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Redox potential of chlorophyll *d in vitro*

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Abstract

Chlorophyll (Chl) *d* is a major chlorophyll in a novel oxygenic prokaryote *Acaryochloris marina*. Here we first report the redox potential of Chl *d in vitro*. The oxidation potential of Chl *d* was +0.88 V vs. SHE in acetonitrile; the value was higher than that of Chl *a* (+0.81 V) and lower than that of Chl *b* (+0.94 V). The oxidation potential order, Chl *b* > Chl *d* > Chl *a*, can be explained by inductive effect of substituent groups on the conjugated π-electron system on the macrocycle. Corresponding pheophytins showed the same order; Phe *b* (+1.25 V) > Phe *d* (+1.21 V) > Phe *a* (+1.14 V), but the values were significantly higher than those of Chls, which are rationalized in terms of an electron density decrease in the π-system by the replacement of magnesium with more electronegative hydrogen. Consequently, oxidation potential of Chl *a* was found to be the lowest among Chls and Phes. The results will help us to broaden our views on photosystems in *A. marina*.

Keywords: *Acaryochloris marina*; Chlorophyll *a*; Chlorophyll *d*; Photosynthesis; Redox potential

1. Introduction

In oxygenic photosynthetic organisms, chlorophyll (Chl) *a* (Fig. 1) is the major pigment that plays the key role in the electron transfer in both photosystem (PS) I and PS II reaction centers (RCs). In 1996, however, a Chl *d*-dominated cyanobacteria *Acaryochloris marina* was discovered [1], and much research on the pigment composition of this unique organism has been performed. In both PS I and PS II the surrounding antenna pigment is Chl *d* (Fig. 1).

In the case of PS I of *A. marina*, Chl *d*-type pigments also function as component(s) of the primary electron donor P740. P740 was initially proposed to be a homodimer of Chl *d* [2], later a homodimer of Chl *d*′ [3] and finally a Chl *d*/*d*′ heterodimer (Fig. 2A) [4–6], just like the Chl *a*/*a*′ for P700 in cyanobacteria and higher plants (Fig. 2B) [7,8]. The primary electron acceptor, A_0, in PS I of *A. marina* is not Chl *d* but was found to be Chl *a* (Fig. 2A) [9]. The midpoint potential, E_m, for P740 was reported to be +335 mV [2], which is significantly negative of ca. +470 mV for P700 in other cyanobacteria [10–14]. Because of this, Chl *d* has been supposed to possess an oxidation potential lower than that of Chl *a*. The wavelength of the Chl *d* Q_Y-band, longer than that of Chl *a*, appears also to support the view that Chl *d* is oxidized more easily than Chl *a*. Such a view, however, still remains speculative. To elucidate the *in vivo* role of Chl *d*, it is of much importance to clarify its redox potential *in vitro* by electrochemical measurements.

In the case of PS II of *A. marina* whether Chl *d* acts as the primary electron donor in PS II is a matter of controversy; it has been suggested that the PS II primary donor is a Chl *d* dimer [13,15,16], a Chl *a* dimer [3–5,17–21], or a Chl *a*/*d* heterodimer (Fig. 2A) [6], while the identity of the primary electron acceptor of PS II in *A. marina* has been well defined as not Phe *d* but Phe *a* (Fig. 2A) [3–6,19,22], like other cyanobacteria (Fig. 2B). Our heterodimer model of Chl *a*/*d* was quite recently supported in part by the difference spectra of the PS II RC of *A. marina* in the blue light region (A. Telfer, personal communication).

Abbreviations: Chl, chlorophyll; CV, cyclic voltammetry; DMF, dimethyl formamide; Phe, pheophytin; PS, photosystem; RC, reaction center; SWV, square wave voltammetry (SWV)

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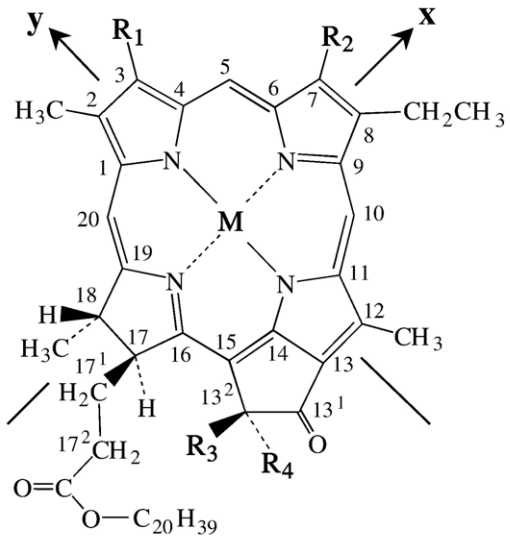

	M	R_1	R_2	R_3	R_4
Chl *a*	Mg	$CH=CH_2$	CH_3	H	$COOCH_3$
Chl *a'*	Mg	$CH=CH_2$	CH_3	$COOCH_3$	H
Phe *a*	2H	$CH=CH_2$	CH_3	H	$COOCH_3$
Chl *b*	Mg	$CH=CH_2$	CHO	H	$COOCH_3$
Phe *b*	2H	$CH=CH_2$	CHO	H	$COOCH_3$
Chl *d*	Mg	CHO	CH_3	H	$COOCH_3$
Chl *d'*	Mg	CHO	CH_3	$COOCH_3$	H
Phe *d*	2H	CHO	CH_3	H	$COOCH_3$

Fig. 1. Molecular structure and carbon numbering of chlorophylls, according to the IUPAC numbering system.

It is well known that six molecules of Chl *a* are present as well as two Phe *a* molecules in the D1/D2/cyt b_{559} complex of Chl *a*-based organisms [23,24]: two corresponding to P680, two corresponding to the accessory, and two peripheral Chls *a* designated Chl a_Z (Fig. 2B). On the basis of pigment analyses, the accessory Chl *a* and Chl a_Z in Chl *a*-type oxygenic organisms are all replaced with Chl *d* in *A. marina* (Fig. 2A) [4–6,19].

Recently, it has been shown that the primary charge separation in PS II is initiated from the excitation of accessory Chl *a*, AccChl *a*, of D1-branch in Chl *a*-type oxygenic organism: P-Acc*-Phe → P-Acc$^+$-Phe$^-$ → P$^+$-Acc-Phe$^-$ [25,26]. Therefore, the replacement of AccChl *a* with AccChl *d* is fundamentally necessary in PS II of *A. marina* (Fig. 2A) [6], because, if Acc was Chl *a*, energy transfer from antenna Chl *d* to AccChl *a* would be difficult because of the extremely uphill process. The primary charge separation initiated from AccChl *d* is hence most likely in the PS II RC of *A. marina* also, after energy transfer from antenna Chl *d* to AccChl *d* (Fig. 2A).

The chemical identity of the primary electron donor of the PS II RC in *A. marina* still remains to be resolved, as mentioned above. This uncertainty is mainly due to the difficulties associated with preparing the photoactive PS II core complexes, also due to the absence of experimental information about the oxidation potential of Chl *d* that is needed to be compared with that of Chl *a*, probably because Chl *d* had not been regarded as being present in any photosynthetic organisms until 1996.

Here we present the redox potentials of Chl *d* in acetonitrile and dimethyl formamide (DMF), comparing them with those of Chls *a, b*, Phes *a, b*, and *d*. In acetonitrile, the first oxidation potential, E^1_{ox}, of Chl *d* (+0.88 V vs. SHE) was more positive than that of Chl *a* (+0.81 V) and more negative than that of Chl *b* (+0.94 V). Corresponding pheophytins showed much higher values; Phe *a* (+1.14 V), Phe *b* (+1.25 V) and Phe *d* (+1.21 V). The E^1_{ox} value of Chl *a* was hence found to be the lowest among Chls and Phes. Note that oxygenic photosynthesis uses Chl *a* for P680 (Fig. 2B), although significantly high oxidation power is needed for water oxidation. The results obtained here will enlarge ones views on photosynthetic mechanisms of *A. marina*.

2. Materials and methods

2.1. Pigment preparation

Chls *a, b* and *d* were extracted and purified as described elsewhere [3,6,27,28]. Briefly, Chls *a* and *b* were extracted from parsley (*Petroselinum crispum*) and Chl *d* from *A. marina* MBIC11017, which were then purified by normal-phase HPLC. Phes *a, b* and *d* were prepared by pheophytinization of Chls *a, b* and *d* respectively, as described before [27].

2.2. Materials purification

Acetonitrile and dimethyl formamide (DMF) (both from Aldrich, anhydrous grade: water < 50 ppm) were deoxidized and dried before use. The solvent was subjected to freeze–pump–thaw cycles at least three times under about 10^{-5} Torr. Under nitrogen atmosphere, the deoxidized solvent was then dried for 24 h with the activated molecular sieves (4A 1/16, Wako), pretreated in vacuo at 473 K over 24 h. Tetra-*n*-butylammonium perchlorate (Bu_4NClO_4, TBAP) (Aldrich, Electrochemical grade: >99.0%) was used as the supporting electrolyte, which was recrystallized from methanol solution and was then dried in vacuo at 333 K over 24 h.

2.3. Electrochemical measurements

The redox potentials of chlorophylls were measured by both cyclic voltammetry (CV) and square wave voltammetry (SWV). Signal-to-noise ratio of SWV is generally better than that of CV, especially for measuring redox couples at such low concentration (ca. 0.5 mM) as the present case [29,30]. Both measurements were performed with an ALS model 620A electrochemical analyzer. Scan speed for CV was 0.1 V/s. Parameters for SWV were V_{step}=5.0 mV, AC signal (V_{pulse})=25 mV, and p–p at 8 Hz. The measurements were carried out in an air-tight electrochemical cell containing small compartment for a sample solution equipped with a glass filter that can be degassed and filled with dry N_2. A platinum disk electrode with 1.6 mm in diameter (outer diameter: 3 mm) was used as the working electrode, and a platinum black wire fabricated in the small compartment (internal diameter: 8.9 mm) as the counter electrode. An Ag/AgCL electrode, chosen for good reproducibility despite possibility of junction potential, was connected through a salt bridge to the outer electrolytic solution of the small components.

The ferrocene–ferrocinium redox couple was used to estimate junction potential changes upon changing solvents. After each measurement, the redox potentials of the ferrocene–ferrocinium were measured as +0.45 V and +0.53 V vs. Ag/AgCL in acetonitrile and DMF, respectively.

3. Results

Typical cyclic voltammogram (CV) and square wave voltammogram (SWV) for Chl *d* in acetonitrile are illustrated