

Note

ESR studies on distortion in the macrocyclic ring of copper(II) chlorophylls

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Abstract

The influence of distortion in the porphine ring on the electronic structure of the central metal ion was studied by means of the ESR spectra of copper(II) chlorophylls (Cu-Chls). A linear correlation between ESR parameters, i.e. g and A values, indicated that the electronic structure of the central copper(II) ion in Cu-Chls is different from that in compounds having the regular porphine skeleton. The weakened Cu–N bond in Cu-Chls was evidenced by a decrease in the bonding coefficient α^2 . These observations on the ESR parameters of Cu-Chls were explained by a partially saturated and distorted macrocyclic structure.

Keywords: ESR study; Copper complexes; Chlorophyll complexes

1. Introduction

The physicochemical property and function of porphyrins have been extensively investigated because of their importance in many biological reactions. So far, most reports have focused on the electronic structure of the porphine skeleton composed of regular and relatively planar tetrapyrrole ligands with D_{4h} symmetry. In recent years non-planar distorted porphyrins with lower symmetry, such as metallochlorins and metallobacteriochlorins in which one or two pyrrole rings are partially saturated, have been studied from the view point of photosynthetic reactions and new types of enzymes, i.e. green heme [1]. A number of papers has been published on the relationship between the distortion of macrocycles and their physicochemical properties [1–3]. The presence of a saturated pyrrole ring and a fused cyclopentanone ring (ring V) in M-Chls should lead to more distortion as compared to compounds having a porphine skeleton [4,5]. In this paper, we have prepared five Cu-Chls (Fig. 1): Cu(II) chlorophyll *a* (Cu-Chl *a*), Cu(II) chlorophyll *b* (Cu-Chl *b*), Cu(II) methylpheophorbide *a* (Cu-MP *a*), Cu(II) chlorin e_6 (Cu-Chl e_6), Cu(II) pyromethylpheophorbide *a* (Cu-PMP *a*), and also synthesized three copper(II) porphyrins for comparison purposes: Cu(II) tetraphenyl-

porphyrin (Cu-TPP), Cu(II) hematoporphyrin (Cu-HP), Cu(II) protoporphyrin IX dimethyl ester (Cu-PP IX). The influence of the distortion in the chlorin ring on the electronic structures of metalloporphyrins has been discussed in terms of the g and A values obtained from the electron spin resonance (ESR).

2. Experimental

2.1. Materials

Organic solvents (reagent grade) were purchased from Junsei Chemical Co. Pheophytin *a* and *b* were obtained from spinach chlorophylls by the literature method [6]. MP *a*, trisodium salt of chlorin e_6 and PMP *a* were purchased from Tama Biochemical Co. TPP and PP IX were purchased from Aldrich Chem. Co. and HP was from Tokyo Kasei Co. Cu-Chl *a* and *b* were obtained from pheophytins by the literature method [6].

Cu-MP *a* was prepared as follows. To 2.0 mg of MP *a* in 10 cm³ of acetone were added 3.9 mg of copper(II) chloride dihydrate (Wako Pure Chem. Ind.) and the mixture was stirred at 5 °C for 1 h in the presence of mercury(II) chloride (7.9 mg) as a catalyst. The reaction mixture was evaporated to dryness, and the residue

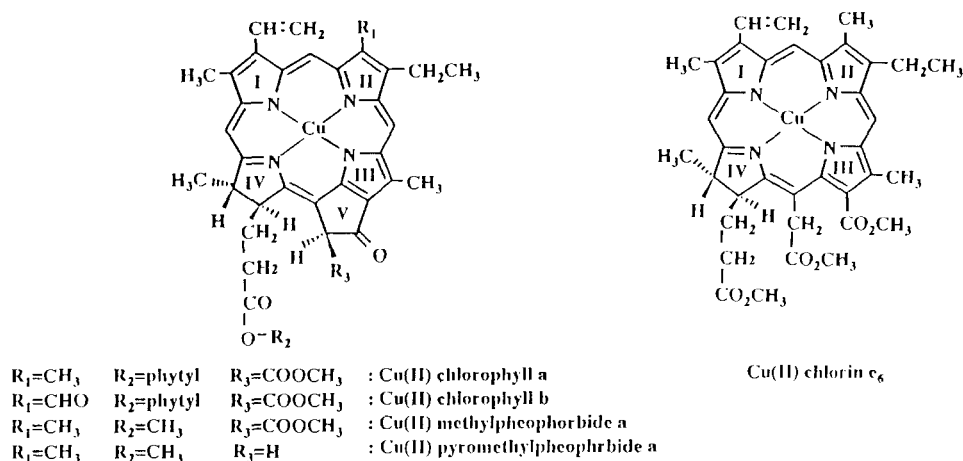


Fig. 1. Structures of Cu-Chls.

was extracted with chloroform, washed three times with water, followed by evaporation to dryness to yield the crude product. Purification by preparative HPLC gave Cu-MP *a* [6]. All operations were carried out under a dark argon atmosphere (yield 80%). *Anal.* Found: C, 63.61; H, 5.75; N, 8.45. Calc. for $C_{36}H_{36}N_4O_5Cu$: C, 63.83; H, 5.52; N, 8.27%. Vis. (acetone): 650, 601, 548, 503, 421 and 399 nm. Capacity factor k' on HPLC: 1.49 (methanol).

Cu-Chl e_6 was prepared as follows. To 2.0 mg of Chl e_6 in 10 cm³ of acetic acid were added 4.2 mg of copper(II) acetate monohydrate (Wako Pure Chem. Ind.) and the mixture was stirred at room temperature for 10 min (yield 60%). *Anal.* Found: C, 57.81; H, 6.19; N, 7.34. Calc. for $C_{34}H_{34}N_4O_6Cu \cdot 3H_2O$: C, 57.32; H, 5.67; N, 7.87%. Vis. (acetone): 628, 498 and 405 nm. k' : 2.27 (methanol/water/acetic acid 97/3/1 (vol./vol./vol.)).

Cu-PMP *a* was prepared by the same procedure as that used for Cu-MP *a* (yield 90%). *Anal.* Found: C, 66.67; H, 5.79; N, 9.10. Calc. for $C_{34}H_{34}N_4O_3Cu$: C, 66.94; H, 5.63; N, 9.19%. Vis. (acetone): 649, 601, 547, 503, 421 and 399 nm. k' : 1.56 (acetone/methanol 50/50 (vol./vol.)).

Copper(II) porphyrins such as Cu-TPP, Cu-HP and Cu-PP IX were prepared by the literature methods [7–9].

2.2. Instruments

Absorption spectra were recorded in acetone on a Hitachi U-2000 UV-Vis spectrometer. The HPLC instrument consisted of a Yanaco L-4000 W pump, a Rheodyne 7120 injector with a 100 mm³ injection loop, a Shodex M-315 variable wavelength detector, and a GL Science Inertsil ODS column (150 × 4.6 mm i.d., 5 μm spherical octadecyl silica). ESR spectra in chloroform/pyridine (99/1, vol./vol.) glass were recorded at -150 °C on a JEOL RE-3X spectrometer equipped

with an Adbantest R-5372 microwave counter, an ESDVT2 digital variable-temperature unit and a JEOL ESPRIT-330 ESR data system. Pyridine was added to the solution to segregate the dimeric Cu-Chls from those of the monomeric state, although copper(II) porphyrins form a five-coordinate complex with pyridine [10]. The ESR parameters of the five-coordinate copper(II) complexes g_{\perp} and A_{\perp} values, were estimated from the g_0 and A_0 at 25 °C.

3. Results and discussion

An ESR spectrum of Cu-Chl *a* is shown in Fig. 2, where the g values are 2.203 and 2.08 for parallel and perpendicular tensors, respectively. Hyperfine couplings with the ⁶³Cu nucleus and superhyperfine couplings with ¹⁴N nuclei are observed. These spectral features are similar to those of copper(II) porphyrins and their derivatives [11,12]. All other Cu-Chls gave much the same type of ESR spectra as that of Cu-Chl *a*.

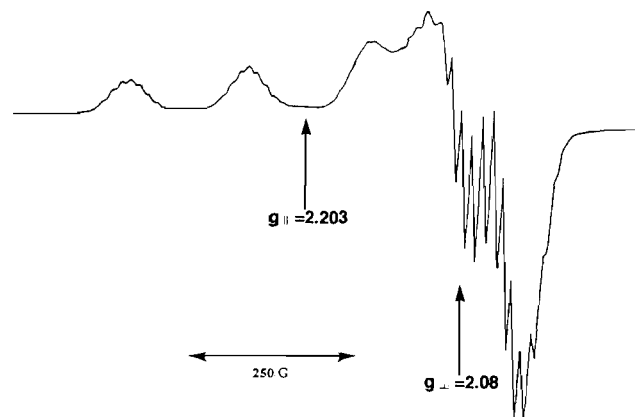


Fig. 2. ESR spectrum of Cu-Chl *a* in $CHCl_3$ /pyridine (99/1 vol./vol.) glass at -150 °C.

The ESR parameters obtained are compared with those of the porphyrins to clarify the differences in the macrocyclic structure. The ESR spectra of the Cu-Chls and copper(II) porphyrins have already been reported¹, but the ESR parameters could not be compared with each other because of the differences in experimental conditions. The diagrams shown in Figs. 3 and 4 are plots of g values versus A values, i.e. hyperfine coupling constants with the ⁶³Cu nucleus. The g and A values of the copper(II) complexes are mainly influenced by the energy separation between the d orbitals, E , and

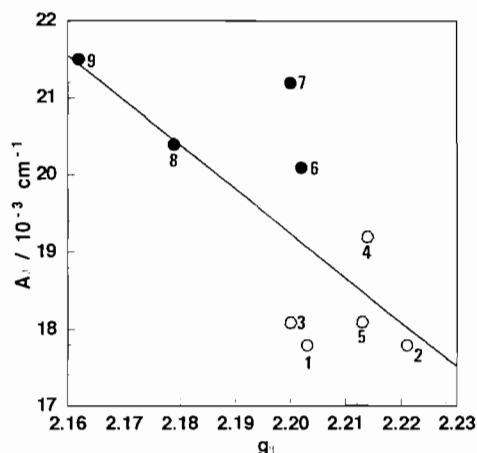


Fig. 3. Correlation diagram of g_{\parallel} and A_{\parallel} for Cu-Chls (O) and copper(II) porphyrins (●): 1, Cu-Chl *a*; 2, Cu-Chl *b*; 3, Cu-MP *a*; 4, Cu-Chl *e*₆; 5, Cu-PMP *a*; 6, Cu-TPP; 7, Cu-HP; 8, Cu-PP IX; 9, Cu(II) phthalocyanine. ESR parameters for compound 9 are from Ref. [7].

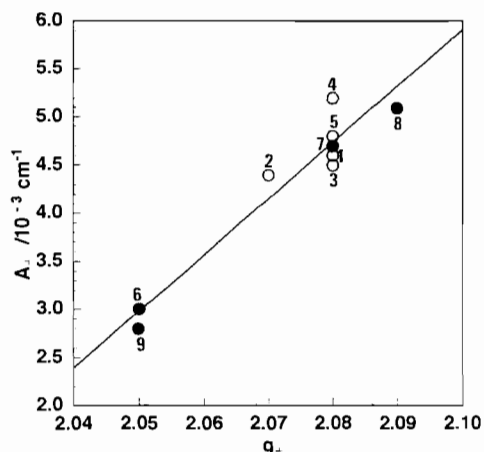


Fig. 4. Correlation diagram of g_{\perp} and A_{\perp} for Cu-Chls (O) and Cu(II) porphyrins (●). The numberings of the compounds are the same as in Fig. 3.

¹Cu-PMP *a*: $g_{\perp} = 2.0660$ in 1-chloro-naphthalene and $g_{\perp} = 2.0776$ in pyridine. Cu-Chl *e*₆: $g_{\perp} = 2.0681$ in 1-chloro-naphthalene and $g_{\perp} = 2.0835$ in pyridine [10]. Cu-TPP: $g_{\parallel} = 2.187$, $g_{\perp} = 2.045$, $A_{\parallel} = 20.2 \times 10^{-3}$ and $A_{\perp} = 3.3 \times 10^{-3}$ cm⁻¹ in H₂-TPP. Cu-PP IX: $g_{\parallel} = 2.192$, $g_{\perp} = 2.054$, $A_{\parallel} = 20.3 \times 10^{-3}$ and $A_{\perp} = 4.3 \times 10^{-3}$ cm⁻¹ in H₂-PP IX [11].

the electron density of the copper(II) nucleus, respectively. An increase in g values indicates a lowering of E and a decrease in A values means a lowering of the electron density on the copper nucleus. In blue copper proteins and their model compounds, it is known that a linear correlation is observed between g_{\parallel} and A_{\parallel} (an increase in g_{\parallel} with a decrease in A_{\parallel}) with a distortion from square planar to tetrahedral structure [13–15]. A similar linear correlation is also observed in the present case of Cu-Chls with a macrocyclic distortion. The correlation between g_{\parallel} and A_{\parallel} values is with a negative slope, while that between g_{\perp} and A_{\perp} is with a positive one. It is noteworthy that Cu-Chls give higher g_{\parallel} and lower A_{\parallel} values compared with copper(II) porphyrins.

The ESR parameters of paramagnetic metallocomplexes provide valuable information about their electronic structure. According to the procedure reported by Lin [11] and Revikant et al. [16], the energy separations between the d orbitals of the copper(II) atom (E), the bonding coefficient of the b_1 molecular orbital α^2 (variable between 0 and 1) and the isotropic part of the hyperfine interaction K were calculated from the ESR parameters obtained in the present experiments. The ESR parameters for axially coordinated copper(II) complexes were calculated by the same procedure used for the four-coordinated species [12].

$$g_{\parallel} = g_e + 8\zeta\alpha^2\delta^2/Ed_{x^2-y^2} \quad (1)$$

$$g_{\perp} = g_e + 2\zeta\alpha^2\gamma^2/(Ed_{x^2-y^2} - Ed_{xz,yz}) \quad (2)$$

$$A_{\parallel} = -K + P[-(4/7)\alpha^2 + (g_{\parallel} + g_e) + (3/7)(g_{\perp} - g_e)] \quad (3)$$

$$A_{\perp} = -K + P[(2/7)\alpha^2 + (11/14)(g_{\perp} - g_e)] \quad (4)$$

In these equations, g_e is the free electron g value, ζ is the spin-orbit coupling parameter and $P = g_e\beta_e g_n \beta_n \langle 1/r^3 \rangle$, in which g_n is the g factor of the copper nucleus and β_n is the nuclear magneton; both latter parameters are for the free Cu⁺ ion ($P = 0.0354$ cm⁻¹ and $\zeta = 830$ cm⁻¹). Energy separations, $Ed_{x^2-y^2}$ and $Ed_{xz,yz}$, are defined as follows; $Ed_{x^2-y^2}$ is the energy separation between $d_{x^2-y^2}$ and d_{xy} and $Ed_{xz,yz}$ is between $d_{xz,yz}$ and d_{xy} . γ and δ are bonding coefficients of the e and b_2 molecular orbitals and they were assumed to be equal to 1.

The effect of the macrocyclic skeleton on the electronic structure of the Cu(II) ion is explained on the basis of E and α^2 . In Cu-Chls, $Ed_{x^2-y^2}$ is calculated to be 20 000–21 500 cm⁻¹, whereas $Ed_{x^2-y^2}$ is 25 800 cm⁻¹ for Cu-TPP and 24 900 cm⁻¹ for Cu-HP. On the other hand, $Ed_{xz,yz}$ is 4000–10 000 cm⁻¹ in all Cu-Chls and porphyrins. The bonding coefficient α^2 is 0.78 for Cu-TPP and 0.74 for Cu-HP, while it is 0.63–0.68 for Cu-Chls. A significant decrease in α^2 gives rise to a lowering in the symmetry of the compounds. A lowering in the

energy level of the antibonding molecular orbital $d_{x^2-y^2}$ and in α^2 means that the Cu–N bond in Cu-Chls is weaker than in copper(II) porphyrins.

The significant difference in the ESR parameters α^2 and E of Cu-Chls and copper(II) porphyrins is interpreted as follows. First, the average bond length between the central metal and macrocyclic nitrogen for M-Chls is the longest in porphine and chlorin compounds. Sange and Smith [17] have indicated that the average Ni–N distance in Ni-PMP *a* is 1.933 Å while it is 1.928 Å in Ni-TPP and 1.929 Å in Ni-OEP. This elongation of the M–N distance characteristic of M-Chls gives rise to a decrease in electron donation from the macrocyclic nitrogen to the central metal ion. Second, the ring IV of the chlorin skeleton is saturated. This saturation of the pyrrole ring leads to a lower electron density on the nitrogen atoms of Chls [18] and hence a reduced electron donation to the central metal ion. As a result both structural and electronic characteristics are reflected strongly in the ESR parameters of Cu-Chls.

Among the nine copper(II) complexes investigated, the A values change significantly, while the g values do not. The peripheral substituents on the macrocyclic chlorin ring affect strongly the A value. The A values of Cu-Chl *a*, Cu-Chl *b*, Cu-MP *a* and Cu-PMP *a* are very similar to each other, indicating that the phytol group or the alkyl chain on ring IV of Chl *a* and *b*, and the methoxycarbonyl group on ring V hardly influence the electronic structure of the central copper(II) ion. An electron-withdrawing formyl group in Cu-Chl *b* had no effect on the ESR parameters, although a significant shift in the electronic spectra of porphyrinic macrocycles is reported [19]. The A_{\parallel} value of Cu-Chl e_6 is larger than that of Cu-MP *a*. The ESR parameter α^2 of Cu-Chl e_6 is the highest in the five Cu-Chls (0.68). The electronic structure of Cu-Chl e_6 is intermediate

between Cu-Chls and copper(II) porphyrins from the view point of their ESR parameters because Cu-Chl e_6 has the reduced ring IV but no ring V.

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