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Coordination Chemistry of Chlorophylls: Which Side of the Chlorin Macrocycle is Favored for the Ligand Coordination?

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Since chlorophyll *a* and bacteriochlorophyll *a* are asymmetric molecules, an external ligand can coordinate to the central Mg atom either from the chlorin macrocycle side where the $C13^2$-methoxycarbonyl moiety protrudes (denoting as the 'back' side) or from the other side (the 'face' side). We investigated which side of the macrocycle is favored for the ligand coordination, by survey of the highly resolved crystal structures of various photosynthetic proteins and theoretical model calculations. It is found that chlorophyll *a* as well as bacteriochlorophyll *a* and *b* in the photosynthetic proteins mostly bind their ligands on the 'back' sides. This finding was confirmed by the theoretical calculations for methyl chlorophyllide *a* and methyl bacteriochlorophyllide *a* as models: the 'back' type ligand-(bacterio)chlorophyll complex was more stable than the 'face' type one. The calculations predicted influence of the $C13^2$-stereochemistry on the choice of the side of the ligand coordination, which is discussed in relation to the presence of the $C13^2$-epimer of chlorophyll *a* in photosystem 1 reaction center [1].

Key words : bacteriochlorophyll, chirality, chlorophyll, chlorophyll *a'*, coordination, photosynthesis, P700

INTRODUCTION

The two axial coordination sites of chlorophylls (Chls; Figure1) and bacteriochlorophylls (BChls) are asymmetric, and two isomers are available around the central magnesium atom: the 'back' and 'face' complexes that bind ligands on the macrocycle sides where the $C13^2$-(*R*)-moiety and the C17-long chain protrude, respectively. For hemoproteins such as myoglobin, differences between the native protein and the protein with the 'reversed' heme (with the 180° rotated macrocycle in the 'back'-to-'face' manner) have been argued [2]. For (B)Chl-proteins, on the other hand, no such a study has been, to our knowledge, reported so far. The coordination chemistry of Chls and BChls should lead to an understanding of the way how these pigments are fixed at the specific sites in the photosynthetic proteins in the folding processes.

Which side of the macrocycle is favored for the ligand binding? How large is the energetic advantage? Here we attempt to address these questions by two approaches of (1) survey of Chls and BChls in structurally determined photosynthetic proteins and (2) computational calculations of model pigments.

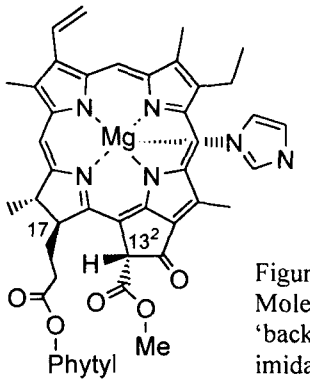

Figure 1
Molecular structure of the 'back' type chlorophyll *a* – imidazole complex.

MATERIALS AND METHODS

The structural data of photosynthetic proteins possessing (B)Chls were provided by Protein Data Bank (PDB) at the Brookhaven National Laboratory. Molecular modelings were performed using a program package HyperChem release 5.1 (Hypercube, Inc.). In the model structures, the C17-phytyl chain was replaced with a methyl group for simplicity. Imidazole was chosen for the fifth ligand. The molecular structures were optimized by molecular mechanics program MM+ and quantum mechanics program PM3 [3]. The potential energy surfaces around the energy-minimized geometries were also explored.

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RESULTS AND DISCUSSION

We reviewed crystal structures of 34 proteins; a reaction center (RC) of photosystem 1 (PS1), an antenna Chl-peridinin-protein, two FMO proteins of green bacteria, two LH2 proteins, one LH3 protein, and 27 RCs of purple bacteria (including RCs of BChl *b*, mutants, *etc.*). It is noted that most of Chls and BChls in these proteins bind their ligands on their 'back' sides. Among 93 Chl *a* molecules in the PS1 protein, whose ligand can be identified, only 14 molecules are ligated from the 'face' side. Two Chl *a* molecules in Chl-peridinin-protein have water molecules as the fifth ligand coordinating to the central Mg atoms from the 'back' sides. There are 14 types of BChls in the BChl-proteins (FMO, 7 types; LH2 and LH3, 3 types; RC, 4 types), and only 4 of 14 types BChls bind the ligands on their 'face' sides: α- and β-B850 (B820) in LH2 (LH3), and two BChls in FMO protein. The side of the macrocycle that bears a smaller ligand, water, indirectly (non-covalently) fixed by protein scaffolds, can be a measure to clarify the favorable side for the ligand coordination: 19 of 20 water-binding (B)Chl molecules are the 'back' type complexes. These findings are consistent with the facts that the crystals of dihydrated methyl and ethyl chlorophyllides *a* (MeChlid *a*, EtChlid *a*) both comprise of the 'back' isomers [4,5]. It is strongly suggested that the 'back' side of the macrocycle is more favorable for the ligand coordinating to the central Mg atoms of (B)Chl *a* and *b*.

We computationally build imidazole-bound MeChlid *a* and methyl bacteriochlorophyllide *a* (MeBChlid *a*) as models, and examined their structures and properties. The obtained geometry of the 'back' type MeChlid *a* complex was bowl-like, in agreement with the crystal structures of the hydrated EtChlid *a* [4,5]. Heat of formation (ΔH_f) of the 'back' complex was lower than that of the 'face' one, not only at the energy-minimized structures (Table 1) but in wide ranges of the potential energy surfaces of the compound obtained upon rotations of the C3-, $C13^2$-, and C17-moieties, and the ligand imidazole. The same tendency was also noted for MeBChlid *a*. These results are consistent with the observations that the crystals are mostly composed of the 'back' isomers.

Further calculations for the $C13^2$-(*S*)-epimer (MeChlid *a*') and the $C13^2$-demethoxycarobonyl compound (MepyroChlid *a*) revealed that inversion and removal of the $C13^2$-moiety reduces the relative stability of the 'back' isomer (Table 1). Presence of the central metal atom that can bear the fifth ligand is one of essential requirements for metallo(bacterio)chlorins to be incorporated into the photosynthetic proteins, and, as a first approximation, coordination of an amino acid residue to the central metal atom may govern the first stage of the formation of the (B)Chl-protein complexes. In this context, the above computational result for MeChlid *a*' conflicts the situation of the P700-Chl *a*' which is coordinated by His residue from the 'back' side [6]. The inconsistency may suggest that interactions other than the Mg – imidazole coordination bonding is decisive for incorporation of Chl *a*' into the unfolded PsaA protein. The observed advantage of the 'face' over the 'back' could, on the other hand, suggest another explanation that Chl *a* epimerizes in the PS1 core protein to be the P700-Chl *a*' (ref. 7 and A. Nakamura, in personal communication).

Table 1. Heat of formation of the energy-minimized structures of the imidazole-bound pigments.

compound	ΔH_f (kJ/mol)		$\Delta\Delta H_f$ [a]
	back	face	(kJ/mol)
MeChlid *a*	−245.6	−242.7	−2.9
MepyroChlid *a*	+76.1	+75.7	+0.4
MeChlid *a*'	−233.0	−241.8	+2.1
MeBChlid *a*	−556.1	−551.5	−4.6

[a] $\Delta\Delta H_f = \Delta H_f$ (back) − ΔH_f (face)

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