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Understanding chlorophylls: Central magnesium ion and phytyl as structural determinants

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ABSTRACT

Phytol, a C_{20} alcohol esterifying the C-17^3 propionate, and Mg^{2+} ion chelated in the central cavity, are conservative structural constituents of chlorophylls. To evaluate their intramolecular structural effects we prepared a series of metal- and phytyl-free derivatives of bacteriochlorophyll *a* and applied them as model chlorophylls. A detailed spectroscopic study on the model pigments reveals meaningful differences in the spectral characteristics of the phytylated and non-phytylated pigments. Their analysis in terms of solvatochromism and axial coordination shows how the central Mg and phytyl residue shape the properties of the pigment. Surprisingly, the presence/absence of the central Mg has no effect on the solvatochromism of (bacterio)chlorophyll π-electron system and the hydrophobicity of phytyl does not interfere with the first solvation shell of the chromophore. However, both residues significantly influence the conformation of the pigment macrocycle and the removal of either residue increases the macrocycle flexibility. The chelation of Mg has a flattening effect on the macrocycle whereas bulky phytyl residue seems to control the conformation of the chromophore via steric interactions with ring V and its substituents. The analysis of spectroscopic properties of bacteriochlorophyllide (free acid) shows that esterification of the C-17^3 propionate is necessary in chlorophylls because the carboxyl group may act as a strong chelator of the central Mg. These observations imply that the truncated chlorophylls used in theoretical studies are not adequate as models of native chromophores, especially when fine effects are to be modeled.

1. Introduction

Chlorophylls (Chls), including bacteriochlorophylls (BChls), are essential functional and structural cofactors in all photosynthetic pigment-proteins involved in oxygenic and anoxygenic photosynthesis (Fig. 1). Due to their vital involvement in photosynthesis, for over a century they have attracted great interest in numerous research laboratories worldwide [1–4]. The uniqueness of these pigments stems from the properties of the extensive π-electron system of the porphyrinic chromophore, which chelates the Mg^{2+} ion in the center and is covalently linked to the hydrophobic residue of an isoprenoid alcohol, in most cases a C_{20} phytyl [4]. Perhaps the most amazing feature of Chls is their versatility, i.e. the fact that a single chemical entity is able to perform several diverse functions in the photosynthetic apparatus. Chls actively participate in photon capturing and excitation energy transfer and storage in the antennae, whereas in the reaction centers in the primary charge separation and electron transfer. This functional versatility of Chls as photosynthetic cofactors is feasible because their π-electron system and its manifestations, i.e. electronic absorption and redox potential, undergo severe modulation upon binding to apoproteins. In effect, the characteristics of isolated free Chls differ substantially from those found in their natural environment [5–10] and in fact the mechanisms of this modulation are not completely understood. Therefore, a more comprehensive view of apoprotein control over the chromophores is necessary in order to identify all key determinants of the relevant Chl properties.

Conformational modulation is among the most important factors involved in the tuning of Chl properties [10–14]. However, the details of Chl structure and conformation have been derived either from crystallographic studies on (B)Chl derivatives devoid of phytyl or on the protein-bound pigments [15–21]. They concern, therefore, chromophores that are far from their relaxed states. Moreover, two things emerge from the crystal structures of photosynthetic complexes. Firstly, Chl molecules are able to make many contacts with apoproteins. Secondly, protein-bound chromophores adopt a wealth of geometries, which means that specific photosynthetic activities of Chls are not circumscribed by unique conformational forms [13]. This lack of a high resolution structure of intact chlorophylls in a relaxed form is one of the major obstacles to achieving full understanding of the functioning of photosynthetic machinery.

The central Mg^{2+} ion and phytyl are very conservative constituents of Chls. This poses a question of the molecular basis for the evolutionary selection of the Mg^{2+} ion as the occupant of the central

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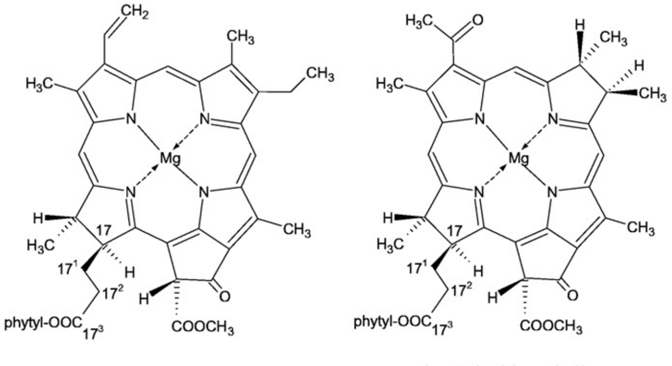
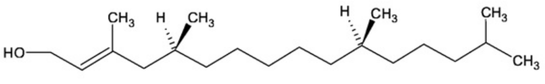


chlorophyll *a*

bacteriochlorophyll *a*



phytol

Fig. 1. Structural formulae of chlorophyll *a* and bacteriochlorophyll *a*, major photosynthetic pigments of higher plants and purple photosynthetic bacteria, respectively. Also the structure of phytol is shown, a long chain isoprenoid alcohol in most chlorophylls esterifying the 17^3 propionic side chain.

cavity in Chls and phytol to become a unit universally associated with the tetrapyrrolic macrocycle. This sparks the further question of how exactly these residues shape pigment properties. The above questions are very relevant because steric factors play a role in virtually all intermolecular interactions which involve Chls. Steric and stereochemical control are crucial for aggregation [22,23], in axial ligand binding to the central metal [24] and in the functioning of photosystem I reactions center [19], and even in interactions with enzymes of the Chl biosynthesis/biodegradation pathway [25]. A coordinatively unsaturated Mg ion chelated by a tetrapyrrole acts as the coordination center and, being a closed shell element, does not electronically perturb the pigment π-electron system [26,27]. Also, this element is light enough not to cause heavy atom effects in the Chl molecule [28,29]. The coordination of nucleophilic amino acid residues (in most cases His) to the central Mg serves to bind Chls to proteins, where pentacoordination (single axial ligand) is most common, while hexacoordination may also occur [30]. The phytyl residue, esterifying the C-17^3 propionic acid side chain (Fig. 1), comprises as much as one third of the molecule in terms of molecular mass [4]. Its presence confers a considerable hydrophobicity to Chls, enhancing their lipophilic character and rendering them practically water-insoluble. In consequence, this residue enhances their aggregative properties and their ability to interact with hydrophobic environment [23,31], serving to position these cofactors within the hydrophobic domains of photosynthetic ensembles [17–20,32]. Regarding the influence of phytyl moiety on the π-electron system of Chls, it is commonly accepted that phytol is neutral and spectroscopically silent. This view is shared in textbooks and other sources, often showing the formulae of Chls with phytyl residue abbreviated to *R*. However, considering the sheer bulk of the phytyl C_{20} chain, it could interfere e.g. with the formation of coordination bonds between Chls and macroligands, i.e. their apoproteins. Indeed, our recent study has shown that the removal of phytyl residue is not without significance to the coordinative and spectroscopic properties of Chl *a*, which indicates the importance of intramolecular steric role of this residue [31].

Bacteriochlorophyll *a* (BChl *a*), which has strong and well separated absorption transitions whose energies show exceptional sensitivity to external factors, has proved a very useful reporter molecule [24,27,33]. To gain a better insight into the intramolecular structural factors that involve the phytyl moiety and the central Mg ion, we prepared a series of bacteriochlorophyll *a* and bacteriopheophytin *a* (free base, BPhe *a*) derivatives, in which phytyl was either removed or substituted for the methyl group, and their ground and excited state properties were compared. Very recently, van der Waals interactions have been suggested to play an important role in the modulation of the electronic properties of Chls [34]. Therefore, a detailed analysis of solvent effects on model pigments has also been done. Our study shows that both Mg and phytyl sterically influence the properties of the macrocycle and have to be considered as important structural determinants of Chls.

2. Materials and methods

2.1. Pigment preparation

BChl *a* was extracted from the wet cells of *Rhodobacter sphaeroides* using methanol and purified by column chromatography on DEAE-Sepharose (Sigma, Germany) following a previously described method [35]. The *R* and *S* diastereoisomers of BChl *a* were separated by HPLC on a semi-preparative silica gel column (Varian) according to a published procedure [25]. For the measurements, only the *R* form of BChl *a* was used.

Bacteriochlorophyllide *a* (BChlide *a*) was obtained by an enzymatic hydrolysis of BChl *a* using the plant enzyme, chlorophyllase, extracted from the leaves of *Fraxinus excelsior*, following a previously described procedure [25,31]. The pigment was purified twice by column chromatography on CM-Sepharose (Pharmacia, Sweden) as described previously [25]. The method yields pure *R* form of the pigment, as confirmed by NMR spectroscopy [25].

Free bases. Bacteriopheophytin *a* (BPhe *a*) was prepared from pure BChl *a* by a short treatment with a small volume of double distilled glacial acetic acid at room temperature, following a previously described method with some modifications [25]. The acid was removed in a stream of nitrogen, the solid residue was dried thoroughly under vacuum and then the product was quickly purified on a short column packed with DEAE-Sepharose equilibrated in acetone. Bacteriopheophorbide *a* (BPheide *a*) was prepared from pure BChlide *a* by a short treatment with glacial acetic acid and the product was purified on CM-Sepharose as above. In both cases, the product showed no detectable level of contamination or degradation.

Methyl esters. Methyl ester of BChlide *a* (Met-BChl *a*) was purified by preparative TLC on silica gel and was a kind gift from Prof. Hugo Scheer (LMU München, Germany). Methyl ester of BPheide *a* (Met-BPheide *a*) was prepared by the transesterification of BPhe *a* in a mixture of methanol and sulfuric acid. The acid was neutralized with solid sodium carbonate and the pigments were extracted using diethyl ether. The product was isolated by column chromatography on DEAE-Sepharose equilibrated in acetone and then purified by isocratic HPLC on reversed-phase silica gel (Varian) using methanol as the eluent. Pure Met-BPheide *a* had a retention time of 17 min.

Because of the high (photo)chemical instability of pigments, all preparative steps were done as quickly as possible and under dim light. Only freshly prepared pigments were used in the experiments and all batches and samples showing any sign of allomerization or oxidation, as checked by TLC and by electronic absorption measurements, were discarded.

2.2. Solvents and reagents

The following solvents were of spectroscopic grade: pyridine (Pyr), dimethylsulfoxide, chloroform, dimethylformamide (DMF), 1-butanol (Merck, Uvasol, Germany). Dichloromethane, tetrahydrofuran (THF), 2-propanol, diethyl ether (DE), acetonitrile, methanol, toluene, *n*-hexane were of HPLC grade (LabScan, Ireland). Triethylamine, acetone (Merck, Germany), ethanol (Roth, Germany) and glacial acetic acid