COMMENTS

Comment on "Acidity of a Cu-bound Histidine in the Binuclear Center of Cytochrome *c* Oxidase"[1]

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Cytochrome *c* oxidase (*CcO*) reduces oxygen in aerobic cells to water and utilizes the free energy of the redox reaction to pump protons across the membrane. In the process, the proton gradient is created which later drives the synthesis of ATP.

In our recent work,[2,3] we have proposed a model of proton pumping by *CcO*, in which one of the histidine ligands to the enzyme Cu_B redox center plays the role of the pump element. The proposal is based on the calculation of the protonation state of the enzyme for different oxidation states of its four redox centers: Cu_A, heme *a*, heme a_3, and Cu_B. We started from semiempirical continuous electrostatic calculations of pK_a values,[2] by a method originally developed by Bashford and co-workers and recently extended calculations to a combination of DFT treatment of a selected QM part of the enzyme with electrostatic Poisson−Boltzmann treatment of the surrounding.[4,5] The key finding is the dependence of the protonation state of the His291 ligand to Cu_B on the redox state of the binuclear center (BNC) of the enzyme: His291 appears to be deprotonated (at pH 7) for partially oxidized states OO, OR, or RO of the binuclear center Fe-a_3/Cu_B and protonated for RR, a fully reduced state of BNC. (Here, O and R refer to a total formal charge of the metal ion and its ligand, such that Cu(II)OH^-, Cu(I)H_2O, Fe(IV)=O^{2-}, and Fe(III)OH^- are all R states, whereas Cu(II)H_2O and Fe(III)H_2O are O states). On the basis of this finding, a model has been proposed in which an incoming chemical proton that converts an OH^- ligand to water in BNC and changes its state from R to O expels a proton from His291, a crucial step in the proton pumping. A detailed catalytic cycle has been developed,[3] which is supported by experimental data on the number of pumped protons during the cycle; the analysis of the kinetics of the membrane potential generated by the enzyme in five different experiments also supports the model.[6] Yet, to prove or disprove the model, a direct experiment that would measure the protonation state of His291 is needed.

The pK_a calculation is a difficult subject. Results can be different depending on how the calculations are done, and our original paper, ref 2, had obvious weaknesses—for example, we had to guess the pK_a of the model Cu_B compound, the reliability of the charges we used were difficult to evaluate rigorously. Our more recent work has attempted to improve the calculations, see, for example, refs 4 and 5. While the ultimate proof or disproof of our proposal can only be obtained in experiment, any additional theoretical work on the subject is of significant interest.

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In a recent publication,[1] Pomes and co-workers reported calculations which are similar to ours, but their conclusion is different: they find that the pK_a of His291 is above 14 for all redox states of BNC, which means that His291 cannot work as a pump element, as we proposed.

Several issues in the above paper show that additional work needs to be done before the matter is settled theoretically.

The optimized geometry of the complex (reported in their Supporting Information) on which calculations were done is much different from the reported crystal structure. For example, the structure of Cu_B ligands is tetrahedral, unlike the one in the protein. In the extended Fe−Cu complex, the distance between the Nϵ2 atoms of His240 X-ray and the structure on which calculations have been done is 0.93 Å. Although Tyr244 is not part of the complex, when the optimized structure is inserted back into protein, the bond distance between the Nϵ2 (His240) atom and Tyr244 (Cϵ2) (cross-link) is 2.02 Å instead of 1.35 Å in the X-ray structure. Some other heavy atoms of His290 are shifted by as much as 0.6 Å.

For the Cu−Fe complex, only the protonated structure was optimized, while the deprotonated was not, that is, the same geometry was used for both protonated and deprotonated states. This is a serious flaw. Our calculation shows that geometry relaxation of the deprotonated structure results in the stabilization of this state by about 15 kcal/mol, which is equivalent to a decrease of pK_a of the site by about 10 p*K* units. (In Table 3, electronic energy of deprotonation is reported to be about 233 kcal/mol. The optimization of the deprotonated structure decreases this energy to 218 kcal/mol, according to our calculations.)

The homogeneous dielectric model of the protein was used in the calculations (the solvent in ref 1, as well in our calculations, is treated as a continuum of dielectric 80). We have reported earlier that results do critically depend on the inhomogeneity of the protein; see Figure 4 of ref 4. For example, for a homogeneous protein model of dielectric 4 (solvent of dielectric 80), or slightly higher, as in ref 1, we do know that the model does not work, that is, the pK_a value of His291 is too high to allow deprotonation of His291, as required by the model. (In their paper, the authors did not mention that we have studied various dielectric models of the protein with cavities and determined the range of parameters for which the proposed pumping scheme would work.)

The charges of the QM system used in the calculations were found without regard to the reaction field of the protein, that is, not in a self-consistent manner. A better procedure for solvation energies would be self-consistent. In such a procedure, the active site complex can be computed by DFT in the presence of a solvent reaction field, which in turn, is evaluated from a solution of the Poisson−Boltzmann equation; the self-consistency between the reaction field potential and the electronic structure of the complex is achieved by iterations.[7,8]

The protonation state of the protein is not properly defined. The charges of the titratable groups are critical for electrostatics. The protonation state depends on the redox state of the protein (i.e., the state of all redox centers). This issue is completely ignored in the present work. The usage of standard protonation

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states of the titratable residues, as in ref 1, would lead to a completely erroneous result. In our estimate, up to 10 kcal/mol error can be made for the deprotonation energy of His291, if an incorrect procedure is used. (We have checked it by doing calculations for both the standard protonation state and the one we have calculated in ref 2.)

The difference between the standard protonation state and the one found in the calculations[2,9] is quite significant. For example, in the OORO redox state, a total charge of the standard protonation state of A + B subunits (including metal ions) is $Q = -19$, whereas the protonation calculation gives $Q = -8.1$ at pH 7. Both subunits have a total negative charge in the standard state: A subunit $= -8$, B subunit $= -11$. Both total charge and its distribution are critical for correct pK_a evaluation.

Also, we find that the membrane needs to be included as a part of the system, since the equilibrium protonation state and the total charge of the enzyme differ depending on the dielectric conditions created by the presence or absence of the membrane.

The pK_a values of metal-ligated histidines have been measured experimentally and accessed theoretically in the recent studies of the iron−sulfur protein of the cytochrome bc_1 complex.[10,11] For this system, it was proposed that the protonation state of the Fe-ligated imidazoles is controlled by the redox changes of the Fe_2S_2 complex, similar to what we proposed for *CcO*. However, the actual pK_a comparison of the reduced iron−sulfur complex, $[(HisH)_2\ Fe^{II}\ S_2\ Fe^{III}\ (Cys^-)_2]$, and oxidized Cu_B center, $[(H_2O)\ Cu^{II}\ (HisH)_3]$, solely on the basis of the same oxidation state of metal atoms, as in ref 1, is invalid—the two complexes have completely different total charges (−1 vs +2, or +1, depending on the protonation state of His291, respectively). Hence, besides the oxidation state of the metals, a ligand charge and ligand type are also very important. In line with this, is a finding that complexes $[(H_2O)\ Cu^{I}\ (HisH)_3]$ and $[(HO^-)\ Cu^{II}\ (HisH)_3]$, despite having a different redox state of metal center, have very similar pK_a values since they bear the same total charge. This finding is consistent with all our previous studies and with a notation of the redox states, which we have introduced in ref 2.

We also disagree that for pK_a calculations in proteins one cannot use the relative scheme, as stated in the paper. For example, this scheme is the basis of MEAD program[12] that the authors used in their calculations.

Unfortunately, the current theoretical methods do not yet allow unambiguous predictions of pK_a values in proteins within few pK_a units, as required for this problem. However, one can hope that a reasonably good calculation can point to some interesting possibilities, which could be targeted for a direct experimental verification and thereby provide guidance to experimental work. The uncertainties of the issues discussed above show that more work is clearly needed on this challenging subject to make theoretical predictions more reliable.

References and Notes

(1) Fadda, E.; Chakrabati, N.; Pomes, R. *J. Phys. Chem. B* **2005**, *109*, 22629.

(2) Popovic, D. M.; Stuchebrukhov, A. A. *J. Am. Chem. Soc.* **2004**, *126*, 1858.

(3) Popovic, D. M.; Stuchebrukhov, A. A. *FEBS Lett.* **2004**, *566*, 126.

(4) Popovic, D. M.; Quenneville, J.; Stuchebrukhov, A. A. *J. Phys. Chem. B* **2005**, *109*, 3616.

(5) Quenneville, J.; Popovic, D. M.; Stuchebrukhov, A. A. *Biochim. Biophys. Acta* **2006**, in press (published online, January 18, 2006; doi: 10.1016/j.bbabio.2005.12.003).

(6) Medvedev, D. M.; Medvedev, E. S.; Kotelnikov, A. I.; Stuchebrukhov, A. A. *Biochim. Biophys. Acta* **2005**, *1710*, 47.

(7) Li, J.; Nelson, M. R.; Peng, C. Y.; Bashford, D.; Noodleman, L. *J. Phys. Chem. A* **1998**, *102*, 6311.

(8) Makhov, D. V.; Popovic, D. M.; Stuchebrukhov, A. A. *J. Phys. Chem. B* **2006**, *110*, 12162.

(9) Kannt, A.; Lancaster, R. D.; Michel, H. *Biophys. J.* **1998**, *74*, 708.

(10) Iwaki, M.; Yakovlev, G.; Hirst, J.; Osyczka, A.; Dutton, P. L.; Marshall, D.; Rich, P. P. *Biochemistry* **2005**, *44*, 4230.

(11) Ullmann, G. M.; Noodleman, L.; Case, D. A. *J. Biol. Inorg. Chem.* **2002**, *7*, 632.

(12) Bashford, D. An object-oriented programming suite for electrostatic effects in biological molecules. In *Scientific Computing in Object-Oriented Parallel Environments*; Ishikawa, Y., Oldehoeft, R. R., Reynders, J. V. W., Tholburn, M., Eds.; Springer: Berlin, 1997; Vol. 1343; p 233.