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The Role of Electrostatic Interactions for Cytochrome *c* Oxidase Function

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In recent years, the enormous increase in high-resolution three-dimensional structures of proteins together with the development of powerful theoretical techniques have provided the basis for a more detailed examination of the role of electrostatics in determining the midpoint potentials of redox-active metal centers and in influencing the protonation behavior of titratable groups in proteins. Based on the coordinates of the *Paracoccus denitrificans* cytochrome *c* oxidase, we have determined the electrostatic potential in and around the protein, calculated the titration curves for all ionizable residues in the protein, and analyzed the response of the protein environment to redox changes at the metal centers. The results of this study provide insight into how charged groups can be stabilized within a low-dielectric environment and how the range of their electrostatic effects can be modulated by the protein. A cluster of 18 titratable groups around the heme a_3–Cu_B binuclear center, including a hydroxide ion bound to the copper, was identified that accounts for most of the proton uptake associated with redox changes at the binuclear site. Predicted changes in net protonation were in reasonable agreement with experimentally determined values. The relevance of these findings in the light of possible mechanisms of redox-coupled proton movement is discussed.

KEY WORDS: Protein electrostatics; Poisson–Boltzmann equation; proton transfer; cytochrome *c* oxidase; *Paracoccus denitrificans.*

INTRODUCTION

In most heme-copper containing terminal oxidases, electron transfer from either ubiquinol or cytochrome *c* to oxygen is coupled to (1) proton uptake from the mitochondrial matrix or the bacterial cytoplasm, the so-called *i*(inner)-side and (2) proton translocation from the *i*-side to the *o*(outer)-side, i.e., the periplasm or the intermembrane space (Wikström, 1977). The two proton transfer processes effectively create a proton and voltage gradient across the membrane that is employed by the F_0F_1-ATP synthase for the formation of ATP from ADP and inorganic phosphate.

The aa_3-type cytochrome *c* oxidase from *Paracoccus denitrificans*[2] consists of four subunits and four redox-active cofactors: a Cu_A center (not present in ubiquinol oxidases) bound to subunit II, a low-spin heme *a*, and a high-spin heme a_3 that, together with Cu_B, forms a binuclear center where oxygen reduction takes place. The crystal structure of the four-subunit enzyme was determined to 2.8 Å resolution (Iwata *et al.*, 1995, cf. Fig. 1) and recently refined to 2.7 Å using a new, improved crystal form containing only subunits I and II (Ostermeier *et al.*, 1997). The structures of the three mitochondrially encoded subunits of

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[2] Amino acid residues are numbered according to the *Paracoccus denitrificans* sequence. If not otherwise indicated, residues belong to subunit I, and residues of other subunits are referred to by Roman numerals between the amino acid type and the sequence number. Cu_A, copper A; Cu_B, copper B; Pra *a*/Prd*a*, ring A/D propionates of heme *a*; Pra a_3/Prd a_3, ring A/D propionates of heme a_3; FTIR, Fourier-transform infrared.

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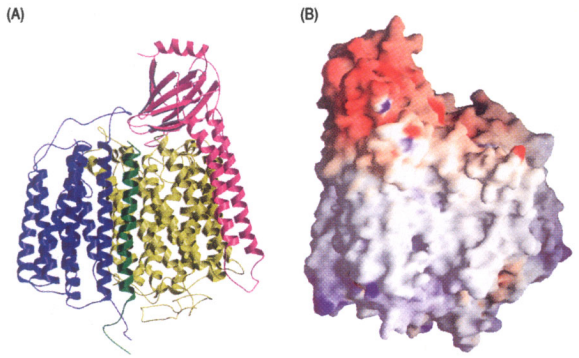

Fig. 1. (A) Ribbon representation of the four-subunit cytochrome *c* oxidase from *Paracoccus denitrificans* (Iwata *et al.*, 1995). Subunits I, II, III, and IV are shown in yellow, magenta, blue, and green, respectively. The figure was prepared with MolScript (Kraulis, 1991) and Raster3D (Bacon and Anderson, 1988; Merrit and Murphy, 1994). (B) Electrostatic potential derived from charged, surface-exposed residues of the enzyme. Note that (A) has been rotated by 180° to give (B) in order to allow a better view of the region of predominantly negative potential facing the periplasm (see text). The image was prepared with GRASP (Nicholls *et al.*, 1991).

the thirteen-subunit bovine-heart cytochrome *c* oxidase, the crystal structure of which was reported recently (Tsukihara *et al.*, 1996; see also Yoshikawa *et al.*, this volume.) were found to be remarkably similar to their bacterial homologues. In this minireview we will focus on the role of ionizable residues in the *Paracoccus denitrificans* protein, how they are distributed over the protein surface, how their charged states are stabilized by the protein environment, how they can influence the redox potentials of the metal centers, and how their pK_a's, in turn, are controlled by the redox state of the cofactors.

CHARGE-COMPENSATING INTERACTIONS

Essentially, there are three ways to stabilize a charge (Gunner *et al.*, 1997): through reorientation of surrounding dipoles, by pre-oriented dipoles in a rigid environment such as the protein interior, or through local neutralization by a counter charge. In a polar solvent, e.g., for surface-exposed charged groups, stabilization is achieved by rearrangement of solvent dipoles that effectively screen the charge and minimize its long-range effect. Within a protein, it can be stabilized by pre-oriented dipoles such as the protein backbone or polar side chains. However, in contrast to a polar solvent, such an environment of fixed dipoles is *polar* but not *polarizable,* meaning that the degree of stabilization of a charge depends upon its position relative to the dipoles and the stabilization/destabilization effect is the opposite for positive and negative charges. Two examples for how the dipolar environment can stabilize or destabilize a positive charge are given in Figs. 2A and 2B. The rigidity of the dipoles and the resulting loss of polarizability is reflected by a smaller dielectric constant ($\varepsilon = 4$) for the protein interior. Thus, buried charges screened by dipoles can have a considerable long-range effect as their potentials decreases with $1/\varepsilon r$ while the dipole potential falls of with $\mu \cos\theta/\varepsilon r^2$ (μ is the length of the dipole, θ the angle of the dipole axis relative to the "observer"). Additionally, the dipoles may be oriented to cancel out their respective effects or even to augment the monopole's potential.

Calculations predict that such long-range interactions with buried charges can have a significant effect on the midpoint potentials of redox centers (Gunner and Honig, 1991) or, vice versa, the pK of such groups may be determined by the charge on the redox center (Beroza *et al.*, 1995; Lancaster *et al.*, 1996; Kannt *et al.*, 1998). For example, the charge–charge interaction energy between Arg-54 (Fig. 2a) and heme *a* has been determined as 23.7 kJ/mol (Kannt *et al.*, 1998), a value that corresponds to an increase in redox potential of the heme by 245 mV or, the other way, a decrease in pK_a of the arginine by 4.1 ΔpK units through interaction with the oxidized heme.

The third possibility is stabilization by a counter charge of opposite sign. In contrast to dipolar stabilization, however, this will diminish the long-range effect as the charge pair effectively creates a dipole, the potential of which falls of with $\mu\cos\theta/\varepsilon r^2$.

DISTRIBUTION OF CHARGED RESIDUES OVER THE PROTEIN SURFACE

The electrostatic potential generated by charged residues on the surface of the *Paracoccus denitrificans* cytochrome *c* oxidase is depicted in Fig. 1b. While the overall charge on the cytoplasmic side of the enzyme, especially in subunits I and II, is rather positive, thus being in line with the "positive inside" rule postulated by von Heijne (1992) for integral membrane proteins, there is a large region of predominantly negative potential on the surface facing the periplasm. This region has been suggested as the binding region for cytochrome *c*,

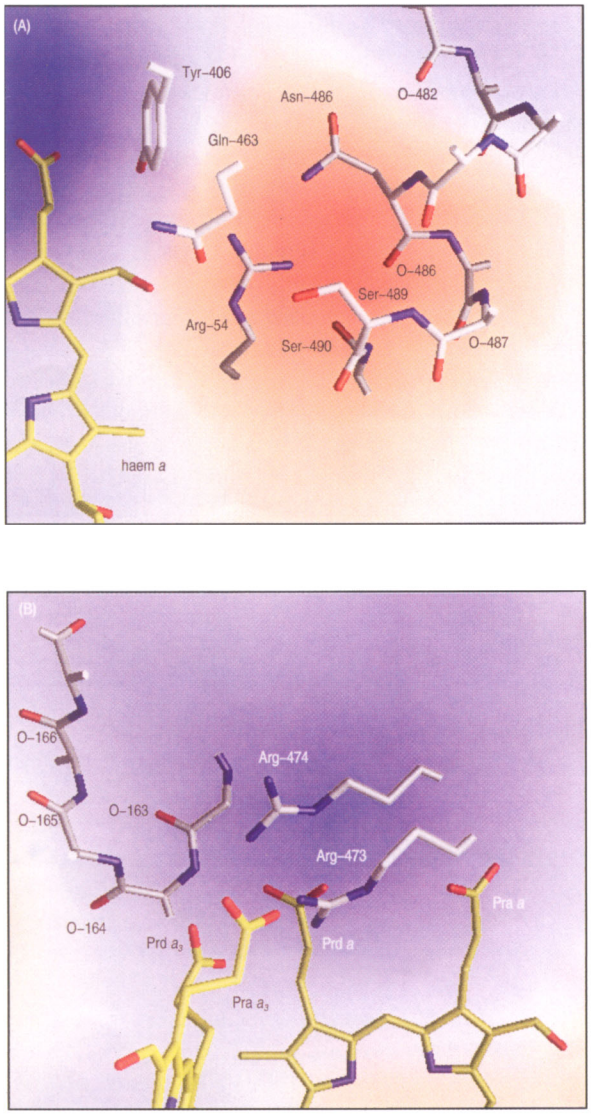

Fig. 2. Contribution of the polypeptide backbone and polar groups to the electrostatic potential. (A) Negative potential stabilizing the charge of Arg-54. Destabilization by the side chain of Asn-486 is overcompensated by favorable interactions with the formyl group of heme *a*, with the polar side chains of Gln-463, Ser-489, and Ser-490, and with the backbone carbonyl dipoles of residues 482, 483, 486, and 487 that are oriented with their carbonyl oxygen atoms pointing toward the arginine. (B) Positive potential destabilizing the charged state of Arg-474, mainly arising from the backbone carbonyl groups of residues 163–166 that are arranged with their carbonyl oxygen atoms pointing away from Arg-473. Note, however, that in this case the contribution of the polar groups will eventually be overcompensated by strong charge–charge interactions with the propionates of hemes *a* and a_3.

as supported by mutagenesis results (Witt *et al.*, 1995). However, whether there are one or two or more binding sites is still a matter of debate (Salamon and Tollin, 1996, and references therein). Considering the size of the negatively charged region, it is tempting to suggest that a rather unspecific, electrostatically favored "encounter complex" is formed first, which then rearranges to a more specific complex that is optimized for electron transfer. It should be noted, however, that complementary charged regions do not necessarily provide low-energy docking complexes as the complex formation involves the removal of water molecules from the surface of either reaction partner, and the associated desolvation energy can overcompensate the electrostatic contribution (Honig and Nicholls, 1995; Novotny and Sharp, 1992).

ELECTROSTATIC INTERACTIONS BETWEEN IONIZABLE RESIDUES WITHIN THE PROTEIN

The role of electrostatic interactions in *Paracoccus denitrificans* cytochrome *c* oxidase has been investigated through calculation of the electrostatic potential and interaction energies of potentially charged groups and analysis of the response of the protein environment to redox changes by using a continuum dielectric model and finite difference technique (Kannt *et al.*, 1998). The finite difference method (Bashford and Karplus, 1990; Yang *et al.*, 1993; Beroza *et al.*, 1995; Lancaster *et al.*, 1996; Gunner *et al.*, 1997) assigns a partial charge and a dielectric constant to each atom in the protein. The atoms, in turn, are then mapped onto a grid thereby assigning a charge and a dielectric constant to each point of the grid. Then the Poisson–Boltzmann equation is solved numerically (Gunner *et al.*, 1997).

The electrostatic free energy of a charged group i in the protein is the sum of three different energy terms (Gunner and Honig, 1992): the reaction field energy (or Born energy) $\Delta G_{r\times n}$ which is the result of the polarization of atoms and electrons in the media stabilizing a charge, the interaction energy ΔG_{pol} with permanent dipoles such as the protein backbone and polar side chains (see above), and the pairwise interaction energies with other ionizable residues j, $\Delta G_{crg}(i,j)$. The latter, in contrast to $\Delta G_{r\times n}$ and ΔG_{pol}, is dependent on pH and a function of the average protonation of all other ionizable groups j in the protein. Thus a Monte Carlo sampling method (Beroza *et al.*, 1995; Lancaster

et al., 1996) was applied to determine the average protonation of all titrating sites in the protein.

Groups of residues with strong pairwise electrostatic interactions and thus interdependent ionization states can be referred to as clusters (Lancaster *et al.,* 1996). Such a cluster has been identified in the *Paracoccus denitrificans* cytochrome *c* oxidase, consisting of altogether 18 titratable groups that were calculated to strongly electrostatically interact with each other and with the cofactors (Kannt *et al.,* 1998; depicted in Fig. 3). The term "strong" is used for interactions that correspond to a change in pK of at least 2 ΔpK units, i.e., with interaction energies larger than 2.74 kcal/mol. Tyrosine residues, with the exception of Tyr-280 (see below), were omitted from the cluster as they were calculated to be neutral over the whole pH range and in all redox states. Despite being buried within the transmembrane part of the protein (cf Fig. 3) and the corresponding large desolvation penalties, most groups of the cluster are fully charged at pH 7.0 in the fully oxidized state of the enzyme. Exceptions are the two strongly-coupled groups Asp-399 and Pra a_3 that were calculated to share a single proton, His-464, Lys-II-191, Lys-354, and Glu-278 (see also below). As suggested by Fann *et al.,* (1995), a hydroxide ion bound to Cu_B was included in the calculations and found to be fully charged in the oxidized enzyme. When this additional copper ligand was omitted, Tyr-280 was calculated to compensate for the loss of the negative charge by becoming partially deprotonated. This is particularly interesting in that the tyrosine has been suggested as the terminal residue of a proton transfer pathway to the binuclear site (Iwata *et al.,* 1995; see below).

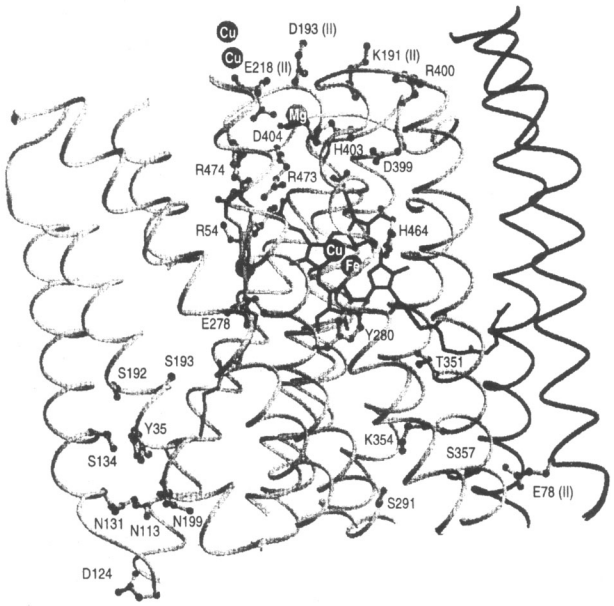

Fig. 3. The cluster of ionizable groups strongly interacting with each other and with the cofactors. Additionally, the apparently electrostatically isolated residues Asp-124 and Glu-II-78 and the residues providing the two putative proton-conducting pathways (Iwata *et al.,* 1995) have been included. See also Kannt *et al.* (1998).

The charge–charge interaction energy between hemes a and a_3 was determined as 2.5 ΔpK units or 145 mV. Thus, reduction of either heme will cause a decrease in midpoint potential and hence lower the affinity for electrons of the other heme (as proposed in the "neoclassical model" by Nicholls, 1974) by 145 mV, a value that is in reasonable agreement with the experimentally determined value of 124 mV (Wilson *et al.,* 1972; Wikström *et al.,* 1976).

REDOX-LINKED PROTONATION CHANGES

As described above, reduction of a cofactor affects the protonation equilibria of ionizable groups in the protein since the loss of a positive charge causes an increase in their pK_a's, i.e., it stabilizes the protonated states of basic residues and destabilizes the charged state of acidic residues. Electron input into the enzyme was calculated to be counterbalanced by an increase in net protonation of 0.7–1.1 H^+/electron at pH 7.0 (Kannt *et al.,* 1998), which is in good agreement with values reported for the mitochondrial enzyme by Oliveberg *et al.* (1991), Rich and co-workers (Mitchell *et al.,* 1992; Mitchell and Rich, 1994), and Capitanio *et al.* (1997). Two-electron reduction of the binuclear center was determined to be coupled to uptake of 1.8 H^+ while 0.8 H^+ was found to be taken up upon reduction of heme a (Kannt *et al.,* 1998).

An increase in net protonation by 1.0 H^+ was calculated to be associated with the formation of the one-electron reduced intermediate at pH 7.0. Apart from the hydroxide ion bound to Cu_B that becomes protonated to water, no other residues contribute significantly to the overall change in protonation. The protonation of the hydroxide ion could be of functional importance as it would significantly raise the redox potential of heme a_3 thereby speeding up the heme a–heme a_3 electron transfer. It was reported by Verkhovsky *et al.* (1995) that this electron transfer reaction is controlled by proton uptake, and a group close to

the binuclear site was proposed whose protonation might trigger electron transfer. The hydroxide ion appears to be a likely candidate. Additionally, its protonation would allow the newly-formed water molecule to dissociate from the binuclear center thereby making way for the oxygen to bind. It was also shown by Ädelroth *et al.* (1995, 1996) and Hallen *et al.* (1994) that reduction of heme a_3 is coupled to protonation of a group close to the binuclear site, and it was speculated that a hydroxide ligand could be the proton binding group.

Further reduction of the heme a_3–Cu_B center was calculated to be coupled to uptake of additional 0.8 H^+, 0.6 H^+ being associated with Lys-II-191 and 0.2 H^+ with Glu-II-78. As the former residue belongs to the cluster of strongly interacting residues, it should be stressed that, within such a cluster, the assignment of protonation changes to individual sites is problematic. Even subtle conformational changes within the cluster may cause a redistribution of protons in the cluster without, however, affecting its net protonation. Slightly differing sets of coordinates will yield different proton uptake profiles for residues within the cluster (for example, when using a slightly lower resolution set of coordinates, the coupled groups Asp-399/Pra a_3 rather than Lys-II-191 were determined to become protonated).

After binding of oxygen to the reduced binuclear site, electron equilibration within the heme a_3–Cu_B center leads to formation of the so-called peroxy species P. Subsequent transfer of a third electron to the binuclear center then produces compound P1 (Verkhovsky *et. al.*, 1994) or P_R (Morgan *et al.*, 1996). The latter reaction is thermodynamically irreversible and traps the oxygen in the binuclear center (Verkhovsky *et al.*, 1994, 1996). This third electron transfer to the binuclear site was calculated to be coupled to a net increase in protonation by 0.7 H^+ when compared to the two-electron reduced enzyme, i.e., the transfer of the electron from heme *a* (whose reduction was determined to be associated with uptake of 0.8 H^+) to the binuclear site was found not to be coupled to a significant change in net protonation (-0.1 H^+). Again, Lys-II-191 and Glu-II-78 were calculated to become more protonated. However, when the Cu_B ligand His-325 was modeled in an alternative conformation with the imidazole ring pointing away from the copper, it was determined to become protonated ($+0.9$ H^+) to its imidazolium form upon transfer of the third electron to the binuclear site and hence the major contributor to the increase in net protonation. Thus, the results of the calculations are in general agreement with the model proposed by Iwata *et al.*, (1995) where His-325 provides the "gate" that prevents the protons that have been taken up from being expelled back into the cytoplasm, and, at the same time, allows substrate protons to enter from the *i*-side. The model, a combination of the histidine cycle mechanism (Wikström *et al.*, 1994) and the electroneutrality principle (Rich, 1995), was proposed on the grounds of a lack of electron density for His-325 in the crystal structure of the oxidized, azide-treated enzyme (Iwata *et al.*, 1995), suggesting the possibility of multiple conformations for the side chain of this residue. In the calculations, however, His-325 was determined to become doubly protonated and hence stabilized in its nonbound conformation only upon transfer of the third electron to the binuclear site, thus differing from the model suggested by Iwata *et al.* where the histidine moves away from the copper already upon two-electron reduction of the enzyme, but agreeing with the original histidine cycle model (Wikström *et al.*, 1994).

Recent structural investigations showed that His-325 is a Cu_B ligand in both the azide-free oxidized (Ostermeier *et al.*, 1997) and the fully-reduced enzymes (Harrenga *et al.*, unpublished). The latter result is difficult to interpret within the framework of the mechanism proposed by Iwata *et al.* (1995).

PROTON TRANSFER PATHWAYS

Supported by site-directed mutagenesis results (Thomas *et al.*, 1993; Garcia-Horsman *et al.*, 1995), two putative proton pumping pathways were identified in the crystal structure of the *Paracoccus denitrificans* enzyme (Iwata *et al.*, 1995). The shorter one, referred to as the K-pathway, leads directly to the binuclear center and involves the conserved residues Ser-291, Lys-354 ("K"), Thr-351, and Tyr-280. Interestingly, the latter has been suggested to be covalently linked to one of the Cu_B histidine ligands (His-276 in *Paracoccus*) in the bovine-heart enzyme (Yoshikawa, personal communication) as well as in the bacterial homologue (Ostermeier *et al.*, 1997), and calculated to be strongly electrostatically coupled to the binuclear site, even to become protonated upon one-electron reduction of the binuclear center when no additional Cu_B ligand was present (Kannt *et al.*, 1998; see above). The K-channel, originally proposed as the pathway for the substrate protons, has recently been shown to be associated with proton uptake linked to reduction of

the binuclear center while having no obvious function in the part of the catalytic cycle where the oxygen chemistry takes place, i.e., the P→O transition (Konstantinov *et al.*, 1997; Brzezinski and Ädelroth, this issue).

The second, longer pathway termed D-pathway starts at the conserved Asp-124 ("D") and, via a number of polar residues (Iwata *et al.*, 1995; Fig. 3), leads to Glu-278, the acidic function of which has been shown to be crucial for proton pumping (Verkhovskaya *et al.*, 1997). From there, the pathway to the binuclear center is less clear but may involve the backbone carbonyl group of Pro-277. Alternatively, a conformational change could bring the glutamate side chain in contact with one of the heme a_3 propionates or, via a chain of water molecules, with one of the Cu_B histidine ligands (Riistama *et al.*, 1997), although it is difficult to imagine why then a heme a_3-bound oxygen species is not protonated directly.

Both Lys-354 and Glu-278 were calculated to be neutral at pH 7.0 for all redox states of the protein, mainly due to the high degree of desolvation and the resulting loss of reaction field energy that, especially in the case of Lys-354, is not compensated for by polar or charge–charge interactions (Kannt et al., 1998). This can be explained in different ways. First, a conformational change, as suggested for Glu-278 on the grounds of FTIR experiments (Puustinen et al., 1997), may be required to allow the residues to participate in redox-coupled proton transfer. However, in our calculations modeling of Glu-278 in an alternative conformation did not yield a different average protonation for the glutamate (Kannt, Lancaster, and Michel, unpublished). Furthermore, if the residues were part of a "proton wire," their protonation states might transiently change to stabilize a specific redox intermediate. However, this could be followed by rapid reprotonation through other residues, yielding the same average protonation in different equilibrium states. This view is supported by recent FTIR results indicating that Glu-278 is protonated in the reduced state of the enzyme (Puustinen *et al.*, 1997) and may also be so in the oxidized state (Lübben and Gerwert, 1996). Finally, in the calculations water-filled cavities were treated as high-dielectric continuum thus neglecting the charge-stabilizing effect of highly-directed hydrogen bonds to bound water molecules. Hence, the calculated protonation states for the two residues that both point into large, possibly water-filled cavities may be artifactual due to the limitations of the computer model.

Close to the cytoplasmic side of the membrane (see Fig. 3), two acidic residues that are electrostatically isolated from other charged groups have been identified (Kannt *et al.*, 1998): Asp-124 and Glu-II-78. Both residues are conserved in ubiquinol and cytochrome *c* oxidases. Their deprotonated states are stabilized through dipolar interactions with the backbone and conserved polar side chains in their vicinity. Such residues with relatively steep titration curves and apparent p*K*'s in the physiologically relevant regions may be of importance as transiently protonatable proton entry sites. And indeed, Asp-124, as the entry point of the D-channel, has been shown to be a key residue for proton pumping (Thomas *et al.*, 1993; Garcia-Horsman *et al.*, 1995; Brzezinski and Ädelroth, this issue). One could speculate that Glu-II-78 might function as a proton entry point for the K-channel. Proton transfer to Lys-354 could occur via Ser-357 (cf. Fig. 3) that is either conserved or an asparagine in other oxidases.

CONCLUSIONS

Theoretical analysis of electrostatic interactions is a valuable tool in studying the effect of buried charges on the midpoint potential of metal centers and the titration behavior of ionizable residues. Electrostatic calculations can help to identify residues or groups of residues involved in redox-coupled protonation reactions. In the *Paracoccus denitrificans* cytochrome *c* oxidase, a cluster of 18 strongly interacting residues was identified that account for most of the protonation changes linked to electron transfer. The overall stochiometry of the calculated protonation changes was found to be in good agreement with experimentally determined values. The results also indicate that apparently isolated sites, such as Asp-124 and Glu-II-78, may be crucial for mediating the effect of redox changes to proton uptake from the cytoplasm.

In the future it may be possible to overcome the current limitations of the computer model by including many different conformations of the protein, minimized for each redox state and at each pH (Alexov and Gunner, 1997). In addition, higher resolution structures will be required to improve the accuracy of such theoretical studies.

REFERENCES

Ädelroth, P., Brzezinski, P., and Malmström, B. G. (1995). *Biochemistry* **34**, 2844–2849.

Ädelroth, P., Sigurdson, H., Hallen, S., and Brzezinski, P. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 12292–12297.
Alexov, E. G., and Gunner, M. R. (1997). *Biophys. J.* **74**, 2075–2093.
Bacon, D. J., and Anderson, W. F. (1988). *J. Mol. Graphics* **6**, 219–220.
Bashford, D., and Karplus, M. (1990). *Biochemistry* **29**, 10219–10225.
Beroza, P., Fredkin, D. R., Okamura, M. Y., and Feher, G. (1995). *Biophys. J.* **68**, 2233–2250.
Capitanio, N., Vygodina, T. V., Capitanio, G., Konstantinov, A. A., Nicholls, P., and Papa, S. (1997). *Biochim. Biophys. Acta* **1318**, 255–265.
Fann, Y. C., Ahmed, I., Blackburn, N. J., Boswell, J. S., Verkhovskaya, M. L., Hoffman, B. M., and Wikström, M. (1995). *Biochemistry* **34**, 10245–10255.
Garcia-Horsman, J. A., Puustinen, A., Gennis, R. B., and Wikström, M. (1995). *Biochemistry* **34**, 4428–4433.
Gunner, M. R., and Honig, B. (1991). *Proc. Natl. Acad. Sci. USA* **69**, 9151–9155.
Gunner, M. R., and Honig, B. (1992). In *The Photosynthetic Bacterial Reaction Center*II (Breton, J., and Vermeglio, A., eds.), Plenum Press, New York.
Gunner, M. R., Alexov, E., Torres, E., and Lipovaca, S. (1997). *J. Biol. Inorg. Chem.* **2**, 126–134.
Hallen, S., Brzezinski, P., and Malmström, B. G. (1994). *Biochemistry* **33**, 1467–1472.
Honig, B., and Nicholls, A. (1995). *Science* **268**, 1144–1149.
Iwata, S., Ostermeier, C., Ludwig, B., and Michel, H. (1995). *Nature* **376**, 660–669.
Kannt, A., Lancaster, C. R. D., and Michel, H. (1998). *Biophys. J.* **74**, 708–721.
Konstantinov, A. A., Siletsky, S., Mitchell, D., Kaulen, A, and Gennis, R. B. (1997). *Proc. Natl. Acad. Sci. USA* **94**, 9085–9090.
Kraulis, P. J. (1991). *J. Appl. Crystallogr.* **24**, 946–950.
Lancaster, C. R. D., Michel, H., Honig, B, and Gunner, M. R. (1996). *Biophys. J.* **70**, 2469–2492.
Lübben, M.,and Gerwert, K. (1996).*FEBS Lett.* **397**, 303–307.
Merrit, E. A., and Murphy, M. E. P. (1994). *Acta Crystallogr.* **D50**, 869–873.
Mitchell, R., Mitchell, P., and Rich, P. R. (1992). *Biochim. Biophys. Acta* **1101**, 188–191.
Mitchell, R., and Rich, P. R. (1994). *Biochim. Biophys. Acta* **1186**, 19–26.
Morgan, J. E., Verkhovsky, M. I., and Wikström, M. (1996). *Biochemistry* **35**, 12235–12240.
Nicholls, A., Sharp, K. A., and Honig, B. (1991). *Proteins* **11**, 281–296.
Nicholls, P. (1974). In: *Dynamics of Energy-Transducing Membranes* (Ernster, L., *et al.* eds.), Elsevier, Amsterdam, pp. 39–50.
Novotny, J., and Sharp, K. A. (1992). *Prog. Biophys. Mol. Biol.* **58**, 203–209.
Ostermeier, C., Harrenga, A., Ermler, U., and Michel, H. (1997). *Proc. Natl. Acad. Sci. USA* **94**, 10547–10553.
Oliveberg, M., Hallen, S., and Nilsson, T. (1991). *Biochemistry* **30**, 436–440.
Puustinen, A., Bailey, J. A., Dyer, R. B., Mecklenburg, S. L., Wikström, M., and Woodruff, W. H. (1997). *Biochemistry* **36**, 13195–13200.
Rich, P. R. (1995). *Aust. J. Plant Physiol.* **22**, 479–486.
Riistama, S., Hummer, G., Puustinen, A., Dyer, R. B., Woodruff, W. H., and Wikström, M. (1997). *FEBS Lett.* **414**, 275–280.
Salamon, Z., Tollin, G. (1996). *Biophys. J.* **71**, 858–867.
Thomas, J. W., Puustinen, A., Alben, J. O., Gennis, R. B., and Wikström, M. (1993). *Biochemistry* **32**, 10923–10928.
Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R., and Yoshikawa, S. (1996). *Science* **272**, 1136–1144.
Verkhovskaya, M. L., Garcia-Horsman, A., Puustinen, A., Rigaud, J.-L., Morgan, J. E., Verkhovsky, M. I., and Wikström, M. (1997). *Proc. Natl. Acad. Sci USA*, **94**, 10128–10131.
Verkhovsky, M. I., Morgan, J. E., and Wikström, M. (1994). *Biochemistry* **33**, 3079–3086.
Verkhovsky, M. I., Morgan, J. E., and Wikström, M. (1995). *Biochemistry* **34**, 7483–7491.
Verkhovsky, M. I., Morgan, J. E., Puustinen, A., and Wikström, M. (1996). *Nature* **380**, 268–270.
von Heijne, G. (1992). *J. Mol. Biol.* **225**, 487–494.
Wikström, M. (1977). *Nature* **266**, 271–273.
Wikström, M., Harmon, H. J., Ingledew, W. J., and Chance, B. (1976). *FEBS Lett.* **65**, 259–277.
Wilson, D. F., Lindsay, J. G., and Brocklehurst, E. S. (1972). *Biochim. Biophys. Acta* **256**, 277–286.
Witt, H., Zickermann, V., and Ludwig, B. (1995). *Biochim. Biophys. Acta* **1230**, 74–76.
Yang, A.-S., Gunner, M. R., Sampogna, R., Sharp, K., and Honig, B. (1993). *Proteins* **15**, 252–265.