In vitro complexes of copper and zinc with chlorophyll

JELENA PETROVIĆ, GORAN NIKOLIĆ[#] and DEJAN MARKOVIĆ[*#]

Faculty of Technology, 16000 Leskovac, Serbia and Montengro
(e-mail: markovic57@info-net.co.yu)

Abstract: Complexes of copper and zinc with chlorophyll, the major photosynthesis pigment, were studied by Vis, FTIR and fluorescence spectroscopy. Two types of complexes were recognized. While copper replaces the central magnesium atom of chlorophyll to form a "central" Cu–Chl complex, this was not proposed in the case of zinc. Instead, the zinc-mediated formation of a 6-membered chelate cycle fused at the periphery of the chlorophyll structure is proposed. The latter event could be ascribed to allomerization reactions of chlorophyll.

Keywords: chlorophyll, copper, zinc, complexes; Vis, FTIR and fluorescence spectroscopy.

INTRODUCTION

Chlorophyll (Chl), a major photosynthesis pigment, in chemical terms is a porphyrin derivative (a cyclic tetrapyrrole with an isocyclic cyclopentanone ring, fused to a pyrrole ring between the C-13 and C-15 positions), where the central metal Mg-atom coordinates with the four symmetric pyrrole rings (Fig. 1). Its major function in photosynthesis is related to light collection and light conversion processes. Significant progress has been made in the field of the *in vitro* properties of Chl and this has contributed to a better understanding of the role of Chl in photosynthesis on the molecular level.[1,2]

Plants easily absorb many toxic heavy metals.[3,4] Once absorbed, they penetrate the plant tissues (including the leaves) and, in higher concentrations, they may inhibit photosynthesis.[5–7] Heavy metals can replace the labile bonded central magnesium atom (Mg) of chlorophyll, to form heavy metal complexes (Chl–HMS).[8–10] The Chl–HMS complexes may cause an impairment of the photosynthetic function and this, as a final consequence, may lead to death of the plant.[11] Detailed consequences of this substitution for higher plants and green algae have been discussed by Küpper.[8,12]
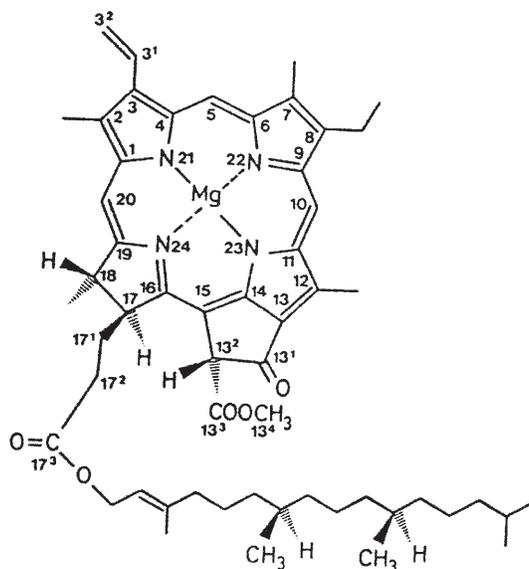

Fig. 1. Structure of chlorophyll *a*, with numbered atoms.

The general reactions of Chl with heavy metals *in vitro*[11] and *in vivo*,[13,14] have been already reported. The Chl–HMS complexes can be prepared by heating Chl with metal salts in acids or organic solvents.[15] Also, acidification of Chl solutions with hydrochloric acid followed by the addition of a solution of a heavy metal leads to formation of Chl–HMS.[7,8,15–17] Küpper performed experiments *in vitro* by adding solutions of heavy metals to 96 % ethanolic grass extracts.[8]

Chl–HMSs show different spectral behavior compared to Chl itself.[8,16–20] This work deals with possible formation of Chl–HMS complexes between isolated Chl-fractions and two chosen heavy metals: zinc (Zn) and copper (Cu). The complexes were then examined by Vis, FTIR and fluorescence spectroscopy.

EXPERIMENTAL

The formation of Chl–HMS may occur under low light ("shade reaction") and under high light conditions ("sun reaction").[12] Since only just a minority of antenna chlorophylls (*in vivo*) is accessible to Chl–HMS formation under high light conditions,[12] the experimental procedure described below was performed under shade conditions as much as possible.

Extraction of plant pigments

Plant pigments were extracted from spinach leaves (*Spinacia oleracea*) using the method proposed by Svec.[21,22] Fresh spinach leaves free of midribs (0.030 kg) were dropped into boiling water (to prevent enzymes-mediated Chl-oxidation) which was quickly replaced after 1–2 min with cold water. After drying, the leaves were separated, placed in a mixture of methanol (30 cm^3) and 40–75 ºC petroleum ether (15 cm^3); the mixture was occasionally agitated. Methanol removes water from the plant material and the petroleum ether takes up the pigments before undergoing secondary reactions. The deep-green extract was decanted through a cotton pad. The leaves were reextracted twice with a mixture containing methanol and 40–75 ºC petroleum ether (2:1, v/v). The extracts were diluted with 60 cm^3 of saturated NaCl solution, to maintain most of the pigments in the petroleum

ether layer. The remaining aqueous methanol layer was reextracted with 18 cm^3 of mixture containing 40–75 ºC petroleum ether and diethyl ether (1:1, v/v), ensuring the solubility of the chlorophyll in the organic phase. The successive extracts were treated in the same manner. The final extract was a mixture of pigments containing a large amount of various Chl-forms (predominantly Chlorophyll a – Chl$a$) as well as accessory pigments, carotenoids (carotenes and xanthophylls).

Isolation of chlorophylls

The Chl-fractions were isolated using a modified procedure of Svec[22] and Backmann,[23] *i.e.*, column chromatography with silica gel (silica gel 60, Merck, 0.063–0.200 mm) as the adsorbent and benzene/acetone mixtures as the eluents. The benzene/acetone ratio was changed from the initial 1:0 to the final 1:0.6, to permit an easier elution of the polar fractions. β-Carotene appears first, followed by the chlorophylls and xanthophylls fractions. The collected Chl-fractions were then analyzed by HPLC, which showed that Chl$a$ was the major component in each fraction with a minor contribution of Chl$b$ (the peak ratio was 8.5:1). The (Chl$a$ – Chl$b$) content in the isolated fractions was calculated as reported[24] and was in the range of $1.0 \times 10^{-4} – 8.0 \times 10^{-6}$ mol/dm^3.

Preparation of the complexes (Cu–Chl and Zn–Chl)

Chl–HMS (Cu–Chl and Zn–Chl) complexes were prepared using a modified method proposed by Küpper.[8] The solvent was removed from the Chl-fraction at room temperature and the remaining solid was dissolved in 5 cm^3 of methanol/water mixture (90:10, v/v). Then 10 cm^3 of aqueous solutions of CuSO$_4$ (or ZnSO$_4$) were added. The metal ion concentration was varied in the range of 0.01 – 0.05 mol/dm^3. The time periods following the beginning of the Chl–HMS formation (t_c) ranged from 2 h to 3 weeks. The complex formation was stopped by dissolving the reaction mixture in cyclohexane. The obtained solutions were dried with anhydrous CaCl$_2$, centrifuged for 20 min at 2500 rpm and allowed to evaporate at room temperature. The samples were dissolved in appropriate solvents. The solutions contained not only Cu–Chl and Zn–Chl, but also unreacted Chl$a$ and Chl$b$, as well as chlorophyll degradation products formed during the preparation.[3,4]

Measurements and methods applied

Vis spectra of Cu–Chl and Zn–Chl in acetone were recorded on a Varian Cary-100 spectrophotometer in the range of 350 to 700 nm with a constant concentration of metal ions (Cu^{2+} or Zn^{2+}) after different t_c periods.

FTIR spectra of Cu–Chl and Zn–Chl in CCl$_4$ were recorded on a BOMEM MB-100 FTIR spectrometer, in the range of 4000–900 cm^{-1} with a resolution of 2 cm^{-1}, using CaF$_2$ cells (0.1 or 0.5 mm) for liquid samples.

Fluorescence spectra of Cu–Chl and Zn–Chl in acetone were recorded on a FLUOROLOG Jobin Yvon Horiba spectrofluorimeter. The excitation wavelength was 430 nm.

The isolated chlorophyll fractions were analyzed by HPLC (Knauer, Germany) on a reverse-phase Lichrosorb C-18 column, with CH$_3$CN as the mobile phase; the flow rate was 1 cm^3/min.

RESULTS AND DISCUSSION

The Vis spectra of the Cu–Chl and Zn–Chl complexes, after different time periods from the beginning of the complex formation (t_c) are shown in Fig. 2a,b, respectively. In both cases, the Chl-fraction (conc. ~ $10^{-4} – 10^{-5}$ mol/dm^3) was the "blank" sample ($t_c = 0$). As can be seen, the Q and B bands absorption maxima of both complexes were shifted towards the blue region with increasing t_c and simultaneously their intensities decreased.

Chlorophylls have two major absorption regions in the visible range: a "red" (Q) band and "blue" (Soret or B) band (Fig. 2).[25,26] Chl–HMS formation was detected by
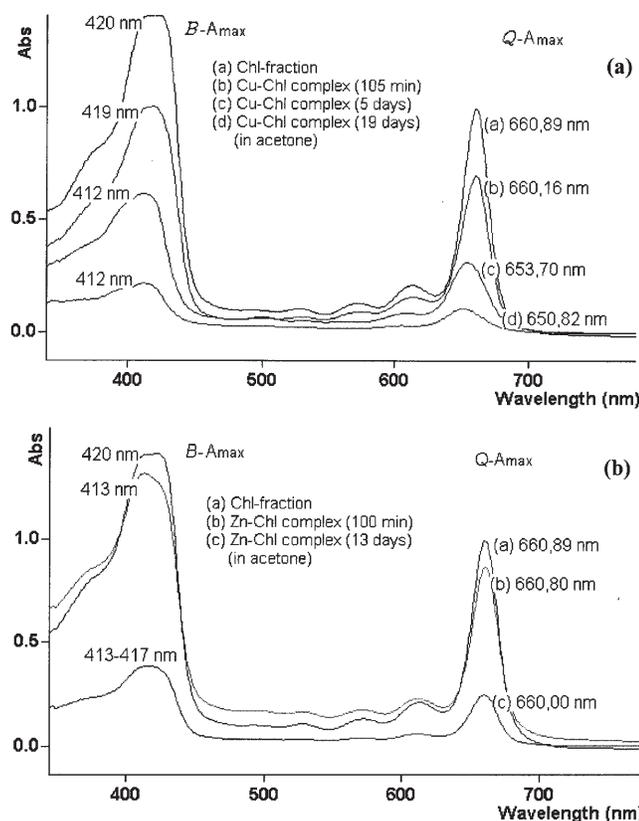
Fig. 2. Vis spectra of Cu–Chl complex (upper), and Zn–Chl complex (lower), for different time periods after the commencement of complex formation (t_c). In both cases, the Chl-fraction (conc. $\sim 10^{-4} – 10^{-5}$ mol/dm^3) is the blank ($t_c = 0$).

Vis spectroscopy *via* both the B and Q-bands. The Q-band absorption maxima (A_{max}) of Cu–Chl showed a remarkable, increasing "blue shift" with increasing reaction time, t_c (Fig. 2a). This effect has already been reported and was explained by the replacement of the central Mg-atom of chlorophyll with a heavy metal atom, *i.e.*, by Chl-HMS formation.[8,17,18] However, with Zn, this was barely visible, at least for the Q-band (Fig. 2b). On the other hand, in both cases, the B-band expressed a clear "blue shift" (Fig. 2a,b). Simultaneously, a pronounced instability of the complexes was seen: the intensities of both bands decrease with increasing t_c (Fig. 2a,b). Hence, it seems that one is dealing with a dynamic equilibrium: the shifts of the absorption maxima of the bands indicate Chl–HMS (Cu–Chl and Zn–Chl) formation; the decrease of the bands intensities with increasing t_c is a mark of their disappearance. Although the reason for the change of direction is not clear, it is certainly not a consequence of pheophytinisation of the complexes, *i.e.*, permanent removal of the central Cu or Zn atom from the complexes, *i.e.*, pheophytin *a* formation as Pheo *a* has a Q-band with an absorption maximum at 665–667 nm.[25,27,28]

The FTIR spectra of Chl-fraction, which plays the role of the "blank" sample, Cu–Chl, and Zn–Chl are shown in Fig. 3a–c, respectively. Such presentation allows comparison of the corresponding bands, existing in all three spectra (posi-
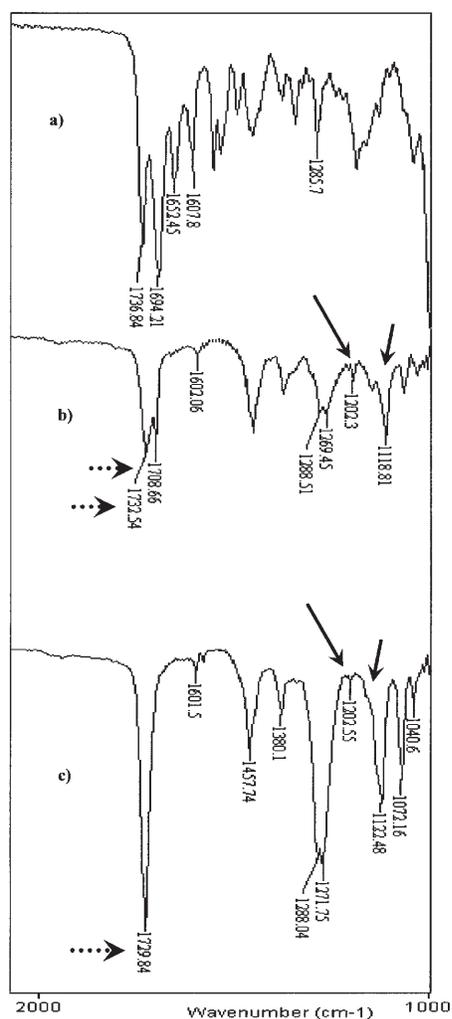
Fig. 3. FTIR spectra of (a) Chl-fraction, which is the "blank" sample, (b) Cu–Chl, and (c) Zn–Chl. The dashed arrows show the shifts of the corresponding bands. New bands are marked by full arrows.

tions, as well as the intensities). It also permits a reliable recognition of new bands (marked by full arrows). Selected stretching frequencies from the three spectra (Fig. 3a–c) are presented in Table I. The values in brackets show a shift of the absorption maxima of a particular band in the two complexes, compared to the position of the same band in Chl*a* ("the blank"). New bands resulting from the formation of Cu–Chl and Zn–Chl are given in bold form.

The FTIR spectrum of Chl*a* in non-polar solvents is shown in Fig. 3a (the rest of the spectrum from 4000–2000 cm^{-1} contains no bands of specific interest). Three major bands in 1800–1600 cm^{-1} region[29–31] were observed. The band at ~1737 cm^{-1} was assigned to stretching vibrations of the two ester-carbonyl groups in the Chl*a* molecule – Fig. 3a and Table I (marked as (C-17^3) and (C-13^3)=O, see Fig. 1). The band at ~1700 cm^{-1} was assigned to the stretching vibration of the free

Table I. Selected stretching frequencies in the FTIR spectra of Chl-fraction, Cu–Chl and Zn–Chl complexes. The values in brackets show the shift of a particular group of absorption maxima in the two complexes, compared to the same band position in Chl$a$ (the "blank" sample). The new bands, corresponding to Cu–Chl and Zn–Chl formation, are given in bold form. s – strong, m – medium, w – weak intensity band

Compound	ν(C17³; C-13³)=O from esters	ν(C-13¹)=O	ν(C-13¹)=O aggregated	ν(C=C) ν(C=N)	ν_{as}(C-13³)–O from esters	ν_{as}(C-17³)–O from esters	ν(C-13¹)–O
Chl	1736.84 cm⁻¹ (s)	1694.21 cm⁻¹ (s)	1652.45 cm⁻¹ (m)	1607.80 cm⁻¹ (m)	1285.70 cm⁻¹ (s)	absent	absent
Cu–Chl	1732.54 cm⁻¹ (s) ($\Delta\nu = -4.3$ cm⁻¹)	1708.66 cm⁻¹ (s) ($\Delta\nu = +14.45$ cm⁻¹)	absent	1602.06 cm⁻¹ (w)	1288.51 cm⁻¹ (s)	1269.50 cm⁻¹ (s)	**1202.30 cm⁻¹ (w)** **1118.81 cm⁻¹ (s)**
Zn–Chl	1729.84 cm⁻¹ (s) ($\Delta\nu = -7.00$ cm⁻¹)	absent	absent	1601.50 cm⁻¹ (w)	1288.00 cm⁻¹ (s)	1271.75 cm⁻¹ (s)	**1202.50 cm⁻¹ (s)** **1122.48 cm⁻¹ (s)**

keto-group (Fig. 3a and Table I), positioned at C-13[1] (and marked as (C-13[1])=O, see Fig. 1). The band at ~1650 cm^{-1} was assigned to the stretching vibration of aggregated C-13[1] keto-groups (Fig. 3a and Table I) (in non-polar solvents, the aggregation occurs with the central Mg-atom of a second Chl-molecule, due to the nucleophilic character of the keto-group providing a fifth ligand to the central Mg-atom in the Chl-molecule[18,29,32]). The band close to 1600 cm^{-1} (Fig. 3a and Table I) was assigned to skeletal C=C and C=N stretching vibrations of the aromatic system in the Chl-molecule.[30]

The FTIR spectrum of Cu–Chl in CCl$_4$ contains the expected bands (Fig. 3b). The band assigned to stretching vibrations of the ester (C-17[3]) and (C-13[3])=O groups was "red" shifted (1732.5 cm^{-1} *vs* 1736.8 cm^{-1} for Chl*a*, Table I) while the band assigned to the stretching vibration of the free keto (13[1]-C)=O group showed a remarkable "blue shift" (1708.7 cm^{-1} *vs* 1694.1 cm^{-1} – Table I). The bands at 1652.5 and 1607.8 cm^{-1} were smaller or absent. This is not surprising, bearing in mind the already reported small affinity of the Cu–Chl complex for self-agregation.[33] However, two new bands emerged in the C–O stretching region, at 1202.3 (weak) and 1118.8 cm^{-1} (strong) – Table I. These bands were absent in the FTIR spectrum of Chl*a* itself (Table I).

The FTIR spectrum of Zn–Chl in CCl$_4$ (Fig. 3c) has a strong band at 1729.8 cm^{-1} ((C-17[3], C-13[3])=O), significantly "red"-shifted compared to Chl*a* itself (1736.8 cm^{-1}) – Table I. A band belonging to the free keto group ((13[1]-C)=O) stretching vibration is absent in the spectrum. Similar to Cu–Chl, the FTIR spectrum of Zn–Chl shows two new bands in the C–O stretching region, at 1202.5 (weak) and 1122.5 cm^{-1} (very strong). In addition, the aggregated and skeletal bands (at 1652.4 and 1607.8 cm^{-1}) were smaller or absent (Table I). The major reason for the disappearance of the aggregated bands could be the low complex concentration, although the self-aggregation ability of Zn–Chl is larger than that of Cu–Chl but smaller than that of Chl*a* itself. It should be seen at higher concentrations,[33] close to 0.01 mol/dm^3, which was not applied in the present study (~10^{-4} – 10^{-5} mol/dm^3).

Strictly speaking, replacement of the central atom was not confirmed by the results of FTIR spectroscopy. The crucial Cu, Zn–N (tetrapyrrole) bonding could not be seen, since the corresponding band lies beyond 500 cm^{-1}, introducing difficulties in its detection. An indirect proof may be seen from the significant "blue shift" of a free keto (C-13[1])=O absorption maxima in Cu–Ch 1 (from 1694.2 cm^{-1} – for Chl*a* – to 1708.7 cm^{-1}), and the absence of the same band in the spectrum of Zn–Chl (Fig. 3b,c and Table I). In the former case, the band of the free keto group and the band of the ester groups (1708.7 cm^{-1} and 1732.5 cm^{-1}, respectively – Table I) have almost equal intensities (Fig. 3b). However, this could be seen as an indirect mark of the different affinities of zinc and copper to form a complex with chlorophyll. A more direct proof comes from the two new bands in C–O stretching

region: at 1202.5 (weak) and 1122.5 cm^{-1} (very strong) for Zn–Chl, and at 1202.3 (weak) and 1118.8 cm^{-1} (very strong) for Cu–Chl (Table I); neither of these bands exists in the FTIR spectrum of Chl$a$ itself (Table I). The bands are assigned to C–O stretching vibrations and provide a proof for a possible formation of a 6-membered chelate cyclic complex, fused at the edge of the 5-membered isocyclic cyclo-pentanone ring, between the position of C-13^1 and C-13^2 (Fig. 4). Precisely, they have been assigned to the (C-13^1)–O stretching vibration, provided by keto-enol tautomerization at the C-13^1 position. In such a complex, the metal ion M^{n+} (Cu^{2+} or Zn^{2+}) bonds one O-atom at the right side, and coordinates to another one at the left side (Fig. 4). The latter one was confirmed by the already mentioned "red shift" of the carbonyl ester group, $i.e.$, of the (C-13^3)=O absorption maxima (Table I); the other carbonyl ester group ((C-17^3)=O) does not play any role in the chelate forma-tion. The (C-13^3)=O…M^{n+} coordination (Fig. 4) makes the double (carbonyl) bond longer and causes a shift toward lower frequencies. The fact that the shift is much more pronounced for Zn–Chl (1729.8 vs 1736.8 cm^{-1} (Chl$a$)) than for Cu–Chl (1732.5 vs 1736.8 cm^{-1} (Chl$a$)) suggests that Zn–Chl has a higher affinity to form a chelate ring than Cu–Chl.
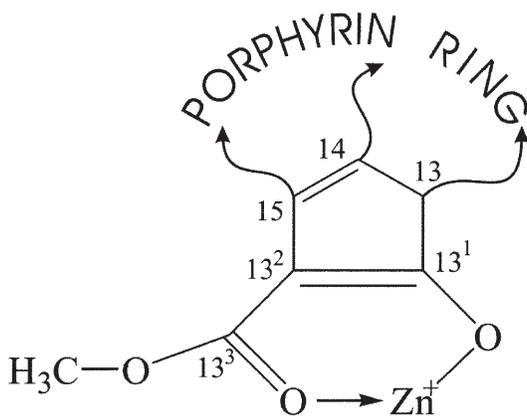

Fig. 4. Structure of a hypothetic 6-membered chelate cycle, fused at the edge of the iso-cyclic cyclopentanone ring of Chl, between the position of C-13^1 and C-13^2, formed in an excess of Zn^{2+} ions.

In addition to the direct proof, chelate formation can be confirmed indirectly. The 1285.7 cm^{-1} band in the Chl$a$ spectrum, assigned to C-17^3 and (C-13^3)–O stretching vibrations, was split in both complexes. In Cu–Chl, the doublet peaks are positioned at 1269.5 and 1288.5 cm^{-1}, while in Zn–Chl they are at 1271.8 and 1288.0 cm^{-1} (Table I). The splitting of the unique band reflects a change in the proximity of the two ester C–O–C stretching vibrations: while the first one remains unchanged ((C-17^3)–O), the other one ((C-13^3)–O) probably undergoes a "red shift" (Table I) on chelate formation.

The fluorescence spectra of the Cu–Chl and Zn–Chl complexes, at two differ-ent time periods after the commencement of the formation of the complexes (t_c : 2
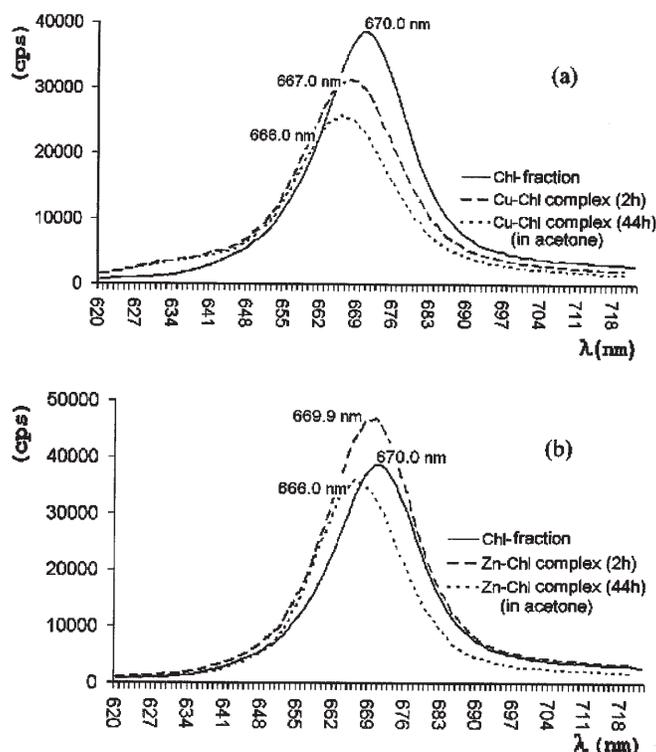
Fig. 5. The Q-band fluorescence spectra of (a) Cu–Chl, and (b) Zn–Chl complex, for two different time periods after the commencement of complex formation (t_c): 2 h and 44.5 h. Chl-fraction (conc. ~10^{-4} – 10^{-5} mol/dm^3) is the "blank" sample ($t_c = 0$).

h and 44.5 h) are shown in Fig. 5a,b, respectively. The fluorescence of the Chl-fraction represents the "blank" sample. Evidently, for both complexes, the fluorescence maximum undergoes a "blue shift" with increasing t_c. The change of the intensity is slightly different for the two complexes.

Taking into account that the Chl-fluorescence represents an intrinsic probe of photosynthesis,[34] it was reasonable to expect another proof for Chl–HMS formation from fluorescence spectroscopy (*in vitro*). Generally, it is well known that Chl–HMS formation leads to a decrease of the fluorescence emission intensity, indicating the formation of an unstable first excitation state, which has a greater tendency to relax thermally; simultaneously the fluorescence maximum of the weakly fluorescing Zn–Chl (compared to Chl*a*) expresses a "blue shift".[12] Both these facts were confirmed in the present experiments (Fig. 5a,b). The maximum of the fluorescent Q-band of Cu–Chl is "blue" shifted and increases with increasing t_c (F_{max} at 667.1 and 666.0 nm, after 2 h and 44.5 h, respectively – compared to F_{max} of Chl*a*, at 670 nm – Fig. 5a). Almost no shift was detected for the F_{max} of Zn–Chl Q-band – compared to Chl*a* – after 2 h: 669.9 *vs* 670 nm, respectively (Fig. 5b). However, after 44.5 h, the shift became remarkably close to that of Cu–Chl, *i.e.*,

666.0 nm (Fig. 5b). This is supporting proof for Zn–Chl formation,[12] much stronger than that concluded from the Vis spectra (Fig. 2b): the "blue shift" of the Q-band A_{max} is less pronounced than that of its fluorescence counterpart. In addition, the F_{max} of both complexes decreased after 44.5 h (Fig. 5a,b), but not as much as expected. The peripheral chelates of methyl pheophorbides a and b have been reported to be non-fluorescent.[19] There is evidently an equilibrium between "central" (replacement of the central atom) and "peripheral" Cu–Chl and Zn–Chl complexes. The "central" ones are thermodynamically favoured, but the "peripheral step" may occur before their formation, and this is more enhanced in the case of Zn–Chl than of Cu–Chl.[19] This is confirmed by the here-presented data: the Vis spectra clearly suggest a stronger tendency of Cu to form a "central" complex, compared to Zn (Fig. 2a,b). Simultaneously, Zn expressed a stronger affinity to form unstable "peripheral" complexes (FTIR data – Table I). The "central" / "peripheral" equilibrium is clearly shifted toward the central complexes for Cu–Chl, and the peripheral ones for Zn–Chl.

Finally, how much this *in vitro* behaviour of the Chl–HMS complexes can mimic the expected *in vivo* situation, where plants and algae pick up copper and zinc from the surrounding environment, permitting their penetration within the photosynthetic apparatus and the formation of Zn and Cu–Chl complexes inside it remains unclear. The main limiting factor is the sensitivity of chlorophyll: Chl can undergo "light" and "dark" reactions. The first ones are mostly photooxidation reactions involving the chlorin structure and changing it to different porphyrin modifications.[35] The second ones involve the periphery of the chlorin structure, without changing the nucleus.[15] While the first ones can be easily prevented by keeping chlorophyll in dark, the other ones can not be avoided and they can potentially influence chelate formation. Peripheral chelate formation is especially sensitive to oxidation reactions at the C-13^2 position (the allomerization reaction) yielding many derivatives.[15] The structures of the allomer products may exclude any possibility for chelate formation. Generally, allomerization products are less abundant in fresh spinach samples than in older ones. In the latter case, prolonged senescence may lead to chlorophyll breakdown and the appearance of monopyrollic compounds.[36]

<div align="center">CONCLUSION</div>

Both copper and zinc form complexes with chlorophyll, but with different affinities. While the Cu–Chl complex is predominantly formed by replacement of the central Mg-atom of chlorophyll ("central complex") with copper, zinc is much more engaged in the formation of a cyclic 6-membered peripheral chelate ("peripheral complex"), playing a coordinating role between two O-atoms. While "central complexes" are better detected by Vis and fluorescent spectroscopy, the chelate existence is firmly confirmed by FTIR spectroscopy, based on the appearance of new bands, reflecting chelate formation.

A dynamic "central"/"peripheral" equilibrium exists for both complexes, undoubtedly shifted toward the "central complex" in the case of Cu–Chl, and toward the "peripheral complex" in the case of Zn–Chl.

ИЗВОД

IN VITRO КОМПЛЕКСИ БАКРА И ЦИНКА СА ХЛОРОФИЛОМ

ЈЕЛЕНА ПЕТРОВИЋ, ГОРАН НИКОЛИЋ и ДЕЈАН МАРКОВИЋ

Технолошки факултет, 16000 Лесковац

У раду су испитивани комплекси бакра и цинка са хлорофилом, главним фотосинтетским пигментом, методама видљиве, инфрацрвене и флуоресцентне спектроскопије. Препозната су два типа комплекса. Док бакар заменом централног магнезијумовог атома хлорофила формира „централни" комплекс, у случају цинка то је мање вероватно. Уместо тога, уз учешће цинка формира се шесточлани хелатни прстен на периферном делу хлорофилне структуре. У овом последњем случају, аломеризационе реакције на ободу хлорофила могу довести до ометања формирања периферног прстена.

REFERENCES

1. G. Sandmann, H. Scheer, in *Photosynthesis*, G. Raghavendra, Ed., Cambridge University Press, Cambridge, 1998, p. 44
2. H. Scheer, in *Light-Harvesting Antennas in Photosynthesis*, B. R. Green, W. W. Parson, Eds., Kluwer Academic Publishers, 2003, p. 29
3. H. Küpper, E. Lombi, F. J. Zhao, G. Wieshammer, S. P. McGrath, *J. Exp. Botany* **52** (2001) 2291
4. H. Küpper, E. Lombi, F. J. Zhao, S. P. McGrath, *Planta* **212** (2000) 75
5. C. Jegerchöld, J. B. Arellano, W. P. Schröder, P. J. M. van Kan, M. Baron, S. Styring, *Biochemistry* **34** (1995) 12747
6. B. D. Hsu, J. Y. Lee, *Plant Physiology* **87** (1988) 116
7. G. Samson, J. C. Morissette, R. Popović, *Photochem. Photobiol.* **48** (1988) 329
8. H. Küpper, F. Küpper, M. Spiller, *J. Exp. Botany* **47** (1996) 249
9. L. F. De Filippis, C. K. Pallaghy, *Z. Pflanzenphysiol.* **78** (1976) 314
10. L. F. De Filippis, *Z. Pflanzenphysiol.* **93** (1979) 129
11. H. Küpper, I. Šetlik, M. Spiller, F. C. Küpper, O. Prášil, *J. Physiol.* **38** (2002) 429
12. H. Küpper, F. Küpper, M. Spiller, in *Chlorophyll* (Series: *Advances in Photosynthesis*), Govindjee, Ed., Kluwer, 2005, in press
13. H. Clijsters, F. Van Assche, *Photosynthesis Res.* **7** (1985) 31
14. C. M. Luna, C. A. González, V. S. Trippi, *Plant Cell Physiol.* **35** (1994) 5
15. P. H. Hynninen, in *Chlorophylls*, H. Scheer, Ed., CRC Press, Boca Raton, FL, 1991, p. 145
16. R. C. White, I. D. Jones, E. Gibbs, L. S. Butler, *J. Agr. Food Chem.* **25** (1977) 143
17. I. D. Jones, R. C. White, E. Gibbs, C. D. Denard, *J. Agr. Food Chem.* **16** (1968) 80
18. L. J. Boucher, J. J. Katz, *J. Am. Chem. Soc.* **89** (1967) 4703
19. H. Scheer, J. J. Katz, *J. Am. Chem. Soc.* **100** (1978) 561
20. H. Scheer, *J. Am. Chem. Soc.* **97** (1975) 3273
21. W. A. Svec, in *Chlorophylls*, H. Scheer, Ed., CRC Press, Boca Raton, FL, 1991, p. 89
22. W. A. Svec, in *The Porphyrins*, D. Dolphin, Ed., Academic Press, 1978, p. 342
23. H. Backman, N. Risch, in *Chlorophylls*, H. Scheer, Ed., CRC Press, Boca Raton, FL, 1991, p. 103
24. H. K. Lichtenthaler, *Methods Enzymol.* **148** (1987) 350

25. A. J. Hoff, J. Amesz, in *Chlorophylls*, H. Scheer, Ed., CRC Press, Boca Raton, FL, 1991, p. 724
26. L. K. Hanson, in *Chlorophylls*, H. Scheer, Ed., CRC Press, Boca Raton, FL, 1991, p. 994
27. S. W. Jeffrey, R. F. C. Mantoura, S. W. Wright (Eds.), *Phytoplankton pigments in oceanography: guidelines to modern methods*, UNESCO Publishing, 1996
28. H. Scheer, in *CRC Handbook of Organic Photochemistry and Photobiology*, W. Horspool, W. Lenci, Eds., CRC Press, Boca Raton, FL, 2003, p. 117(1)
29. K. Balschmiter, J. J. Katz, *Nature* **220** (1968) 1231
30. M. Lutz, W. Mäntelle, in *Chlorophylls*, H. Scheer, Ed., CRC Press, Boca Raton, FL, 1991, p. 855
31. J. J. Katz, G. L. Closs, F. C. Pennington, M. R. Thomas, H. H. Strain, *J. Am. Chem. Soc.* **85** (1963) 3801
32. J. J. Katz, L. L. Shipman, T. M. Cotton, T. R. Janson, in *The Porphyrins*, D. Dolphin, Ed., Academic Press, New York, 1978, p. 401
33. L. J. Boucher, J. J. Katz, *J. Am. Chem. Soc.* **89** (1967) 1340
34. U. Schreiber, W. Bilger, H. Hormann, C. Neubauer, in *Photosynthesis*, G. Raghavendra, Ed., Cambridge University Press, 1998, p. 320
35. H. Scheer, in *CRC Handbook of Organic Photochemistry and Photobiology*, W. M. Horspool, P.-S. Song, Eds., CRC Press, Boca Raton, FL, 1994, p. 1402
36. C. Peisker, T. Duggelin, D. Rentsch, P. Matile, *J. Plant Physiol.* **135** (1989) 428.