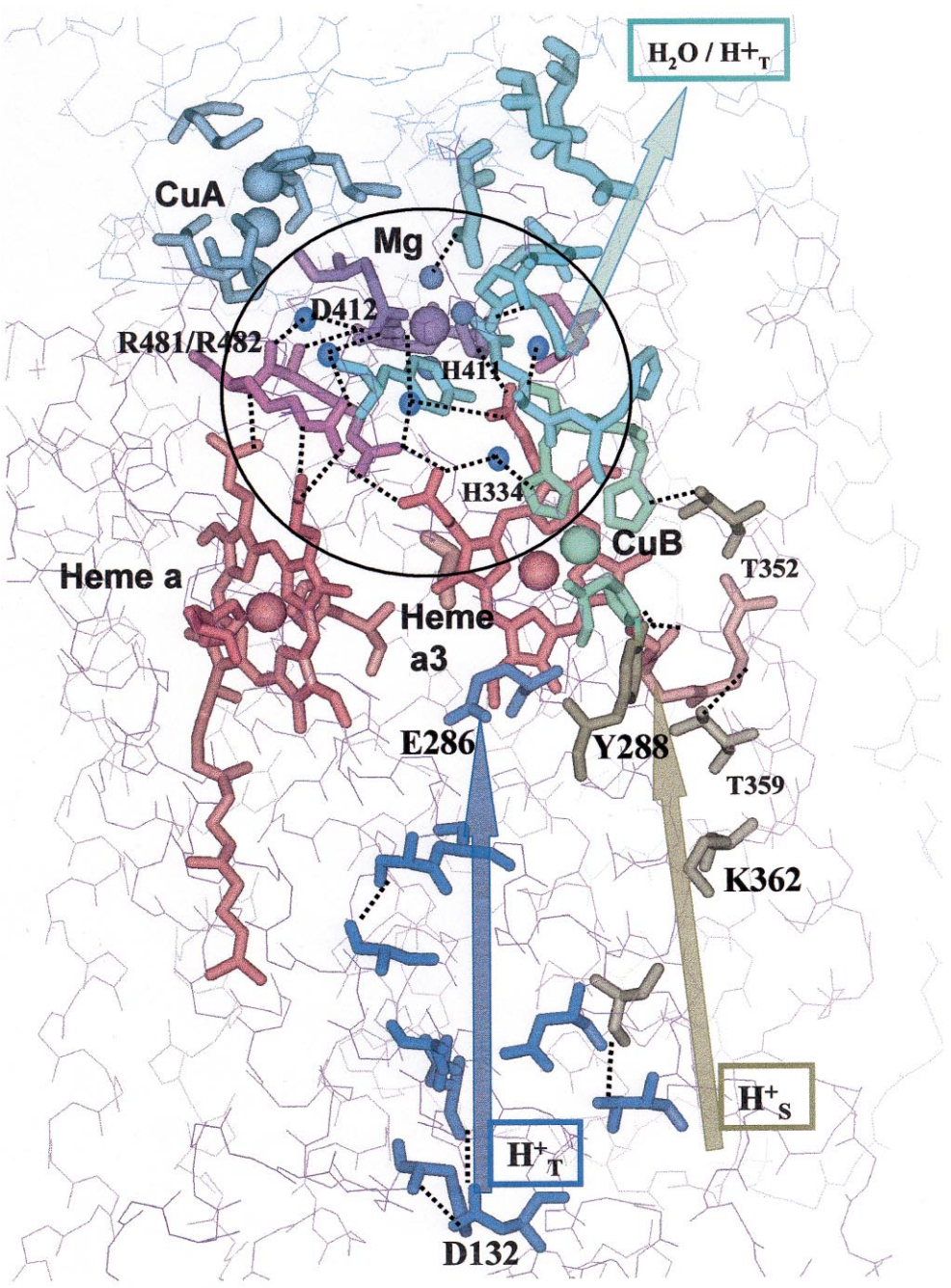
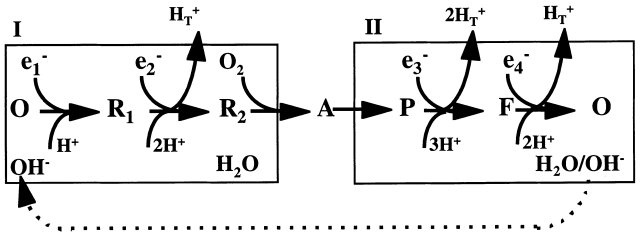


Fig. 1. Structure of the metal centers and key residues from the coordinates of the bovine heart cytochrome *c* oxidase [10] showing proposed proton pathways and the subunit I/II interface region above the hemes. Cu_A+ligands (blue); Cu_B+ligands (green); Mg+ligands (purple); hemes+Fe (red); K channel residues (olive); D channel residues (dark blue); H_2O (blue). Circle indicates hydrogen-bonded network surrounding Mg and involving the heme *a* and a_3 propionates with hydrogen bonds depicted as dotted lines.

by using peroxide as substrate (approx. 50-fold at 5 mM H_2O_2), although the K_m for peroxide is high (50 mM) [22]. This is true for purified and reconstituted enzyme, but no significant respiratory control is observed with peroxide (Mills, unpublished). The activity with peroxide has led to the conclusion that the K channel protons are only required in the initial reductive half of the reaction cycle ($O \rightarrow R \rightarrow P$) (Scheme 1) [20]. Evidence for the non-involvement of the K channel for the remaining substrate protons



Scheme 1. O = oxidized state; R_1, $R_2 = 1e^-$, $2e^-$ reduced; $A = O_2$ bound form; P = peroxy intermediate (at the level of $Fe^{4+} = O$); F = ferryl-oxo intermediate; H_T^+ = pumped proton. Proton uptake and release are shown as proposed in the Michel model [1].

($P \rightarrow F \rightarrow O$) also comes from flow-flash studies of the fully reduced enzyme [25] where single turnover events proceed normally, and from other studies using peroxide as a substrate [20,22]. However, in the flow-flash studies it is difficult to know how many protons are pre-loaded, and in all these studies, it cannot be determined whether the peroxide itself provides the protons necessary for the oxygen chemistry when the K channel is blocked [19,21]. Nevertheless, our results and others show that there is general agreement that the lysine is important for allowing reduction of heme a_3, which explains why blocking the K channel has a drastic effect on the ability of the enzyme to bind and utilize oxygen. Whether this is due to blockage of only one, or several, substrate protons is not clear.

3. What is the proton specificity of the D channel?

The D channel was originally designated as the proton pumping channel. An aspartate (D132) in the loop between helix II/III of subunit I (Fig. 1) is required for proton pumping [2,4,5,26]. The question remains whether protons from the D channel are also required as substrate protons in the second half of the reaction cycle ($P \rightarrow F \rightarrow O$) under normal conditions. There is evidence that this latter part of the reaction cycle (phase II) is responsible for all proton pumping [27,28]. However, this has been contested recently by Michel [1] who proposes that at least one proton is pumped during the initial (phase I) reduction. New evidence from Wikström and colleagues confirms an important role of the reductive phase in at least the release of pumped protons [29]. Results with the K362 mutants suggest that the D channel not only supplies all the pumped protons but also the last two substrate protons [25,30]. An alternative explanation is that only when the K channel is compromised are substrate protons supplied by the D channel [1], or by pre-loaded protons [19,21], or by H_2O_2 [21] (and see above). The evidence for either model is not yet compelling.

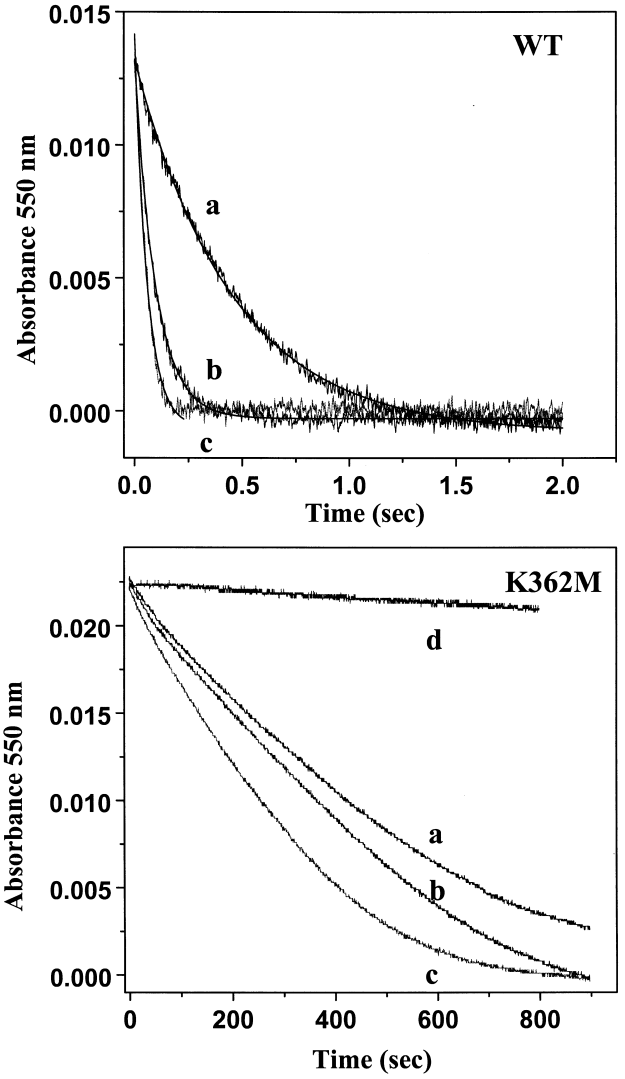


Fig. 2. Cytochrome *c* oxidation by K362M shows normal respiratory control. Measurements were made in an Olis-rsm stopped flow spectrophotometer. Wild-type cytochrome *c* oxidase (0.16 μM) vesicles (COVs) were mixed with 2 μM cyt c^{2+} with: (a) no ionophore (coupled), (b) +2 μM valinomycin, (c) as b +5 μM CCCP (uncoupled). K362M COVs (0.32 μM aa_3) with 3.3 μM cyt. c^{2+} as above and (d) in the absence of enzyme, i.e. a measurement of cyt. c^{2+} autooxidation.

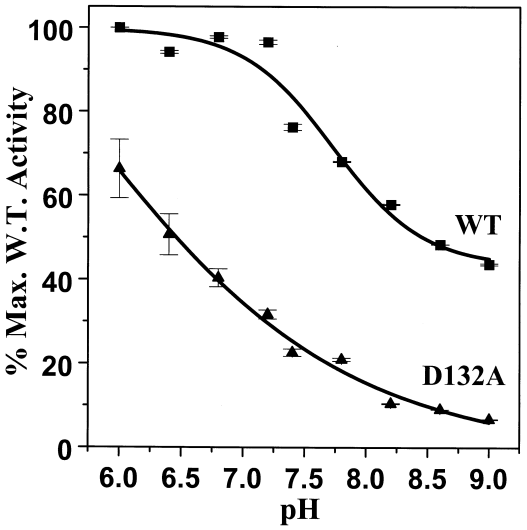


Fig. 3. D132A is rate-limited by the accessibility of protons from the exit path. Cytochrome *c* oxidation measurements were made as in Fig. 2 but with a rapid pH change in the stopped-flow by using different pH buffers with cyt. c^{2+}, mixed with the COVs (0.08 μM aa_3) in low buffer (50 μM HEPES-KOH, 45 mM KCl, 44 mM sucrose, 1 mM EDTA pH 7.4) but keeping the ionic strength constant using KCl.

When D132 is mutated to any non-carboxylate residue, the residual activity is only 5% of wild-type in the *R. sphaeroides* aa_3 oxidase. This is substantially higher than the activity of the K362 mutants, suggesting a less, rather than more, essential role, or that the mutation does not provide as complete a block of the pathway. The latter argument appears true in the case of the *Escherichia coli* bo_3 oxidase where nearly 45% activity is retained by the equivalent mutant, but still with no proton pumping. However, unlike the *E. coli* bo_3 mutants, or the K362 mutants, the residual activity of D132A/N is inhibited rather than stimulated by removal of a membrane potential. Considerable evidence supports the conclusion that this 'reverse respiratory control' is due to uptake of protons from the exterior side of the membrane driven by a negative-inside membrane potential [4,21,24,31].

Consistent with this conclusion is the finding that the activity of the D132A mutant can also be increased by increasing the proton concentration outside. This has been measured in a stopped-flow spectrophotometer using a pH jump with the reconstituted enzyme (Fig. 3). The normal pK_a seen for the wild-type (pH 7.8) is not evident in D132A: the rate increases exponentially with decreasing pH, showing that availability of protons from the outside is rate-limiting in this mutant. Presumably, reversal of proton uptake is not observed in the native enzyme because protons are much more rapidly supplied from the inside through the D channel. However, regulation of reversibility of the proton exit path could explain how 'intrinsic uncoupling' [32], or lowering of the normal proton to electron stoichiometry, might occur under physiological conditions [33].

Are D channel protons normally utilized as both substrate and pumped protons? If so, it would appear that both functions can be satisfied, in a kinetically limited way, by protons coming from the outside when the D channel is blocked. It is relatively easy to imagine the exit channel supplying sites A and B envisioned in the Michel model [1] but access to the active site chemistry is harder to visualize, unless E286 or histidine 334 can pick up protons in their 'out' positions. Even more difficult to conceive is how an indirect coupling model [10] would allow external protons to supply the oxygen chemistry. It is important to note that unlike the K channel mutants [6,20], the steady-state reduction of the hemes of the D132 mutants is not inhibited. D132 mutants also have normal internal electron transfer from heme *a* to heme a_3, or the reverse, when CO is flashed off the CO-inhibited mixed-valence enzyme in the absence of O_2 in single electron transfer experiments [25]. However, D channel protons are required for kinetically competent complete turnover, and at least for the neutralization of an electron movement from CuA to heme *a* in the fully reduced enzyme in flow-flash transient absorption spectroscopy measurements [34]. But where exactly the D path protons are delivered is not clear, nor whether that delivery route is different at different stages of the oxygen chemistry.

4. Are the propionates above the hemes involved in proton exit?

There is an extended hydrogen-bonded network above the hemes that involves the arginine pair (R482, R481) (Fig. 1) and water [8,9,13] and the non-redox active Mg/Mn site at the subunit I/II interface. Mutation of the Mg/Mn ligand H411 to Q

alters the structure of the metal site, and D412 to N or A causes loss of the metal [35]. When the Mg/Mn is lost, activity is reduced to 40% of wild-type and redox properties of the heme a_3 are altered. Kinetic analysis shows changes in the pH dependence of the overall activity of the D412A mutant along with a change in rate of reduction of heme a_3, suggesting an altered protonation event that is necessary for normal rates of heme a_3 reduction – perhaps secondary to a change in the reducibility of Cu_B. This is reminiscent of the K channel mutants where reduction of heme a_3 is also strongly inhibited, suggesting some relationship between these sites. Such a relationship would be consistent with the Michel model, where a proton coming from the D channel to site B (in the vicinity of the a_3 propionate D) facilitates Cu_B reduction, while a proton from the K channel is required to allow a_3 reduction.

Interestingly, some of the arginine (R481,482) mutants also lack a Mg/Mn metal site and have characteristics somewhat similar to the Mg/Mn ligand mutants (J. Qian, D.A. Mills, S. Ferguson-Miller, unpublished results). Although proton pumping is not abolished in any of these mutants, except where structure is extremely disturbed as in R482P, alterations of the arginines lower the H^+/e^- stoichiometry (J. Qian, D.A. Mills, S. Ferguson-Miller, unpublished results) [36]. It is difficult to determine which changes caused by these mutations may be more critical: (a) alteration of the pK_a of the propionates to which the arginines and the Mg/Mn ligands are normally hydrogen-bonded [36]; (b) destruction of the hydrogen-bonded network which may be important for a proton relay pathway that is part of the exit route; or (c) conformational changes due to the charge redistribution. In all cases, it is an interesting question whether protons reaching the region of the propionates are already 'out'. The Michel model implies that they are not [1], since further energy input from the oxygen chemistry is needed to displace them. The Wikström histidine shuttle model and a Glu-286 shuttle model discussed by Hofacker and Schulten [37] and Iwata et al. [7] imply that they are, since they are outside the gate provided by the movement of the shuttling residue in question.

The effects of mutants in the vicinity of these propionates can be interpreted to indicate that this region is indeed 'out'; that is, beyond an important gating mechanism, because their effects are not as dramatic as might be expected if the region were critical to the pumping mechanism. But all the mutants which have lost the Mg/Mn show an *increased* sensitivity to the pH gradient as illustrated in Fig. 4, where the rate of respiration is not strongly stimulated by valinomycin (loss of the electrical potential, $\Delta\Psi$) in D412A compared to the effect of the uncoupler CCCP (loss of ΔpH). Thus this region of the protein may be important in the mechanism of respiratory control.

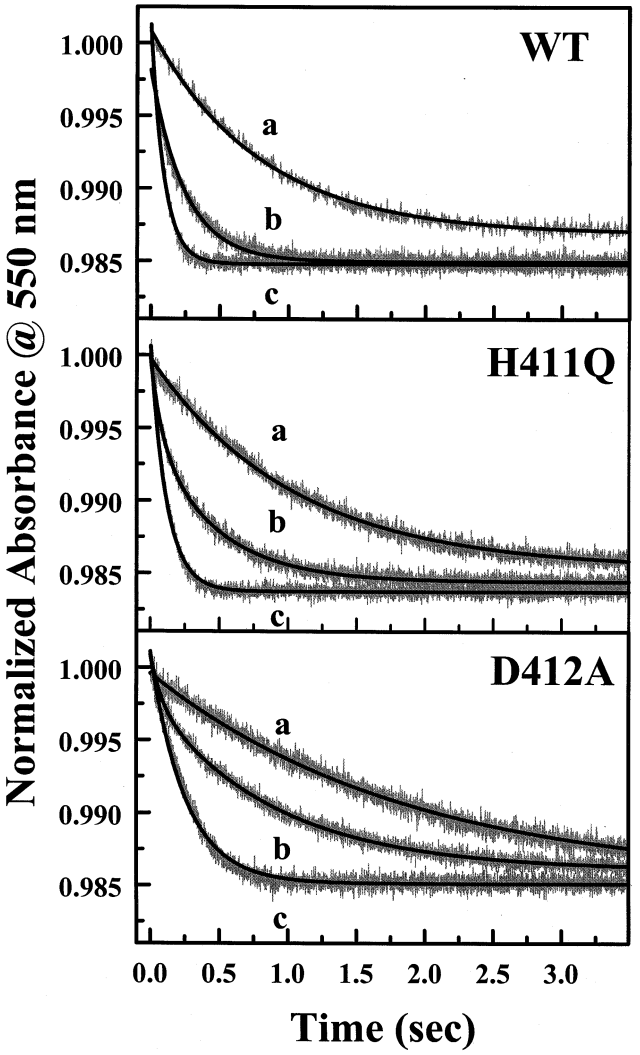


Fig. 4. The release of the membrane potential by valinomycin has a less stimulatory effect on D412A cytochrome c^{2+} oxidation compared to wild-type or H411Q. Reconstituted oxidase at 0.08 μM in asolectin vesicles (as in Fig. 2) with 2 μM cyt. c^{2+} with: (a) no ionophore (coupled), (b) +2 μM valinomycin, (c) as b +5 μM CCCP (uncoupled).

5. Conclusion

Both the D and K channels appear to have the required structure to form a hydrogen-bonded network for proton uptake from the inside of the membrane if predicted waters are included. In both channels there are polar residues (serines, threonines) and water-organizing residues (arginines). Bacteriorhodopsin has a similar hydrogen bonded network controlled by key residues (aspartate, glutamate, threonine and arginine) [38]. In cytochrome *c* oxidase, as in bacteriorhodopsin, the D channel appears to be supplied by a proton collecting antenna that is at the inner surface of the protein. However, in the *Rhodobacter sphaeroides* oxidase, D132 is essential to ensure a kinetically efficient supply of protons through the D pathway. In the absence of this carboxyl, proton reversal through an external path is revealed, demonstrating a connection between the D channel and the exit path. The conclusion can thus be drawn that protonation of a group (or groups) rapidly accessible from the D channel and slowly accessible from the exit channel is involved in proton pumping and permitting electron transfer to proceed.

The K channel is not connected to the exit path because the residual activity of lysine 362 mutants is not increased by the presence of a membrane potential. But the K channel protons are required for efficient reduction of heme a_3. For normal reduction of heme a_3 during turnover, protonation of a site(s) above the heme is also required. The results from mutants in this region (arginines and Mg/Mn ligands) support the idea that protonation of a propionate of heme a_3 could be one of the groups involved, the suggested site B in the Michel model. Since the propionate on ring D in question is hydrogen bonded through a water to His-334, a ligand of Cu_B, it is a reasonable candidate for a site whose protonation could influence reducibility of Cu_B and secondarily heme a_3. Alternatively, in the histidine shuttle mechanism, His-334 itself is a proton acceptor whose behavior could be influenced by modifications that result in loss of Mg.

The current model of Wikström and colleagues suggests that release of protons during the reductive phase of the oxidase reaction is not due to a reduction-driven pump [1], but to energy stored in the oxidative phase [29]. However, a mechanism of 'storing' the oxidative energy (conformational?) is yet to be described. (Proton pumping by conformational events induced by oxygen chemistry is also part of the indirect coupling model proposed by Yoshikawa and colleagues [10].) The storage of protons in a region where they are beyond an energetic gate, but still structurally sequestered almost seems to be required, raising the critical issue of where is 'out' and when do protons get there. Is the hydrogen bonded network which is anchored by Mg and involves the heme propionates 'in' or 'out'? Or is it the region where the protons can be temporarily sequestered? Some of the studies reviewed here would favor the idea that this region is beyond an important gate, since disruption of major elements do not drastically affect the pumping behavior. However, there is also evidence that loss of Mg significantly affects control of activity by the pH gradient ([35,39]; J. Qian, D.A. Mills, S. Ferguson-Miller, unpublished results). Further analysis of mutants in this region may clarify these issues and elucidate the mechanism of regulation of electron and proton transfer.

Acknowledgements

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References

[1] H. Michel, Proc. Natl. Acad. Sci. USA 95 (1998) 12819–12824.
[2] J.P. Hosler, S. Ferguson-Miller, M.W. Calhoun, J.W. Thomas, J. Hill, L. Lemieux, J. Ma, C. Georgiou, J. Fetter, J.P. Shapleigh, M.M.J. Tecklenburg, G.T. Babcock, R.B. Gennis, J. Bioenerg. Biomembr. 25 (1993) 121–136.
[3] J.W. Thomas, L.J. Lemieux, J.O. Alben, R.B. Gennis, Biochemistry 32 (1993) 11173–11180.
[4] J.R. Fetter, J. Qian, J. Shapleigh, J.W. Thomas, J.A. García-Horsman, E. Schmidt, J. Hosler, G.T. Babcock, R.B. Gennis, S. Ferguson-Miller, Proc. Natl. Acad. Sci. USA 92 (1995) 1604–1608.
[5] J.A. García-Horsman, A. Puustinen, R.B. Gennis, M. Wikström, Biochemistry 34 (1995) 4428–4433.
[6] J.P. Hosler, J.P. Shapleigh, D.M. Mitchell, Y. Kim, M. Pressler, C. Georgiou, G.T. Babcock, J.O. Alben, S. Ferguson-Miller, R.B. Gennis, Biochemistry 35 (1996) 10776–10783.

[7] S. Iwata, C. Ostermeier, B. Ludwig, H. Michel, Nature 376 (1995) 660–669.
[8] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, S. Yoshikawa, Science 272 (1996) 1136–1144.
[9] C. Ostermeier, A. Harrenga, U. Ermler, H. Michel, Proc. Natl. Acad. Sci. USA 94 (1997) 10547–10553.
[10] S. Yoshikawa, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, E. Yamashita, N. Inoue, M. Yao, M.J. Fei, C.P. Libeu, T. Mizushima, H. Yamaguchi, T. Tomizaki, T. Tsukihara, Science 280 (1998) 1723–1729.
[11] A. Puustinen, J.A. Bailey, R.B. Dyer, S.L. Mecklenburg, M. Wikström, W.H. Woodruff, Biochemistry 36 (1997) 13195–13200.
[12] P. Hellwig, J. Behr, C. Ostermeier, O.M. Richter, U. Pfitzner, A. Odenwald, B. Ludwig, H. Michel, W. Mantele, Biochemistry 37 (1998) 7390–7399.
[13] A. Kannt, C. Roy, D. Lancaster, H. Michel, Biophys. J. 74 (1998) 708–721.
[14] M. Lubben, A. Prutsch, B. Mamat, K. Gerwert, Biochemistry 38 (1999) 2048–2056.
[15] P. Rich, B. Meunier, R. Mitchell, R. Moody, Biochim. Biophys. Acta 1275 (1996) 91–95.
[16] M. Wikström, A. Bogachev, M. Finel, J.E. Morgan, A. Puustinen, M. Raitio, M.L. Verkhovskaya, M.I. Verkhovsky, Biochim. Biophys. Acta 1187 (1994) 106–111.
[17] G. Buse, T. Soulimane, M. Dewor, H.E. Meyer, M. Bluggel, Protein Sci. 8 (1999) 985–990.
[18] G.T. Babcock, M. Espe, C. Hoganson, N. Lydakis-Simantiris, J. McCracken, W. Shi, S. Styring, C. Tommos, K. Warncke, Acta Chem. Scand. 51 (1997) 533–540.
[19] S. Junemann, B. Meunier, R.B. Gennis, P.R. Rich, Biochemistry 36 (1997) 14456–14464.
[20] T.V. Vygodina, C. Pecoraro, D. Mitchell, R. Gennis, A.A. Konstantinov, Biochemistry 37 (1998) 3053–3061.
[21] D.A. Mills, S. Ferguson-Miller, Biochim. Biophys. Acta 1365 (1998) 46–52.
[22] D. Zaslavsky, R. Gennis, Biochemistry 37 (1998) 3062–3067.
[23] D.A. Mills, J. Qian, C. Hiser, J.A. Fee, S. Ferguson-Miller, Protein Sci. 7 (Suppl. 1) (1998) 137 486T.
[24] Y. Zhen, D. Mills, C.W. Hoganson, R.L. Lucas, W. Shi, G. Babcock, S. Ferguson-Miller, in: S. Papa, F. Guerrieri, J.M. Tager (Eds.), Frontiers of Cellular Bioenergetics: Molecular Biology, Biochemistry and Physiopathology, Plenum Press, New York, 1999, pp. 157–178.
[25] P. Adelroth, R.B. Gennis, P. Brzezinski, Biochemistry 37 (1998) 2470–2476.
[26] J.W. Thomas, A. Puustinen, J.O. Alben, R.B. Gennis, M. Wikström, Biochemistry 32 (1993) 10923–10928.
[27] M.L. Verkhovskaya, A. Garcia-Horsman, A. Puustinen, J.L. Rigaud, J.E. Morgan, M.I. Verkhovsky, M. Wikstrom, Proc. Natl. Acad. Sci. USA 94 (1997) 10128–10131.
[28] M. Wikström, Nature 266 (1977) 271–273.
[29] M.I. Verkhovsky, A. Jasaitis, M.L. Verkhovskaya, J.E. Morgan, M. Wikström, Nature 400 (1999) 480–484.
[30] A.A. Konstantinov, S. Siletsky, D. Mitchell, A. Kaulen, R. Gennis, Proc. Natl. Acad. Sci. USA 94 (1997) 9085–9090.
[31] J.R. Fetter, M. Sharpe, J. Qian, D. Mills, S. Ferguson-Miller, P. Nicholls, FEBS Lett. 393 (1996) 155–160.
[32] S. Ferguson-Miller, Biophys. J. 76 (1999) A1.
[33] S. Ferguson-Miller, G. Babcock, Chem. Rev. 96 (1996) 2889–2907.
[34] I.A. Smirnova, P. Adelroth, R.B. Gennis, P. Brzezinski, Biochemistry 38 (1999) 6826–6833.
[35] L. Florens, C. Hoganson, J. McCracken, J. Fetter, D.A. Mills, G.T. Babcock, S. Ferguson-Miller, in: G. Peschek (Ed.), The Phototropic Prokaryotes, Kluwer Academic, Plenum, New York, 1999, pp. 329–339.
[36] A. Puustinen, M. Wikstrom, Proc. Natl. Acad. Sci. USA 96 (1999) 35–37.
[37] I. Hofacker, K. Schulten, Proteins Struct. Funct. Genet. 30 (1998) 100–107.
[38] H. Luecke, H.-T. Richter, J.K. Lanyi, Science 280 (1998) 1934–1937.
[39] L. Florens, J. McCracken, S. Ferguson-Miller, Biophys. J. 76 (1999) A238.