Available online at www.sciencedirect.com

ScienceDirect

Journal of Photochemistry and Photobiology B: Biology 87 (2007) 154–162

Journal of Photochemistry and Photobiology B:Biology

www.elsevier.com/locate/jphotobiol

Photophysical and photochemical studies of chlorophyll *a* and cobalt(II)tetraphenylporphyrin in poly(L-glutamate)-decylammonium ion complex

Pascaline Ngweniform, Yoshihumi Kusumoto *, Miyuki Ikeda, Shouichi Somekawa, Bashir Ahmmad, Junichi Kurawaki, Katumitu Hayakawa

Department of Chemistry and Bioscience, Faculty of Science, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890-0065, Japan

Received 4 January 2007; received in revised form 20 March 2007; accepted 21 March 2007

Abstract

Spectroscopic studies were carried out on chlorophyll *a* and cobalt(II)tetraphenylporphyrin solubilized in a poly(L-glutamate) (Poly(Glu))-decylammonium chloride (DeAC) complex system, in the presence of methylviologen (MV^{2+}). The cooperative binding occurred between the anionic Poly(Glu) and the cationic DeAC, leading to the formation of micelle-like hydrophobic clusters of DeAC and also the change in conformation of the Poly(Glu) from the random coil to the α-helix. All of the absorption spectra, the fluorescence quantum yields and the fluorescence lifetimes indicated the existence of equilibrium between the aggregated biofunctional molecules in the bulk phase and the monomeric species in the complex phase of the Poly(Glu)–DeAC solution. The fluorescence quenching of the biofunctional molecules by methylviologen indicates that the conformation-dependent electron transfer occurs in the complex phase.

Keywords: Chlorophyll *a*; Cobalt(II)tetraphenylporphyrin; Poly(L-glutamate); Conformation change; Cooperative binding; Electron transfer

1. Introduction

Porphyrins belong to a class of cyclic tetrapyrrole compounds that play a vital role in nature. This is exemplified by the layered thylakoid membranes of chloroplast which contain chlorophyll molecules bound to proteins to form chlorophyll–protein complexes. Here, chlorophyll–protein complexes play a central role in the stabilization and physiological functions of the chloroplast [1–5]. In addition, the biological importance of vitamin B_{12} has prompted many investigations on the catalytic efficiency of square planar cobalt porphyrin species [6,7]. The role of porphyrin is not limited to biological systems, but includes various fields of science (physics, chemistry, geology and medicine). Porphyrins generally have intense π–π^* absorption bands in the visible region [8]. This unique property of porphyrins is exploited in the construction of artificial photosynthetic models and in systems that convert solar light to chemical energy [1,9]. The conversion of solar energy to chemical energy generally needs porphyrins as well as suitable catalysts. However, biofunctional molecules such as porphyrins are insoluble in water or aggregate in aqueous solutions [10,11]. A number of techniques have been devised in order to solubilize insoluble porphyrins and also to enhance electron- and/or energy-transfer reactions between porphyrins and acceptors [10,12]. Among these techniques is the use of polymer–surfactant complexes to solubilize dye or porphyrin molecules [12–14]. This solubilization takes place as a result of the formation of hydrophobic domains in the polymer–surfactant complex, in which the dye or porphyrin can reside [12]. The solubilization of chlorophyll *a* (Chl) into copolypeptide–surfactant complexes has been reported [13–16]. This solubilization leads to the

---

* Corresponding author. Tel./fax: +81 99 285 8914.
E-mail address: Kusumoto@sci.kagoshima-u.ac.jp (Y. Kusumoto).

1011-1344/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotobiol.2007.03.007

concentration of Chl in the copolypeptide–surfactant complex. We also reported previously the photoinduced hydrogen production using cobalt(II)tetraphenylporphyrin (CoTPP) and Chl in a poly(L-glutamate) (Poly(Glu))-decylammonium chloride (DeAC) complex [17,18]. However, the detailed spectroscopic characteristics of the systems were not given in the foregoing studies. In connection with polypeptide-surfactant complexes, it is of interest to investigate in detail the behavior of Chl and CoTPP in the Poly(Glu)–DeAC complexes and also the possible photochemical reaction that may occur in these complexes.

In this paper, we report our observations on the spectroscopic characterization of chlorophyll *a* and cobalt(II)-tetraphenylporphyrin in a complex system formed between a homopolypeptide, poly(L-glutamate) (Poly(Glu)) which carries a negative charge and a cationic decylammonium (DeAC) chloride surfactant. The cooperative binding occurred between the anionic Poly(Glu) and the cationic DeAC, leading to the formation of micelle-like hydrophobic clusters of DeAC and also a change in the conformation of the Poly(Glu) from the random coil to the α-helix. The absorption and fluorescence measurements indicated the existence of equilibrium between the aggregate biofunctional molecules in the bulk phase and the monomeric species in the complex phase of the Poly(Glu)–DeAC system. The fluorescence quenching of the biofunctional molecules by methylviologen (MV^{2+}) indicated that the conformation-dependent electron transfer occurred in the Poly(Glu)–DeAC complex system.

2. Experimental

2.1. General

Chlorophyll *a* (Chl) from Chlorella, cobalt(II)tetraphenylporphyrin (CoTPP) (Wako, Osaka, Japan, 99%) and methylviologen (MV^{2+}) (Sigma, Kawasaki, Japan, 99%) were used as received. Poly(L-glutamate) (Poly(Glu)) (Peptide Institute, Minoh, Osaka, Japan, molecular weight = 8000) was dissolved in 0.06 M (1 M = 1 mol dm^{-3}) NaOH and dialyzed against redistilled water. The concentration of the Poly(Glu) was determined by colloid titration with standard potassium poly(vinyl sulfate) solution [19]. Decylammonium chloride (DeAC) was prepared by titration of decylamine (Nacalai Tesque, Kyoto, Japan, 99%) with hydrochloric acid [20,21]. The resultant salt was recrystallized twice from ethanol and washed with ethyl ether. Benzene (Wako, Osaka, Japan, 99.8%), ethanol (Wako, Osaka, Japan, 99.5%), 2-methyl-2-propanol (Wako, Osaka, Japan, 99%), acetone (Dotite, Kumamoto, Japan, 99%) were used as received. Laboratory deionized water was distilled twice and used throughout the experiment.

2.2. Preparation of the samples for spectroscopic measurements

Stock solutions of Chl and CoTPP contained in the Poly(Glu)–DeAC complexes and also in the presence of MV^{2+}(in some cases) were prepared to give concentrations of 1×10^{-4} M and 1×10^{-5} M for the Poly(Glu) and Chl or CoTPP, respectively, through out the experiment. An aliquot of CoTPP (in benzene) introduced in a 10 mL volumetric flask was dried with nitrogen gas and redissolved in 100 μL of acetone, whereas that of Chl (in ether) was added to 100 μL 2-methyl-2-propanol in a similar flask and the ether was removed by drying with nitrogen gas. To each of the sample solution, Poly(Glu), DeAC and MV^{2+} (fluorescence quenching samples only) were added and the flask was filled to the marked line with redistilled water. The resultant mixture was sonicated for 10 min. The pH of solutions was not controlled but was found to be about 6.5. It is well known that the pheophytinization and allomerization of chlorophylls occur in acidic and basic homogeneous solutions, respectively [22]. Here it should be noted that Chl was stable and did not undergo the pheophytinization or allomerization.

2.3. Spectroscopic measurements

Circular dichroism (CD) spectra were measured with a JASCO (Hachioji, Tokyo, Japan) J-720 spectropolarimeter and digitized data were transferred to a microcomputer and processed. Absorption and Fourier transformed infrared (FT-IR) spectra were recorded on a Shimadzu (Kyoto, Japan) MPS-2000 and a Perkin–Elmer (Yokohama, Japan) FT-IR spectrophotometer (Spectrum one–B), respectively. Steady-state fluorescence spectra were recorded on a Shimadzu (Kyoto, Japan) RF-5000 spectrofluorophotometer (excitation wavelength: 420 nm for CoTPP and 435 nm for Chl). Relative fluorescence quantum yields of CoTPP were determined indirectly using Chl in ethanol (quantum yield = 0.3) as the reference [23,24]. Fluorescence lifetime (τ) measurements were carried out with a Horiba (Kyoto, Japan) NAES 1100 time-resolved spectrofluorometer that employs a time-correlated single photon counting technique. All measurements were carried out at about 25 °C.

3. Results and discussion

3.1. Conformation of Poly(Glu) in aqueous DeAC

Fig. 1a shows the CD spectra of Poly(Glu) in the 190–250 nm region, in the presence of increasing concentrations of DeAC. In the absence of DeAC (spectrum A of Fig. 1a), a small positive band at 217 nm and a strong negative band around 196 nm which are typical of random coil Poly(Glu) are observed. With increasing [DeAC], the CD spectra adopt a double minimum at 222 nm and 209 nm (spectra E and F of Fig. 1a) which is typical of α-helix. The [DeAC] dependence of the molar ellipticities at 222 nm ($[\theta]_{222}$) is shown in Fig. 1b. A decrease in $[\theta]_{222}$ can be observed as the concentration of DeAC increases. This observation indicates the electrostatic attraction between the anionic Poly(Glu) and cationic DeAC, resulting in the change in the conformation of the Poly(Glu) from the random coil