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Research paper

Statistical and quantum-chemical analysis of the effect of heme porphyrin distortion in heme proteins: Differences between oxidoreductases and oxygen carrier proteins

Yusuke Kanematsu[a,*], Hiroko X. Kondo[a,1], Yasuhiro Imada[b], Yu Takano[a,b,*]

[a] Graduate School of Information Sciences, Hiroshima City University, Hiroshima 731-3194, Japan
[b] Institute for Protein Research, Osaka University, Suita 565-0871, Japan

HIGHLIGHTS

- Statistical and quantum-chemical analysis of heme distortion was performed.
- PCA implied oxidoreductases preferred ruffling distortions to regulate redox potentials.
- LDA provided a characteristic heme distortion separating structural distributions.
- We computed redox potentials and oxygen affinities of hemes with the characteristic distortions.
- This distortion is related to the characteristic properties of oxidoreductases and oxygen carriers.

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ABSTRACT

We studied the effects of heme distortions on the chemical properties in two protein classes, oxidoreductases and oxygen carrier proteins, in the Protein Data Bank by a combination of statistics and quantum chemistry. A statistical analysis of heme distortion provided the characteristic distortions. They were reasonably correlated with the redox potentials and oxygen affinities evaluated by quantum chemical calculations, implying that heme proteins utilize these distortions to enhance their functions. The combination of statistical analysis and quantum chemical calculations is useful for the elucidation of the structure-function relationships.

1. Introduction

Heme proteins are involved in various biochemical functions, such as oxygen transport, electron transfer, molecular sensing, and redox reactions [1–3]. Their diverse functions are performed by a heme cofactor, consisting of an iron ion and a porphyrin molecule. Since the pioneering work on hemoglobin in the mid-19th century, many studies have been conducted to investigate the biological and chemical functions of heme and heme proteins, by both experimental and computational approaches. About 5000 heme protein structures have been deposited in the Protein Data Bank (PDB) [4], and numerous heme-specific data, such as redox potentials, have been reported [5]. Given the recent increase in the available data, statistical analyses of heme proteins will pave the way toward elucidating how they efficiently use their heme cofactors to control their functions.

The diversity of heme functions can be attributed to the functional groups on the porphyrin periphery and the axial ligands on the iron atom. Hemes are classified into various types according to the heme periphery modification, which fine-tunes their electrochemical properties [6]. In addition, heme is typically bound to protein side chain groups as an axial ligand, which modulates the spin state, the electronic structure, and the reactivity of heme [7]. These modulations have been statistically analyzed and compared to build a framework for the design of *de novo* heme proteins [8,9].

Recently, protein-induced distortions of a skeletal porphyrin ring (Fig. 1) have also attracted keen attention. Some out-of-plane distortions are conserved in functionally related proteins in spite of the energetic preference for the planar structure, implying their biological significance [10]. Analyses by pH titration, UV–Vis spectroscopy, nuclear magnetic resonance, vibrational spectroscopy, and quantum chemical calculations suggested that for some proteins, in-plane (breathing) and/or out-of-plane (ruffling, saddling, and doming)

* Corresponding authors at: Graduate School of Information Sciences, Hiroshima City University, Hiroshima 731-3194, Japan (Y. Kanematsu and Y. Takano).
E-mail addresses: ykane@hiroshima-cu.ac.jp (Y. Kanematsu), ytakano@hiroshima-cu.ac.jp (Y. Takano).
[1] Current address: School of Regional Innovation and Social Design Engineering, Kitami Institute of Technology, Kitami 090-8507, Japan.

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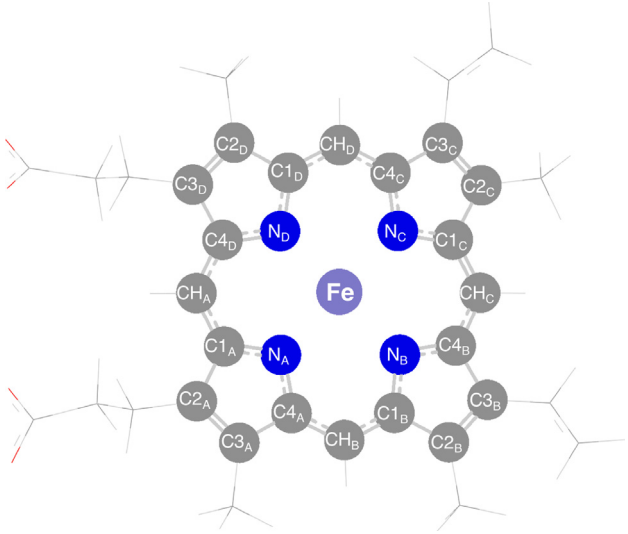

Fig. 1. Atomic components in the skeletal porphyrin ring of heme.

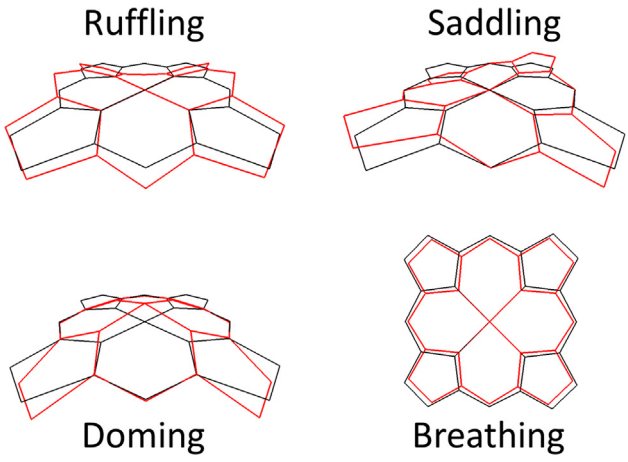

Fig. 2. Important distortions of the porphyrin ring.

distortions (Fig. 2) control the redox potential, the enzymatic activity, and the ligand binding affinity [11–15]. To capture more general trends, the statistics of porphyrin distortions were introduced by Imada et al. [16]. They demonstrated that the first and second principal components of the structural distribution of porphyrin distortions extracted from the PDB corresponded to the two lowest normal modes. In addition, they elucidated the relationship between the ruffling and saddling distortions and the redox potentials of hemes. Although their previous analysis was limited to the redox potential, the statistical investigation of the structural distribution is expected to reveal the structure-function relationships of heme proteins.

In the present study, we performed statistical analyses combined with DFT calculations of the structural distribution of porphyrin skeletons in heme proteins available in the PDB, to elucidate the effects of porphyrin distortions on the diverse functions. We focused on the difference between oxidoreductases and oxygen carrier proteins, which were assumed to have exclusive characteristics. Our results revealed a reasonable relationship between heme distortion and function, implying the significance of such a combinational study to analyze the molecular mechanism of the functional control of heme proteins.

2. Computational methods

To obtain the structural distribution of heme, we extracted 9723 heme structures in 3748 PDB entries. In addition, we classified oxidoreductases and oxygen carrier proteins according to the PDB annotations. We regarded heme proteins with 1 at the first digit of the enzyme classification (EC) number, *i.e.* 1.x.x.x, as oxidoreductases. Heme proteins with the annotation of “OXYGEN STORAGE” or “OXYGEN TRANSPORT” in the KEYWDS or HEADER records in the PDB file were treated as oxygen carrier proteins. The total numbers of oxidoreductases and oxygen carrier proteins were 4191 and 1688, respectively.

The structural distortions of the Fe-porphyrin moieties of hemes were statistically investigated by a principal component analysis (PCA) [17] and a Fisher linear discriminant analysis (LDA) [18]. The significant displacements from the equilibrium structure and the corresponding principal components were evaluated, as described previously [16]. LDA is a statistical method to obtain the feature vector on which the distributions of different classes are linearly separated from each other. The separation of two distributions is achieved by maximizing the Fisher criterion:

$$J = \frac{(m_2 - m_1)^2}{s_1^2 + s_2^2}, \tag{1}$$

where m_i and s_i^2 are the mean and the variance of the i-th distribution. LDA was performed on oxidoreductases and oxygen carrier proteins. To exclude the noise from the structural distribution, we used the first 24 principal components from the PCA before performing the LDA. These components account for 97% of the total variance.

All quantum chemical calculations were performed with the Gaussian09 program packages [19]. We used the Fe-porphyrin molecule as the model of heme without axial ligands. The coordinates of the hydrogen atoms were optimized with the semiempirical PM6 method [20]. We utilized the PBE0 functional [21,22] and the 6-31G(d) basis set for the evaluation of the normal modes. The redox potential and oxygen affinity were evaluated by using the OLYP functional [23,24] with the def2-TZVPP basis set [25]. The OLYP functional provided a better evaluation of the oxygen affinity than the hybrid functionals, such as PBE0 [26]. In the computation of the redox potential and the oxygen affinity, the conductor-like polarizable continuum model (CPCM) [27,28] with a dielectric constant of 4.0 was used to incorporate the electrostatic environment of a protein. Based on the previous studies [6,16,29], the spin states of Fe(II)- and Fe(III)-porphyrin, and the oxygen-bound Fe(II)-porphyrin were assumed to be singlet, doublet, and singlet, respectively.

The computation of the “accurate” redox potential is difficult, owing to the problems of the treatment of the charged system, the standard hydrogen electrode (SHE) potential, the solvation effect, and so on [30,31]. To obtain basic insights into the heme distortion effects on the redox potential, the relative redox potentials ($\Delta E_{\mathrm{redox}}$) between the Fe(III) and Fe(II) states of the Fe-porphyrins were evaluated as the differences between the electronic energies in the oxidized and reduced states in the gas phase, according to:

$$\Delta E_{\mathrm{redox}} = E(\mathrm{ox}) - E(\mathrm{red}) - E_{\mathrm{redox}}^{\mathrm{ref}} \tag{2}$$

where $E(\mathrm{ox})$ and $E(\mathrm{red})$ are the energies of the Fe(III) and Fe(II) states, and $E_{\mathrm{redox}}^{\mathrm{ref}}$ ($= 4.07$ eV) represents the corresponding energy difference for the mean structure. The relative oxygen affinity ($\Delta E_{\mathrm{O_2}}$) was similarly calculated as the energy difference between the oxy and deoxy forms of the Fe-porphyrins:

$$\Delta E_{\mathrm{O_2}} = E(\mathrm{oxy}) - E(\mathrm{deoxy}) - E_{\mathrm{O_2}}^{\mathrm{ref}} \tag{3}$$

where $E(\mathrm{oxy})$ and $E(\mathrm{deoxy})$ are the energies of the oxygen-bound and unbound Fe(II) states, and $E_{\mathrm{O_2}}^{\mathrm{ref}}$ ($= 1.10$ eV) represents the corresponding energy difference for the mean structure. The oxygen molecules of the oxy forms were optimized at the OLYP/def2-TZVPP level of calculation.