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First-principles molecular dynamics study of proton transfer mechanism in bovine cytochrome *c* oxidase

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Abstract

Density functional based first-principles molecular dynamics calculations, performed on a model system extracted from the bovine cytochrome *c* oxidase, have been performed in an attempt to inspect the proton transfer mechanism across a peptide group. Our model system includes the specific Tyr440-Ser441 peptide group involved in a novel proton transfer path and shows that the Y440-S441 enol peptide group [–C(OH)=N–], which is a structural isomer of a keto form [–CO–NH–], is the product of the deprotonation of an imidic acid [–C(OH)–NH–] occurring in the vicinity of the deprotonated aspartic acid residue. For the subsequent enol-to-keto tautomerization, a direct H^+ transfer path in the Y440-S441 peptide group has been identified, in which the transition state takes a distorted four-membered ring structure.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Proton transfer inside proteins is a fundamental step of the proton-pumping function in biological systems. For instance, in cellular respiration, protons are pumped across the mitochondrial membrane by transmembrane proteins such as cytochrome *c* oxidase (CcO) to create an electrochemical proton gradient, which is the driving force for ATP synthesis [1]. The general proton transfer pathways for any proton-pumping proteins include an extended hydrogen bond network formed between side chains of particular amino acid residues, such as aspartate and histidine, and crystallographically ordered water molecules [2]. A general picture that has been drawn is that the proton transfer inside proteins consists of a series

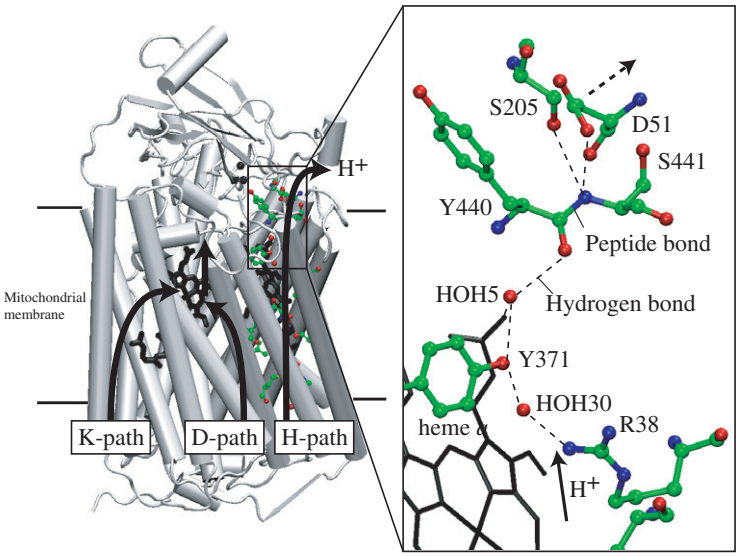

Figure 1. Scheme of three proposed proton transfer paths in bovine CcO. Subunits I and II of the x-ray structure of the fully oxidized bovine CcO (PDBid, 1V54); four redox-active metal sites are depicted in black, whereas grey cylinders represent the protein helicies (left panel). A part of the H-pathway (right panel); the main heavy atoms are shown as sticks and balls, hydrogen bonds as dashed lines and residue names are indicated with the related standard notations. The dotted arrow indicates the movement of the Asp51 amino acid residue.

of protons hopping along the hydrogen bonds of these networks via a standard proton wire mechanism [3, 4].

At variance with these pathways, which are generally referred to as K-pathway and D-pathway (figure 1) [5, 6], recent experiments performed on bovine CcO have suggested the existence of an alternative proton transfer path (H-pathway) involving a peptide (covalent) bond [7–9]. The CcO is the terminal enzyme in the respiratory chain of mitochondria or aerobic bacteria. Its main function consists of proton pumping across the membrane coupled to the reduction of O_2. According to the mechanism proposed for the H-pathway, the peptide group connecting tyrosine Y440 and serine S441 residues is part of the global hydrogen bond network responsible for the proton propagation and dividing the whole path into two parts (figure 1). The first part starts from one side of the mitochondrial membrane and ends at a crystal water molecule (HOH5) located in the vicinity of the peptide carbonyl oxygen. The second part, instead, is composed of a serine (S205) and aspartate (D51) residues; the D51 amino acid residue is the final proton acceptor in the H-path and, upon reduction of the enzyme, it undergoes a significant displacement toward the other side of the membrane.

One proposed scenario for proton transfer across peptide groups has been inferred from a sequential reaction (figure 2) in which an enol peptide group [–C(OH)=N–], a structural isomer of a keto form [–CO–NH–], is formed via an imidic acid peptide group [–C(OH)–NH–] and subsequently tautomerizes to the keto [9]. Namely, a proton can reach the peptide carbonyl oxygen via a proton wire mechanism, producing an imidic acid peptide group, and the amide proton is then released from the peptide group in a basic environment, leading to the formation of an enol form. A subsequent enol-to-keto tautomerization is accompanied by a proton transfer across the peptide group. This picture is consistent with the experimental data on bovine CcO [9, 10] in the sense that the deprotonated carboxyl group (COO^-) of D51 is

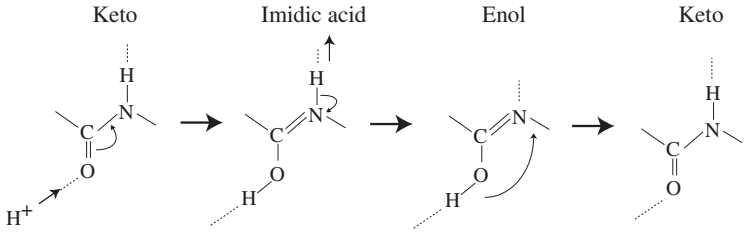

Figure 2. Schematic representation of a sequential reaction of peptide groups.

located in the vicinity of the amide (figure 1), and this can promote the transition to the enol form. Furthermore, according to mutagenesis experiments, the mutants, in which the Y440-S441 peptide group lacks its amide proton by replacing S441 with the proline (S441P), do not show any proton-pumping activity.

In this work, we investigate whether or not a series of these reactions can occur in bovine CcO, via first-principles molecular dynamics simulations performed on the CcO model system including the Y440-S441 peptide group. The results show that the imidic acid peptide group is a metastable state, and it turns out to be in an enol form at room temperature in the presence of the deprotonated aspartic acid residue. For the subsequent enol-to-keto tautomerization, a direct transfer path in the Y440-S441 peptide group has been identified via metadynamics simulations. Although the whole proton transfer process leaves the active region substantially unchanged and does not induce permanent large conformational changes of the backbone structure, the transition state turns out to be a distorted four-membered ring structure. A careful inspection of the CcO x-ray structure suggests the existence of catalytic factors in the vicinity of the Y440-S441 peptide group which are likely to promote such a reaction.

2. Computational details

The CcO model system used in these calculations is extracted from the x-ray data of fully oxidized bovine CcO (Brookheaven Protein Data Bank, accession code 1V54 [9]) and includes the Y440-S441 peptide group and its surrounding amino acid residues and crystal water molecules: Glu40, Leu41, Gly45, Thr46, Asp51, Tyr440, Ser441, Asp442, His204, His205, HOH5, HOH25, and HOH69. In order to represent the propionate of heme *a* and the phenolic group of Tyr371, an acetic acid and a 1-propen-2-ol are also included in the model. To reduce the computational costs, five amino acid residues were replaced with Ala or Gly: Y440A, D442A, H204A, L41G, and T46G. The resultant CcO model has a net charge $Q = +1e$ and amounts to 120 atoms. All the geometry optimizations were performed by a standard conjugated gradient procedure until the residual forces were less than 0.6 kcal mol^{-1} Å^{-1}. The position of several atoms, such as the terminal ones, far from the active reaction centre were kept fixed to the experimental crystallographic positions. In the dynamical simulations, these same atoms were allowed to oscillate around their experimental position by bounding harmonic potentials, whose force constants were set to be 6.5 or 26.0 kcal mol^{-1} Å^{-2}. Similarly, the Ψ torsion angle of Y440A was constrained to maintain its x-ray structure value via a harmonic force constant of 62.8 kcal mol^{-1} in order to mimic the presence of the surrounding Tyr54 residue.

First-principles molecular dynamics simulations [11] were performed within the Car–Parrinello (CP) scheme in the framework of density functional theory (DFT) [12], including gradient corrections on the exchange and correlation functional, after Hamprecht, Cohen, Tozer

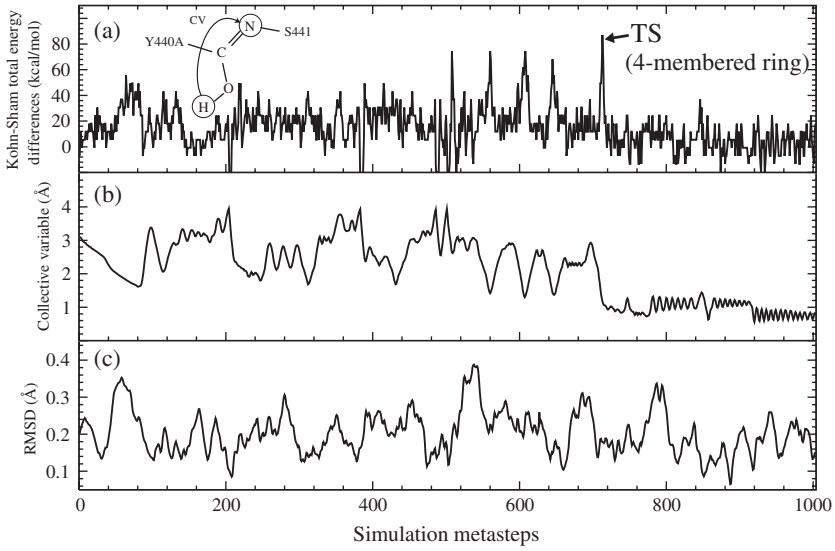

Figure 3. Evolution of the Kohn–Sham total energy differences (a), the collective variable (b) and the root mean square deviation (RMSD) values (c) during the metadynamics simulations. The origin along the energy axis corresponds to the reference value given by the time average ([0.58, 1.15] ps) of the Kohn–Sham total energy during standard CP molecular dynamics. The RMSD values were measured for the backbone heavy atoms (C_α, C, O, and N) of the Y440A-S441-D442A segment.

and Handy (HCTH) [13]. The interaction between core and valence electrons is described by Troullier–Martins norm-conserving pseudopotentials [14] and, in the case of Na, semi-core states, 2s and 2p, are included. Valence orbitals are expanded in a plane-wave basis set with an energy cutoff of 80 Ryd. The ionic temperature is controlled by velocity rescaling and kept at 300 ± 40 K. The MD time step of 0.097 fs and a fictitious electron mass of 400 au were adopted. The reaction paths were sampled via the metadynamics approach [15, 16]. In this study, we selected as a collective variable the N–H distance in the Y440-S441 peptide group (see figure 3 for details); this accounts for the formation of the N–H bond. Fictitious effective mass $M_\alpha = 12.0$ au and harmonic coupling constants $k_\alpha = 0.24$ were used. The width and height of the Gaussian penalty potential functions are sampled in the intervals [0.132, 0.265] Å and [1.1, 1.8] kcal mol^{-1}, respectively.

3. Results and discussion

Before starting any dynamical simulation, we performed geometry optimization of our CcO model system, assuming, as an initial state, the deprotonated carboxyl group of D51 (COO^-) and the protonated HOH5 water molecule (H_3O^+). This corresponds to the situation in which a pumped proton accesses the HOH5 water molecule via a proton wire mechanism after the deprotonated carboxyl group of D51 returns inside the protein. This optimization allows for the elimination of residual bond stress that could wrongly bias the reactions and show that an additional proton in the H_3O^+ transfers to the carbonyl oxygen of the keto Y440A-S441 peptide group, producing an imidic acid Y440A-S441 peptide group. On the other hand, the carboxyl group of D51 remains in the deprotonated form, indicating that the imidic acid Y440A-S441 peptide group is a metastable state. Unconstrained CP molecular dynamics simulations were then performed, at $T = 300$ K, on the fully relaxed system. This simulation has shown that the

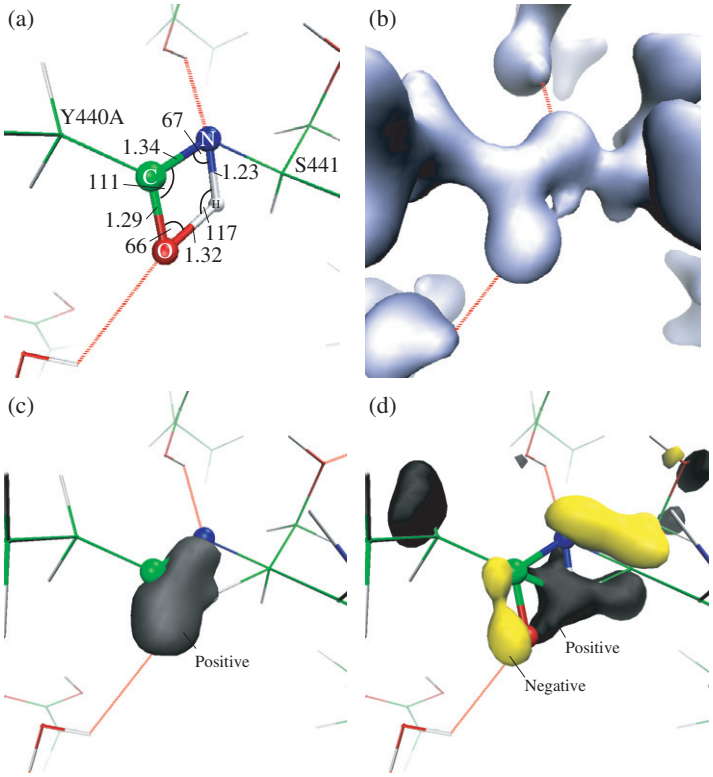

Figure 4. Atomic configuration and related electron states of the four-membered ring structure. (a) Geometry of the ring; distances and angles are expressed in Å and degrees, respectively. The main atoms are shown as balls labelled with the corresponding chemical symbol. The dotted lines indicate hydrogen bonds. (b) Isodensity contour at 0.94 (e Å^{-3}) of the total electron density. (c) Isodensity contour (black clouds) at +0.34 (e Å^{-3})$^{1/2}$ of the occupied Kohn–Sham (KS) orbital corresponding to the 158th state, starting from the highest occupied KS orbital (HOKS-158). (d) Isodensity contour at +0.18 (black clouds) and −0.18 (grey clouds) (e Å^{-3})$^{1/2}$ of the occupied KS orbital (HOKS-102).

deprotonated carboxyl group of D51 approaches the imidic acid Y440A-S441 peptide group and extracts a proton with a negligible activation barrier, converting the peptide group into an enol form. The surrounding serine residues, such as S205, can favour this reaction without undergoing large conformational changes. These results indicate that the formation of the enol peptide group via imidic acid can be a viable process in bovine CcO.

The reaction paths for the enol-to-keto tautomerization were sampled via metadynamics, starting from the equilibrated ionic configuration and velocities obtained from the previous dynamics. The collective variable was chosen to be the N–H distance in the Y440A-S441 peptide group (inset in figure 3). The result of the simulation is summarized in figures 3 and 4. Namely, during the metadynamics simulations (figure 3) the system undergoes, several times, a rotation of the C–O–H moiety of the Y440A-S441 peptide group around its C–O bond, and eventually the enol-to-keto tautomerization occurs via a transition state (TS) characterized by a four-membered ring structure of atoms belonging to the Y440A-S441 peptide group. Panel (a) in figure 4 shows the geometry of the TS. The proton originally bound to the carbonyl O of the Y440A-S441 peptide group is transferred to the N atom from below the peptide backbone. This induces a lot of bond stress and local deformations that make this particular proton

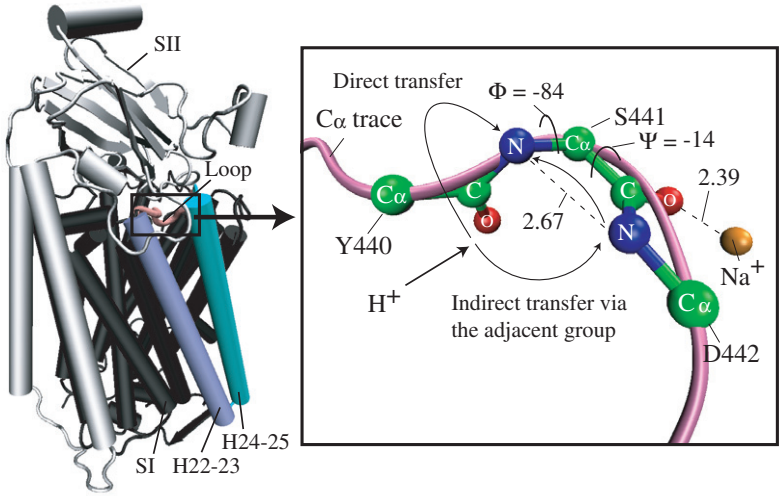

Figure 5. X-ray structure of the fully oxidized bovine CcO around the Y440-S441 peptide group (PDBid, 1V54). Labels SI and SII stand for subunits I and II, respectively. A Ω-like group segment (from Gly435 to Tyr443) labeled as 'Loop' is shown in detail in the right panel, while helices 22 and 23 (from Asn406 to Ser434) and helices 24 and 25 (from Pro444 to Lys479) are labelled as H22-23 and H24-25, respectively. The Y440-S441 and S441-D442 peptide groups in the Ω-like loop segment are reported in the right panel where distances and angles are expressed in ångstroms and degrees, respectively.

transfer highly unstable and hence energetically demanding; in fact, the activation barrier is about 72 kcal mol^{-1}. Similar trans ring structures are also realized in the TS of enol-to-keto tautomerization in polypeptide systems [17, 18]; the estimated activation barriers are comparable to that in the present case, suggesting that the common feature of a distorted four-membered ring structure determines the value of the activation energy and accounts for a general rate-limiting step in this class of processes.

Activation barriers are generally underestimated in any DFT approach, and are very sensitive to the exchange–correlation functional. The HCTH functional adopted here represents an improvement in comparison with respect to the more popular Becke–Lee–Yang–Parr (BLYP) functional in terms of dissociation energies, and its performance is comparable to that of the hybrid B3LYP functional [13]. For some biochemical reactions involving proton transfer, the accuracy of the HCTH functional has been shown to be within 10–15% [19, 20].

Panels ((b)–(d)) of figure 4 show the total electron density and the corresponding Kohn–Sham (KS) states around the ring structure. As can be seen, the departing H atom displays a non-negligible electron density around its nucleus (figure 4(b)). Through an analysis of the KS states, we found that the two electronic states characterized by the largest amplitudes on the departing H are mainly originated by a 2s orbital of O (figure 4(c)) or 2p orbitals of atoms belonging to the peptide backbone (figure 4(d)). A Mulliken population analysis, performed on the ring structure, gives a value of +0.379 for this specific H, smaller than the value for a bare proton. These results indicate that the departing H is not completely ionized in the distorted four-membered ring structure.

As shown in our previous calculation on polyglycine [17], the flexibility of the backbone conformation around the peptide group allows us to realize another enol-to-keto

tautomerization path. Indeed, in the isolated polyglycine case, the most favourable path is characterized by a cis/trans isomerization of the C–N peptide bond. Yet, in the present CcO case, the x-ray data indicate that the segment involving the Y440-S441 peptide group is relatively rigid. This segment is located at the interface between subunits I and II and sandwiched between them (figure 5), thus having, as inferred from isotropic temperature factors (B-factors), a relatively high rigidity. Therefore, it is expected to be difficult for the cis/trans isomerization path to become favourable.

By estimating the root mean square deviation (RMSD) from the CcO crystal structure (figure 3(c)), we found that these values, evaluated for the peptide backbone involving the Y440A-S441 peptide group, remain small all along the simulations. This indicates that the elementary transfer process along the present path does not induce permanent conformational changes. This feature favours the proton transfer inside proteins. Yet, as mentioned, the process is still energetically demanding due to the formation of a distorted four-membered ring configuration in the TS. A close inspection of the bovine CcO crystal structure suggests that one possible catalytic factor is the adjacent S441-D442 peptide group. Indeed, we found that the segment involving the Y440-S441 and S441-D442 peptide groups takes a Ω-like loop conformation and, due to this turn conformation, the amide N of the S441-D442 peptide group points to that of the Y440-S441 peptide group (figure 5); furthermore, the carbonyl O of the S441-D442 peptide group coordinates with the Na^+ metal ion, contributing to keep the N–H orientation. Thus, in the enol-to-keto tautomerization, an indirect proton transfer pathway can be realized by using two adjacent peptide groups. Calculations still in progress have shown that this pathway is more favourable than the direct transfer discussed here. Namely, in the indirect path, larger ring structures of atoms belonging to the Y440-S441 and S441-D442 peptide groups are formed. They do not induce big structural modifications and bond stresses, thus leading to a pathway characterized by a lower activation barrier. This stresses the importance of the physiological CcO tertiary structure around the Y440-S441 peptide group in promoting proton transfers across the peptide group.

Acknowledgments

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References

[1] Voet D and Voet J G 2004 *Biochemistry* 3rd edn (New Jersey: Wiley) p 797
[2] Decoursey T E 2003 *Physiol. Rev.* **83** 476
[3] Agmon N 1995 *Chem. Phys. Lett.* **244** 456
[4] Pomès R and Roux B 1996 *J. Phys. Chem.* **100** 2519
[5] Brzezinski P and Larsson G 2003 *Biochim. Biophys. Acta* **1605** 1
[6] Yoshikawa S 2002 *Adv. Prot. Chem.* **60** 341
[7] Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R and Yoshikawa S 1996 *Science* **272** 1136
[8] Yoshikawa S *et al* 1998 *Science* **280** 1723
[9] Tsukihara T *et al* 2003 *Proc. Natl Acad. Sci. USA* **100** 15304

[10] Shimokata K, Katayama Y, Murayama H, Muramoto K, Suematsu M, Tsukihara T, Yoshikawa S and Shimada H 2004 *Biochim. Biophys. Acta* EBEC Short Reports vol 13, p156
[11] CPMD, Copyright IBM Corp. 1990–2006, Copyright MPI für Festkŕperforschung Stuttgart 1997–2001
[12] Kohn W and Sham L J 1965 *Phys. Rev.* **140** A1133
[13] Hamprecht F A, Cohen A J, Tozer D J and Handy N C 1998 *J. Chem. Phys.* **109** 6264
[14] Troullier N and Martins J L 1991 *Phys. Rev.* B **43** 1993
[15] Laio A and Parrinello M 2002 *Proc. Natl Acad. Sci. USA* **99** 12562
[16] Iannuzzi M, Laio A and Parrinello M 2003 *Phys. Rev. Lett.* **90** 238302
[17] Kamiya K, Boero M, Shiraishi K and Oshiyama A 2006 *J. Phys. Chem.* B **110** 4443
[18] Takano Y and Nakamura H 2006 *Chem. Phys. Lett.* **430** 149
[19] Gervasio F L, Boero M and Parrinello M 2006 *Angew. Chem. Int. Edn* **45** 5606
[20] Boero M, Ikeda T, Ito E and Terakura K 2006 *J. Am. Chem. Soc.* **128** 16798