We focus on a variant of the heme protein, i.e., the recently discovered neuroglobin (NHb) and cytoglobin (CHb), looking for clues with regard to possible applications in polymer electrolyte fuel cells (PEFCs), in a quest to design nanomaterials that would replace the expensive platinum catalysts used in PEFC electrodes. The active heme sites of these two new members of the globin superfamily display a functionally-relevant heme iron atom, whose sixth coordination site is occupied by an endogenous (internal) protein ligand, in the absence of exogenous (external) ligands, such as O₂, CO, and NO. Density Functional Theory (DFT)-based calculation results indicate a possible means of controlling the adsorption and desorption on adsorbed O₂ through the endogenous protein ligand occupying the sixth coordination site of the central iron atom in the heme structure. [DOI: 10.1380/ejssnt.2005.233]

Keywords: Polymer electrolyte fuel cells; Cathode electrode; Density functional calculations; Catalysis; Chemisorption; Iron; Oxygen; Biological compounds; Biological molecules-proteins; Biological aspects of nanostructures

Polymer electrolyte fuel cells (PEFCs) are among the most promising alternative electric power generators for vehicles and residences in the near future. However, despite several related technological advances, commercial obstacles to the widespread use of PEFCs still remain. One outstanding problem is finding alternative materials to replace the widely used expensive platinum (Pt) catalysts. Some notable candidates are, e.g., Pt-based alloys and Pt-group metals (cf., eg., [1] and references therein). However, despite much effort, cheaper substitutes exhibiting reactivities comparable to that of platinum have yet to be discovered.

Realizing that new breakthroughs for catalyst-design necessitate deviation from conventional ideas, we have recently turned our attention to the best designer, Nature, for clues. For example, Nature has provided us with the most efficient producers of hydrogen on Earth: bacteria including the well studied escheria coli, found in the human intestine. Over the span of a few billion years, ancient bacteria have evolved the ability to make hydrogen from water and then oxidize it as fuel, developing enzymes now known as hydrogenases specifically for this purpose (cf., e.g., [2] and references therein). Nature has also provided us with the most efficient oxygen storage and transport agents on Earth, viz., heme proteins, esp., hemoglobin (Hb), myoglobin (Mb), and cytochromes (Cym), through the heme structure found incorporated in these proteins (cf., e.g., [3–6] and references therein). One thing common to the hydrogenases and the heme proteins is that the corresponding active sites involve iron (Fe), a rather cheap material compared to Pt (cf., [2–6] and references therein). Thus, we have recently considered the possibility of using hydrogenase-based nanomaterials in the anode electrodes of PEFCs, and showed that such a material, if realized, would exhibit a catalytic reactivity (with respect to H₂ dissociation) equivalent to that of the well-known Pt catalyst [2]. For the cathode electrodes of PEFCs, we considered nanomaterials based on the heme active sites of Hb, Mb, and Cym [3–6]. We found that nanomaterials having the basic structure of an iron-porphyrin (FeP) [3] can be made catalytically comparable (with respect to O₂ adsorption and dissociation) to that of Pt, either by tuning the orientation [8] of the incoming O₂ [4] or by inducing spin polarization [5]. Comparing the O₂-FeP interaction with that of O₂ and other various metalloporphyrins, e.g., manganese-porphyrin (MnP), cobalt-porphyrin (CoP), and nickel-porphyrin (NiP), our results indicate that FeP possesses the optimum electron configuration for O₂ dissociation [6]. One can view this as Nature’s way of showing how one could, through the porphyrin, locally change the properties of Fe, so as to exhibit a particular function, i.e., simulate the catalytic reactivity of Pt [9].

Here, we focus on a variant of the heme protein, i.e., the recently discovered neuroglobin (NHb) and cytoglobin (CHb) [10–13]. The active heme sites of these two new members of the globin superfamily are different from that found in Hb, Mb, and Cym in that they are reportedly found to display a functionally-relevant hexacoordinated heme iron atom, whose sixth coordination site is taken to be an endogenous (internal) protein ligand, in the absence of the exogenous (external) ligands such as O₂, CO,
and NO, i.e., they contain bis(histidine) hemes. Although the corresponding functions of these new members of the globin superfamily are still under active study and debate, our purpose here is to explore the possibility of utilizing the distal (replaceable) endogenous protein ligand mentioned for PEFCs applications, particularly as a possible alternative to the Pt-based cathode electrode catalyst.

To represent the bis(histidine) heme, we consider a bis(imidazole) iron porphyrin (ImFeP) complex, as shown in Fig. 1. One could consider this an extension of the ImFeP model we adopted earlier to represent the heme protein in Hb and Mb [3–6]. In addition to the proximal (permanent) Im ring found attached at one side of the FeP plane [3–6], there is a distal imidazole occupying the sixth coordination site of the heme Fe atom, at the other side of the FeP plane (cf., Fig. 1(a)). The configuration we adopted for the case when the distal histidine is not bonded to the heme.

FIG. 1: The bis(imidazole) iron porphyrin (ImFeP) complex used to represent the bis(histidine) heme. One could consider this an extension of the ImFeP model we adopted earlier to represent the heme protein in Hb and Mb [3–6]. The configuration shown is based on available X-ray crystallographic data [14]. (a) In addition to the proximal (permanent) Im ring found attached at one side of the FeP plane [3–6], there is a distal imidazole occupying the sixth coordination site of the heme Fe atom, at the other side of the FeP plane. (b) The configuration we adopted for the case when the distal histidine is not bonded to the heme.

For comparison, we also considered a mono(histidine) heme-O2 complex, represented by a mono(imidazole) iron porphyrin-ImFeP–O2 complex, as shown in Fig. 2 (cf., e.g., [3–6] and references therein). In addition to the proximal (permanent) Im ring found attached at one side of the FeP plane [3–6], there is an O2 occupying the sixth coordination site of the heme Fe atom, at the other side of the FeP plane (cf., Fig. 2(a)). In Fig. 2(b), we also show the configuration we adopted for the case when the distal histidine is not bonded to the heme.

FIG. 2: (a) The mono(imidazole) iron porphyrin (ImFeP) complex used to represent the mono(histidine) heme, with O2 occupying the sixth coordination site of the heme Fe atom, at the other side of the FeP plane [3–6]. (b) The configuration we adopted for the case when the O2 is not bonded to the heme.

We performed all calculations based on the density functional theory (DFT) [15, 16], with the Beck-Lee-Young-Parr (B3LYP) exchange-correlation functional, and the 6-31G* basis set, as implemented in the Gaussian03 program suite [17]. We also relaxed the geometry of the distal imidazole. For more details regarding the computational techniques, cf., [2–6].

Our results indicate that the Im2FeP (Fig. 1(a)) is ≈ 0.96—1.06 eV more stable than ImFeP+Im (Fig. 1(b)), taking into account relaxation. The results agree with recent experimental findings (activation energies Ea in the range of 19.5—23.5 Kcal/mol, or ≈ 0.846—1.019 eV) [18, 19].

We looked into the Mulliken charges of each atom and group them together as the Fe center, porphyrin ring and the imidazole ligands. In the Im2FeP system, the Fe2+ has a net charge of only +1.30, indicating that it accepted partial electronic charges from the attached ligands. The highly conjugated porphyrin ring around Fe has a net charge of −1.48. This indicates electron donation to the Fe center. (The ring should, otherwise, have a charge of −2.) The two imidazoles have the same net charges of 0.09. The electron donation of imidazole is much less when compared to each of the four nitrogen-containing pyrroles that comprise the porphyrin ring; this is expected due to lesser conjugation in imidazole. For the ImFeP system, the central Fe2+ has a net charge of +1.17, the porphyrin has a net charge of −1.29, and the proximal imidazole has a net charge of +0.12. Upon formation of the ImFeP+Im system, negative charge is mainly transferred from the porphyrin ring to the dissociated (distal) imidazole. The central Fe2+ and the proximal imidazole remain practically unaffected.

Mulliken spin density analyses reveal that in the formation of the high-spin (S = 2) ImFeP+Im system, the spin density is concentrated at the Fe atom, consistent with earlier findings (cf., e.g., [3–6] and references therein). The four highest occupied molecular orbitals (viz., HOMO, HOMO-1, HOMO-2, HOMO-3) are occupied with single alpha electrons, mostly coming from the Fe, with their corresponding beta orbitals are empty, again consistent with earlier findings (cf., e.g., [3–6] and references therein).
We relaxed the Im$_2$FeP energetically by varying the bond angles, the dihedral angles, and the bond lengths of the distal imidazole. However, it should be noted that in the actual NHb molecule, the distal imidazole may not be able to achieve its most relaxed conformation, as it is bonded to the proteins polypeptide chain. Our purpose is to determine whether it is possible to observe geometrical effects on the affinity for cleavage of the distal imidazole, as we have observed earlier for O$_2$ [4]. The calculation shows that the energy difference between the relaxed and unrelaxed geometries is only $\approx 0.1$ eV, and the energy of both systems are within the range of experimental $E_a$ values.

Following earlier studies [3–6], we consider both the low spin and high spin states ($S = 0$ and $S = 1$, respectively) for ImFeP-O$_2$ (Fig. 2(a)). ImFeP-O$_2$ is $\approx 0.20$ eV and 0.64 eV more stable than ImFeP$+$O$_2$ (Fig. 2(b)), respectively for $S = 1$ and $S = 2$. Note that $\approx 1.01$ eV will evolve from the binding of distal imidazole (vide supra). This energy is enough to liberate another O$_2$. Our results also indicate that O$_2$ may rebind, but it is not energetically favored over the distal imidazole.

For the low-spin ($S = 0$) and high-spin ($S = 1$) ImFeP-O$_2$, the central Fe$^{2+}$ has a net charge $> 1.3$, the porphyrin has a net charge $< -1.1$, the imidazole has a net charge $> 0.12$, with the O$_2$ having a net charge $< -0.3$. This difference in charge distribution—when compared to ImFeP$+$can be explained by the electron withdrawing effect of the O$_2$ ligand. The electronegative O$_2$ causes the rest of the molecule to be more positively charged.

As expected, Mulliken spin density analyses reveal that, for the high-spin ($S = 1$) ImFeP-O$_2$ system, spin density is mainly distributed between Fe and O$_2$, consistent with earlier findings (cf., e.g., [3–6] and references therein). The two highest occupied molecular orbitals (viz., HOMO, HOMO-1) are occupied with single alpha electrons, mostly coming from the Fe and O$_2$, with their corresponding beta orbitals being empty, again consistent with earlier findings (cf., e.g., [3–6] and references therein).

In summary, we focussed on a variant of the heme protein, i.e., the recently discovered neuroglobin (NHb) and cytoglobin (CHb), looking for clues with regard to possible applications in polymer electrolyte fuel cells (PEFCs), in a quest to design nanomaterials that would replace the expensive platinum catalysts used in PEFC electrodes (in this case, as an alternative to the Pt-based cathode electrode catalyst). The active heme sites of these two new members of the globin superfAMILY display a functionally-relevant heme iron atom, whose sixth coordination site is occupied by an endogenous (internal) protein ligand, in the absence of exogenous (external) ligands, such as O$_2$, CO, and NO, i.e., they contain bis(histidine) hemes. To represent the bis(histidine) heme, we considered a bis(imidazole) iron porphyrin (Im$_2$FeP) complex, as shown in Fig. 1. Density Functional Theory (DFT)-based calculation results indicate a possible means of controlling the adsorption and desorption on adsorbed O$_2$ through the endogenous protein ligand occupying the sixth coordination site of the central iron atom in the heme structure, i.e., the distal histidine (represented by imidazole). With the extra distal imidazole ligand, one could control the occupation of the active Fe site, freeing it for use in succeeding relevant reactions, otherwise rendered inactive by exogenous ligands, e.g., O$_2$, NO, CO, and even O. Further studies are underway to consider the necessary detailed dynamics involved [20].

This work is partly supported by: a Grant-in-Aid for Scientific Research on Priority Areas (Developing Next Generation Quantum Simulators and Quantum-Based Design Techniques) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT); the 21st Century Center of Excellence (COE) Program "Core Research and Advance Education Center for Materials Science and Nano-Engineering" supported by the Japan Society for the Promotion of Science (JSPS); the New Energy and Industrial Technology Development Organization (NEDO) Materials and Nanotechnology program. Some of the calculations were done using the facilities of the Yukawa Institute Computer Facility (Kyoto University), the Institute for Solid State Physics (ISSP) Supercomputer Center (University of Tokyo), the Information Technology Based Laboratories Project of the Japan Atomic Energy Research Institute (ITBL, JAERI).

[1] N.M. Marcović, P.N. Ross, Surf. Sci. Rep. 45, 117 (2002).
[2] M. Tsuda, W.A. Diño, H. Kasai, Solid State Commun. 133, 589 (2005).
[3] M. Tsuda, W.A. Diño, H. Nakanishi, H. Kasai, e-J. Surf. Sci. Nanotech. 2, 226 (2004).
[4] M. Tsuda, W.A. Diño, H. Nakanishi, H. Kasai, Chem. Phys. Lett. 402, 71 (2005).
[5] M. Tsuda, W.A. Diño, H. Kasai, Jpn. J. Appl. Phys. 44, L57 (2005).
[6] M. Tsuda, E.D. Sy, H. Kasai, J. Chem. Phys. 122, 244719 (2005).
[7] D.M. Smith, M. Dupuis, E.R. Vorpagel, T.P. Straatsma, J. Am. Chem. Soc. 125, 2711 (2003).
[8] M. Okada, K. Moritani, A. Yoshigoe, Y. Teraoka, H. Nakanishi, W.A. Diño, H. Kasai, T. Kasai, Chem. Phys. Lett. 301, 315 (2004).
[9] W.A. Diño, J. Phys.: Condens. Matter 14, 4379 (2002).
[10] T. Burmester, B. Weich, S. Reinhardt, T. Hankeln, Nature 407, 520 (2000).
[11] N. Kawai, D.B. Kristensen, K. Asahina, K. Nakatani, Y. Minamiyama, S. Seki, K. Yoshizato, J. Biol. Chem. 276, 25318 (2001).
[12] T. Burmester, B. Ebner, B. Weich, T. Hankeln, Mol. Biol. Evol. 19, 416 (2002).
[13] J.T. Trent 111, M.S. Hargrove, J. Biol. Chem. 19, 19538 (2002).
[14] A. Pesce, S. Dewilde, M. Nardini, L. Moens, P. Ascenzi, T. Hankeln, T. Burmester, M. Bolognesi, Structure 11, 1087 (2003).
[15] P. Hohenberg, W. Kohn, Phys. Rev. B 136, 864 (1964).
[16] W. Kohn, L.J. Sham, Phys Rev. A 140, 1133 (1965).
[17] M.J. Frisch et al., Gaussian 03, Revision C.02, Gaussian, Inc., Wallingford CT, 2004.
[18] J. Uzan, S. Dewilde, T. Burmester, T. Hankeln, L. Moens, D. Hamdane, M.C. Marden, L. Kiger, Biophys J. 87, 1196 (2004).
[19] L. Kiger, J. Uzan, S. Dewilde, T. Burmester, T. Hankeln, L. Moens, D. Hamdane, V. Baudin-Creuza, M.C. Marden, http://www.sssj.org/ejssnt (J-Stage: http://ejssnt.jstage.jst.go.jp)
IUMB Life 56, 709 (2004).

[20] E.S. Dy, W.A. Diño, M. Tsuda, H. Kasai, in preparation.