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Proton pumping mechanism and catalytic cycle of cytochrome *c* oxidase: Coulomb pump model with kinetic gating

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Abstract Using electrostatic calculations, we have examined the dependence of the protonation state of cytochrome *c* oxidase from bovine heart on its redox state. Based on these calculations, we propose a possible scheme of redox-linked proton pumping. The scheme involves His291 – one of the ligands of the Cu_B redox center – which plays the role of the proton loading site (PLS) of the pump. The mechanism of pumping is based on ET reaction between two hemes of the enzyme, which is coupled to a transfer of *two* protons. Upon ET, the first proton (fast reaction) is transferred to the PLS (His291), while subsequent transfer of the second "chemical" proton to the binuclear center (slow reaction) is accompanied by the ejection of the first (pumped) proton. Within the proposed model, we discuss the catalytic cycle of the enzyme.

Keywords: Cytochrome *c* oxidase; Proton pumping; Coupled electron–proton transfer; Catalytic cycle

1. Introduction

Cytochrome *c* oxidase (CcO) is a redox driven proton pump that utilizes free energy of oxygen reduction for creation of proton gradient across the mitochondrial membrane; subsequently, the proton gradient drives the synthesis of ATP [1–5]. Although the structure of CcO has been solved for several organisms, the molecular mechanism of its pumping remains unknown.

In our recent study [6], electrostatic calculations were employed to investigate the dependence of the protonation state of CcO from bovine heart [7] on its redox state. On the basis of electrostatic calculations, here a possible scheme of redox-linked proton pumping is proposed. The scheme involves His291 – one of the ligands of the Cu_B redox center – as the proton loading site (PLS) of the pump [6]. The pumping mechanism is based on ET reaction between two hemes of the enzyme coupled to a transfer of *two* protons. Upon ET, the first proton (fast reaction) is transferred to the PLS (His291), while subsequent transfer of the second "chemical" proton to the binuclear center (BNC) (slow reaction) is accompanied by the ejection of the first proton. An essential part of the pumping mechanism is kinetic gating [8].

2. The pumping mechanism

The proposed pumping mechanism is based on the following key observations made in previous studies (for structural features see Fig. 1):

1. There are two chains of water molecules connecting Glu242 to propionate D of heme a_3 ($PRDa_3$) and to the catalytic site of the enzyme (i.e., a hydroxide ion bound to either Fe or Cu metal centers in BNC) [9,10].
2. $PRDa_3$ has a strong salt bridge with a nearby Arg438. No proton transfer to $PRDa_3$ from Glu242 is possible, unless Arg438 is deprotonated [6].
3. There is a water molecule, Wa3, both predicted in [9] and visible in crystal structure [11,12], which is located between two propionates of heme a_3, and hydrogen bonded to δ1 nitrogen of His291 – the "top" ligand of Cu_B center. With this molecule there is proton connectivity between Glu242 and Nδ1 of His291 via the Glu242–$PRDa_3$–Arg438–Wa3–His291 chain.
4. According to electrostatic calculations [6], Nδ1 position of His291 is protonated only when both Fe and Cu_B centers are in formally reduced states (i.e., when the total charge on the metal ion plus the ligand is 2+ for Fe-a_3, and 1+ for Cu_B). When one of the metal centers of BNC is oxidized (or, equivalently, when one of the ligands in BNC accepts a proton), His291 becomes deprotonated.

The following mechanism of proton pumping is proposed (see Fig. 1).

During the cycle, the stable state of the catalytic center, before an additional electron is supplied to the system, is that in which one of the metal centers is formally oxidized, e.g., Fe^{3+}–H_2O, or Cu^{2+}–H_2O. This state is established in a previous step of the cycle, when a chemical proton is accepted by one of the hydroxy ligands of the BNC. In this state, His291 (i.e., Nδ1 position) is deprotonated and Glu242 is protonated.

(1) and (2) An electron is supplied to the system via cyt *c* and Cu_A, which is transferred to heme a and then to heme a_3 – Cu_B BNC. One of the metal ions is reduced and the overall charge of the BNC becomes one charge unit more negative. The driving force of electron transfer is about 20 meV [6].

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Abbreviations: CcO, cytochrome *c* oxidase; PLS, proton loading site; $PRAa_3$, $PRDa_3$, propionates of heme a_3, the numbering refers to bovine CcO

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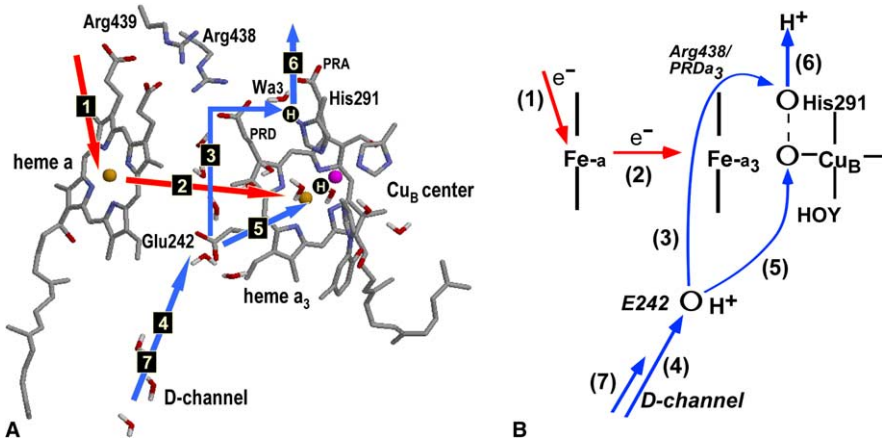

Fig. 1. (A) The key structural elements of the proposed pumping mechanism of CcO and the sequence of transitions during one pumping cycle. Two protonation sites, PLS and one in BNC, are shown as H-circles. PT and ET steps are shown by blue and red arrows, respectively. (B) The schematics of the model. The key assumption of the model is that the proton transfer rate upon ET between the hemes (step 2) is much higher along the pumping channel 3 than along the chemical channel 5 ($k_3 \gg k_5$), in other words step 3 occurs before step 5.

(3) In response to the increased negative charge of the BNC, the proton from Glu242 has now a driving force to move closer to the BNC. There are two pathways leading from Glu242 to two possible sites: one is leading to the BNC itself, the second is leading to His291 Nδ1 position – the PLS. The assumption (kinetic gating) is made that the rate of proton transfer to His291 is much higher than that of transfer to the BNC (the basis for this key assumption is discussed later in the paper). Therefore, the protonation of His291 occurs *before* that of the BNC. The fast proton transfer from Glu242 to His291 occurs by Grotthuss mechanism via Arg438 and PRDa$_3$, details of the transfer are discussed below. The driving force for this transfer is about 100 meV [6]. With this transition, His291 becomes protonated, as equilibrium electrostatic calculations predict for a redox state of the BNC in which both metal centers are now in formally reduced state.

(4) In this step Glu242 is reprotonated.

(5) Now the second chemical proton can be transferred to the BNC, using the second path connecting Glu242 and the catalytic center of CcO. The driving force for this transition is about 250 meV [6]. A significant driving force, despite the presence of the proton on a nearby His291, is due to the formation of water molecule in BNC – which is the main source of energy in the process. This transition occurs after the first proton has moved to His291 PLS. The formed state of BNC is such that one of the metal ions is formally oxidized (one positive charge now added). In this redox state, at equilibrium, His291 has to be deprotonated [6].

(6) The previously formed state has two additional protons present in two closely located sites: PLS (His291) and BNC, but only one additional electron residing on one of the metal ions of BNC. This state is metastable because of significant proton repulsion. The state is stabilized, therefore, by the expulsion of the proton from the His291 PLS site. The additional energy of stabilization gained is about 250 meV [6]. The expulsion of the proton from His291, from a state in which one of the metal centers is formally oxidized, is predicted by electrostatic calculations, which show that in this redox state, His291 has to be deprotonated.

(7) Glu242 is reprotonated again and one turnover of the cycle is complete. The formed state is stable until the next electron is passed through the system. This last step of reprotonation of Glu242 may be correlated with the expulsion from His291 and/or subsequent re-reduction of heme a in the next turnover in the cycle.

Each time a proton is pumped, in a single turnover of the pump, a stable state is formed until the next electron is injected into the system. Thus, for each electron passing through the chain, there is one pumped proton. For each oxygen molecule, four electrons are required to form two water molecules; therefore maximum four protons can be pumped.

2.1. Kinetic gating, absence of a mechanical gate

The above model is based on the energetics of the system described in [6], and a key kinetic assumption that upon the reduction of BNC, the first (pumping) proton is transferred to the PLS of the pump, and later the second, chemical proton, arrives to the BNC. The first proton transfer is fast, however it leads to a state (proton on PLS), which is not most favorable energetically [6]. The state that is energetically most favorable one (protonation of OH^- in the BNC) is achieved by a slow proton transfer via the chain of water molecules between Glu242 and BNC. Since PLS and BNC sites are closely located, due to electrostatic repulsion, the two protons cannot co-exist, and the first proton is expelled for the sake of achieving energetically more stable state. Since both chemical and pumping protons are derived from the same source – protonated Glu242, [1] and due to the special arrangement of the two channels leading to PLS and to BNC, the transfer of the second chemical proton blocks the return (back transfer) of the first proton to Glu242.

The difference in rates of proton transfer along the "fast" pumping channel leading from Glu242 to His291 and the "slow" chemical channel leading from Glu242 to BNC is based on the structural arrangements of the key groups. The protonated Arg438 is located in the immediate vicinity of His291,

[1] One chemical proton presumably comes via the K-channel Lys319, see [13,14].

so that when the proton is needed on His291, $Arg438H^+$ quickly donates the proton to this residue. This proton transfer is achieved via a water molecule Wa3. Immediately after that, the chain of water molecules connecting $PRDa_3$/Arg438 site with Glu242 provides a proton from Glu242 to reprotonate Arg438. The net result of these transitions is that a proton from Glu242 is quickly transferred to His291.

For the proper function of the pump (i.e., to pump protons in the right direction, against the membrane electrochemical gradient), another key requirement should be fulfilled, namely, that the protonation of the PLS occurs by a proton from the negative side of the membrane with low chemical potential, and not from the opposite side with high chemical potential. Otherwise, the protons would flow in the wrong direction. Closely related to this requirement is the one that is usually assumed in the form of a mechanical gate that would prevent the leak of protons through the pump between the pumping events.

The proposed model does not require a mechanical gate. It is based solely on the kinetic principles. The pump may work against the leaking, if the rate of pumping is higher than that of the leaking. A detailed study of passive proton leaking through the pumping chain His291–Arg438–$PRDa_3$–Glu242 (in the opposite direction to the pumping) is given in our upcoming paper [15], where it is shown that the leaking rate is indeed low, primarily due to protonation state of Arg438 and Glu242, and biased orientation of water chain between them.

The position of the proton on His291 is stable, in the sense that it cannot easily reach the catalytic site and short-circuit the process. This is achieved because of geometrical restrictions for water molecules in this region (examined in MD simulations, [9]) and the related lack of hydrogen bond connectivity, despite the short distance, between the histidine Nδ1 position and hydroxyl groups in the catalytic site. Besides, the negative charges on both nearby propionates create a local potential well that restricts the position of the proton to Nδ1 site. In the ideal model of the Coulomb pump, the PLS and the BNC should be completely isolated for proton transfer, and communicate only via Coulomb interaction at a distance.

3. The catalytic cycle of the enzyme

Fig. 2 shows the proposed catalytic cycle of the enzyme which is based on the described model.

The overall reaction in the enzyme is

$$O_2 + 4e^- + 8H^+_{(in)} \rightarrow 2H_2O + 4H^+_{(out)}$$

where (in) and (out) denote protons on inner and outer sides of the membrane. The overall scheme is close to those discussed earlier in the literature [2,16–19], and includes $R \rightarrow A \rightarrow P \rightarrow F \rightarrow O \rightarrow E \rightarrow R$ transitions. Below we only comment on the most significant differences, and characteristic features, which results from the assumptions of the proposed model.

3.1. $A \rightarrow P_m \rightarrow P_p$

In the A to P transition, if electron is available on heme a, the P_r state [20–22] is formed. If heme a is oxidized, the electron along with the proton is provided by adjacent Y244 [4,23], and P_m state is formed. The P_m state is characterized by $Cu_B(2+)$, tyrosyl radical and oxoferryl state of iron of heme a_3 [24], $Fe^{4+}{=}O^{2-}$. The protonation state of His291 in the P_m however is unknown, as is the nature of the ligand to $Cu_B(2+)$ ion (it can be OH^- or H_2O).

Here, the notation P_m (adopted in Fig. 2) is reserved for a state in which the protonation state of His291 is the same as in the R state, i.e., His291 is protonated, and the ligand to $Cu_B(2+)$ is OH^-. The Figure shows a hypothetical transition from such a P_m state in a situation when an electron is available on heme a. If electron transfer from heme a occurs before a chemical proton is delivered into BNC, the transient P_r state is formed. No pumping occurs at this step, because the addition of a negative charge to BNC does not change protonation state of (already protonated) His291. The formed state is unstable (for protonation) and will further evolve into F and later to F_p state, as shown in Fig. 2. Here, from R to F_p only one proton is pumped.

The P_m state, however, as we characterize it by a protonated His291 and OH^- ligand to $Cu_B(2+)$, is itself relatively unstable, according to our calculations [6]. Therefore, if the electron arrival from the heme a is delayed, a chemical proton will be transferred to the BNC first, which will cause the expulsion of the proton from His291, and formation of a stable state, which we call P_p, see Fig. 2. This state is characterized by $Cu_B(2+)$ with H_2O bound to it, and $Fe^{4+}{=}O^{2-}$. One metal center is formally reduced, another is formally oxidized, therefore His291 is deprotonated. The difference between P_m and P_p is in the protonation state of His291 and the nature of the ligand to Cu_B. If an electron now is injected into the system, the state F and then F_p is formed, as shown in Fig. 2. Here, from R_2 to F_p, two protons are pumped.

Here and below, we use X_p notation for stable, pumped states, see Fig. 2. Following the logic of the model, these states correspond to experimentally observed "X" states (e.g., "F" = F_p, "P_m" = P_p, etc.).

3.2. "$P \rightarrow F$" ($P_p \rightarrow F_p$)

The evolution of the P_m state depends on the sequence of electron and proton transfers to BNC in this state, as shown in Fig. 2. In both cases the F state is formed [25]. Similar to P_m state, there is an ambiguity in the protonation state of the ligand on $Cu_B(2+)$ and the His291 in the F state. We characterize the F state as shown in Fig. 2: by a protonated state of His291 and a hydroxide ion ligand on $Cu_B(2+)$. Both metal ions are formally reduced (Fe=O)(2+), (Cu–OH)(+1), and therefore His291 is protonated. This state is unstable. The subsequent transfer of a chemical proton results in the expulsion of the proton on His291 and the formation of a stable F_p state. Formally, the difference between F and F_p is in the position of one proton: in the F state the proton is on His291, and in the F_p state, the proton is on the hydroxide ion in the BNC (i.e., the ligand is H_2O instead of OH^-).

Upon the transition between two stable states, P_p to F_p, one electron is added to BNC and one proton is pumped. If one starts from P_r, again one proton is pumped in P_r to F_p transition.

The kinetics of the transition P_r to F was studied experimentally in great detail [16,26,27]. However, the protonation state of the ligands in the experimentally formed state is unknown. According to our model, the experimentally observed "F" state most likely corresponds to our stable state F_p.

As shown in Fig. 2, the first step in P_p to F_p transition is ET from heme a to $Y^{\cdot}$ forming the tyrosinate and, as in all other pumping steps (along the catalytic cycle), it is coupled to

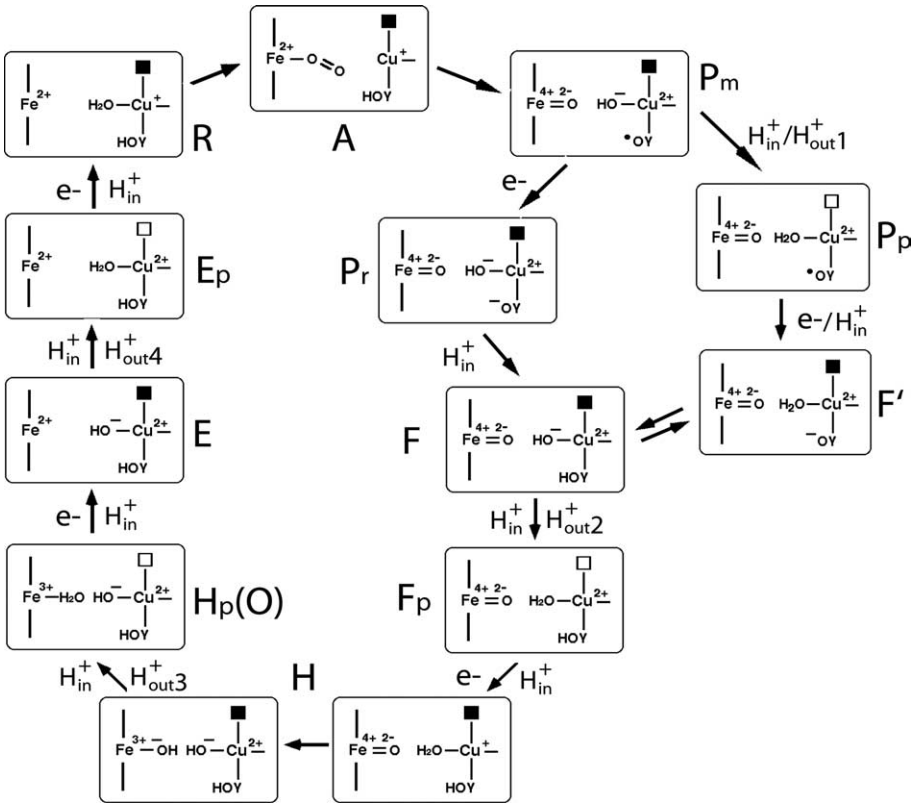

Fig. 2. Proposed catalytic cycle of cytochrome oxidase, based on the model shown in Fig. 1. The schematic drawing shows the redox states of heme a_3 and Cu_B centers together with the ligands; a square represents the His291 PLS, which can be protonated (filled square), or deprotonated (empty square). The two branches during the P_m to F transition are shown: left – the formation of the P_r state following the reduction of the P_m state, and right – alternative route where in the P_m state a proton transfer to hydroxyl group occurs *before* reduction of the tyrosyl radical. In the second branch, the formation of the P_r state is by-passed and two protons will be pumped during the A to F_p transition. Through the left branch only one proton would be pumped.

reprotonation of His291. The formed intermediate state, F′, has a proton on Nδ1 atom of His291, and a water molecule bound to Cu_B, which is also hydrogen-bonded to Y^-244. Due to the higher pK_a of tyrosine compared to water, a proton is shifted along the H-bond to tyrosinate forming the neutral YH. The unstable F-state is formed and the P_r state is by-passed. The proposed bypass of the P_r state is qualitatively similar to the one discussed in the literature by Michel [17,19].

3.3. "F → O" (F_p → H_p)

In the F_p state, heme a_3 is in oxoferryl state whereas the oxidized Cu_B^{2+} has an H_2O bound to it. The His291 site is empty. The electron transfer to BNC in this state is favorable and leads to generation of H-state. H denotes a state with a hydroxo-ligand on ferric Fe-a_3 [28]. Electron transfer is accompanied by reloading the His291 site with a proton. At this step internal proton and electron rearrangement in BNC can lead to formation of the hydroxyl groups on both metals.

The next step in the reaction is transfer of a chemical proton via the D-channel and protonation of OH^- bound to Fe-a_3. It results in the pumping of the third proton and generation of a stable O (in our notation H_p) state [25,29]. One proton is pumped in the F_p to H_p transition.

3.4. "O → R" (H_p → E_p → R)

The H_p (O) → E transition is shown in Fig. 2. It involves heme a to a_3 ET and a coupled PT to reprotonate His291. In E state both metal ions are formally reduced and therefore His291 is protonated. The formed state E is unstable and will subsequently lead to E_p state with one proton pumped. In this step, the chemical proton that converts OH^- group to water is thought to be delivered via the K-channel [30–32].

The last electron transfer completes the catalytic cycle and leads to regeneration of the R state.

The developed scheme of the catalytic cycle, which follows from the pumping model described in the previous sub-section, has a symmetric form shown in Fig. 3. For each of the intermediate states discussed in the literature, and observed experimentally, we distinguish two sub-states: one unstable, or metastable, such as P_m, F, H, E and their corresponding stable counterparts: P_p, F_p, H_p (O), and E_p. The p-states are formed after the pumping event, in which one chemical proton is added to BNC and one proton is expelled from PLS (His291). The difference between the pumped p-state and its meta-stable counterpart is that in the pumped stable states, His291 is deprotonated, and in the BNC it has one more proton. Thus, formally these states differ in the position of one proton. In our scheme a total of four protons are pumped, three of them in the oxidative ($P_m \rightarrow P_p$, $F \rightarrow F_p$, $H \rightarrow H_p$) and one in the reductive half (O → E, or $H_p \rightarrow E_p$ in our new notation) of the cycle.

Many features of the described model are supported by recent experiments. In [33], $1e^-/1H^+$ pumping stoichiometry is observed, as predicted by our model. When a fully reduced enzyme is mixed with oxygen, according to our model two protons are pumped, and that is what is observed in [34]. In several recent experimental studies, e.g. [33], upon injection of

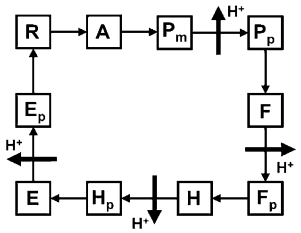

Fig. 3. A simplified schematic representation of the catalytic cycle of cytochrome oxidase.

an electron three potentiometric phases are observed, one electronic and two protonic phases, with an approximate ratio of 1:1:5. This is exactly what our model predicts.

Some other features, however, disagree with current interpretation of experimental data. Thus, in [35] and [33], it is proposed that one proton is pumped during "$E \rightarrow R$" transition (our $E_p \rightarrow R$), however, our model suggests instead that one proton is pumped during $R \rightarrow P_p$ state, see Figs. 2 and 3, and there is no pumping $E_p \rightarrow R$. Also, in [33] it is suggested that during the catalytic cycle special high-energy intermediates, O_H and E_H, are formed. Our model does not require any special high-energy intermediates, see Fig. 2, but suggests that if one starts from fully oxidized enzyme prepared in anaerobic conditions (no OH^- ligands are present in BNC) no pumping would be observed during reductive transitions $O \rightarrow E \rightarrow R$. The identity of the experimentally observed intermediate states is not known exactly, in particular the protonation of these states is unclear. Thus, it is difficult to be certain about the correspondence between our stable X_p states and experimentally observed states, and more work is needed in this regard.

The described model contains many of the elements that have been presented in previous models. For example, electroneutrality principle, which is at work in our model, was suggested earlier by Rich et al. [36]; the suggestion of a rapid proton delivery to form a metastable state is similar to Michel's model [19], and the protonation of His291 is also part of the various histidine cycle models [16], previously proposed by Wikstrom albeit in a very different context. The described combination of these elements, however, and the support of the electrostatic calculations are certainly new and represent a new self-consistent model worthwhile of experimental verification.

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References

[1] Wikström, M. (1998) Curr. Opin. Struct. Biol. 8, 480–488.
[2] Gennis, R.B. (1998) Proc. Natl. Acad. Sci. USA 95, 12747–12749.
[3] Michel, H., Behr, J., Harrenga, A. and Kannt, A. (1998) Annu. Rev. Biopys. Biomol. Struct. 27, 329–356.
[4] Babcock, G.T. and Wikström, M. (1992) Nature 356, 301–309.
[5] Ferguson-Miller, S. and Babcock, G.T. (1996) Chem. Rev. 7, 2889–2907.
[6] Popovic, D.M. and Stuchebrukhov, A.A. (2004) J. Am. Chem. Soc. 126, 1858–1871.
[7] Yoshikawa, S., Shinwaza-Itoh, K., Nakashima, R., Yaono, R., Yamashita, E., Inoue, N., Yao, M., Fei, M.J., Libeu, C.P., Mizushima, T., Yamaguchi, H., Tomizaki, T. and Tsukihara, T. (1998) Science 280, 1723–1729.
[8] Stuchebrukhov, A.A. (2003) J. Theor. Comp. Chem 2, 91–118.
[9] Zheng, X., Medvedev, D.M., Swanson, J. and Stuchebrukhov, A.A. (2003) Biochim. Biophys. Acta 1557, 99–107.
[10] Wikström, M., Verkhovsky, M.I. and Hummer, G. (2003) Biochim. Biophys. Acta 1604, 61–65.
[11] Ostermeier, C., Harrenga, A., Ermler, U. and Michel, H. (1997) Proc. Natl. Acad. Sci. USA 94, 10547–10553.
[12] Svensson-Ek, M., Abramson, J., Larsson, G., Tornroth, S., Brzezinski, P. and Iwata, S. (2002) J. Mol. Biol. 321, 329–335.
[13] Karpefors, M., Ädelroth, P., Aagaard, A., Sigurdson, H., Svensson-Ek, M. and Brzezinski, P. (1998) Biochim. Biophys. Acta 1365, 159–169.
[14] Ruitenberg, M., Kannt, A., Bamberg, E., Fendler, K. and Michel, H. (2002) Nature 417, 99–102.
[15] Popovic, D.M. and Stuchebrukhov, A.A. (2004) J. Am. Chem. Soc. (to be published).
[16] Wikström, M. (2000) Biochim. Biophys. Acta 1458, 188–198.
[17] Michel, H. (1998) Proc. Natl. Acad. Sci. USA 95, 12819–12824.
[18] Wikström, M., Morgan, J.E. and Verkhovsky, M.I. (1998) J. Bioenerg. Biomembr. 30, 139–145.
[19] Michel, H. (1999) Biochemistry 38, 15129–15140.
[20] Han, S., Ching, Y.C. and Rousseau, D.L. (1990) Proc. Natl. Acad. Sci. USA 87, 8408–8412.
[21] Verkhovsky, M.I., Morgan, J.E. and Wikstrom, M. (1994) Biochemistry 33, 3079–3086.
[22] Morgan, J.E., Verkhovsky, M.I. and Wikström, M. (1996) Biochemistry 35, 12235–12240.
[23] Proshlyakov, D.A., Pressler, M.A., DeMaso, C., Leykam, J.F., DeWitt, D.L. and Babcock, G.T. (2000) Science 290, 1588–1591.
[24] Fabian, M., Wong, W.W., Gennis, R.B. and Palmer, G. (1999) Proc. Natl. Acad. Sci. USA 96, 13114–13117.
[25] Adelroth, P., Karpefors, M., Gilderson, G., Tomson, F.L., Gennis, R.B. and Brzezinski, P. (2000) Biochim. Biophys. Acta 1459, 533–539.
[26] Jasaitis, A., Verkhovsky, M.I., Morgan, J.E., Verkhovskaya, M.L. and Wikström, M. (1999) Biochemistry 38, 2697–2706.
[27] Wikström, M. (2000) Biochemistry 39, 3515–3519.
[28] Rousseau, D.L. (1999) Nature 400, 412–413.
[29] Karpefors, M., Ädelroth, P., Aagaard, A., Smirnova, I.A. and Brzezinski, P. (1999) Isr. J. Chem. 39, 427–437.
[30] Konstantinov, A.A., Siletsky, S., Mitchell, D., Kaulen, A. and Gennis, R.B. (1997) Proc. Natl. Acad. Sci. USA 94, 9085–9090.
[31] Brzezinski, P. and Ädelroth, P. (1998) J. Bioenerg. Biomembr. 30, 99–107.
[32] Wikström, M., Jasaitis, A., Backgren, C., Puustinen, A. and Verkhovsky, M.I. (2000) Biochim. Biophys. Acta 1459, 514–520.
[33] Bloch, D., Belevich, I., Jasaitis, A., Ribacka, C., Puustinen, A., Verkhovsky, M.I. and Wikström, M. (2004) Proc. Natl. Acad. Sci. USA 101, 529–533.
[34] Verkhovsky, M.I., Jasaitis, A., Verkhovskaya, M.L., Morgan, J.E. and Wikström, M. (1999) Nature 400, 480–483.
[35] Ruitenberg, M., Kannt, A., Bamberg, E., Fendler, K. and Michel, H. (2002) Nature 417, 99–102.
[36] Rich, P.R., West, I.C. and Mitchell, P. (1988) FEBS Lett. 233, 25–30.