Fine tuning of copper(II) – chlorophyll interactions in organic media. Metalation versus oxidation of the macrocycle.

SCHOLARONE™
Manuscripts

Journal Name

ARTICLE

Fine tuning of copper(II) – chlorophyll interactions in organic media. Metalation versus oxidation of the macrocycle

Łukasz Orzeł,[a]* Bartłomiej Szmyd,[a] Dorota Rutkowska-Żbik,[b] Leszek Fiedor,[c] Rudi van Eldik[a,d]* and Grażyna Stochel[a]

The nature of chlorophyll interactions with copper(II) ions varies considerably in organic solvents, depending on the dominant coordinative form. Besides formation of the metallo tetrapyrrolic complex, Cu(II) ions can cause oxidation of the pigment, reversible or irreversible, leading to the destruction of the macrocyclic structure. All these reaction types can be distinguished within a quite narrow range of reaction conditions. The ability to form new metallo derivatives in either metalation or transmetalation reactions is obviously limited by the concentration of the potential oxidant, but can be secured below this level via suitable composition of the reaction system. The decisive factor in the selection of a specific reaction pathway is the presence of a potential ligand that can affect the reactivity of Cu(II) for example by shifting its redox potential. Spectroscopic and electrochemical studies were performed in order to determine the predominant species of Cu(II) in methanol, nitromethane and acetonitrile in the presence of chloride and acetate ions, as well as to assign their appropriate oxidizing ability. This allowed us to estimate the boundary conditions for the electron transfer processes in chlorophyll - Cu(II) systems. Chlorophyll and its free base can undergo both types of electron transfer processes, however, they reveal different susceptibilities that make this class of ligands quite versatile markers in tuning the reactivity of metal ions in solutions.

Introduction

Interactions of chlorophylls (Chls; including chlorophyll *a,* Chl*a*, its free base pheophytin *a*, Pheo*a*, and other derivatives) with metal ions, although not strictly related to their basic biological functions, are important for proper metabolism of the photosynthetic pigment. They are decisive not only in biosynthesis of Chl*a* (Fig. 1) at the stage of enzyme-driven Mg^{2+} incorporation into protoporphyrin IX **(**3,7,12,17-tetramethyl-8,13-divinyl-2,18-porphinedipropionic acid**),**[1-4] but also in the spontaneous substitution of metal ions occurring in plants growing on soils contaminated by heavy metals.[5] The latter effect, observed clearly for Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}, results in the irreversible loss of photosynthetic abilities.[5-7] In recent years, deeper insight into the mechanisms of metal ion – Chl interactions has become important also for the development of procedures for modification and synthesis of new tetrapyrrolic complexes in terms of their potential biomedical applications.[8-10] Although the primary function of chlorophylls is related to electron transfer processes, their occurrence in biological systems is not due to pigment interactions with redox-active metal ions. Properties of natural photosystems, including the composition of endogenous ligands, prevent degradation of the pigment's macrocyclic structure in the presence of Cu^{2+} and other potential metallic oxidants.
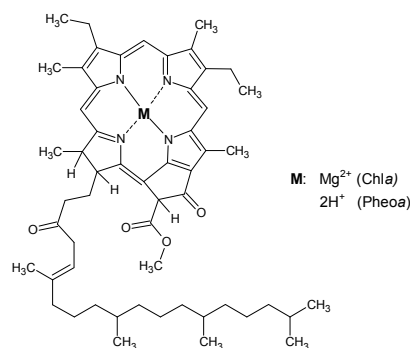

Figure 1. Structure of Chl*a* and Pheo*a*.

Displacement of the chlorophyllic central ion *in vivo* is limited to a few metals by their abundance, water solubility and ability to adapt to the operation of the plants' ion transport system. *In vitro*, the site of Mg^{2+} can be occupied spontaneously by most divalent transition metal ions, through transmetalation or, above

all, metalation conducted in acidic medium.[11] The hydrophobicity of the pigment introduces significant restrictions on the reproduction of the conditions in natural photosystems. As long as the native structure of Chla is retained, the studies can be carried out only in organic solvents. Such a forced approach, belittling the possibility of referring the results directly to biological conditions, is still justified in the context of the development of strategies for the synthesis of metallotetrapyrrolic complexes in terms of their potential biomedical applications. According to our studies, organic media provide a wider variety of potential reaction courses than natural photosystems. In the presence of certain metal ions, such as Cu^{2+}, two types of electron transfer processes can be distinguished in the formation of new metallo derivatives.[12] Whereas the one-electron oxidation of the tetrapyrrolic ring appears to be reversible, thus not preventing further central ion substitution, the multi-electron process leads directly to the degradation of the macrocyclic system.[13]

Each of the mentioned reaction pathways is accessible for Chl – copper(II) systems within a quite narrow range of reaction conditions, as the switching between reaction pathways takes place on changing only one of three factors, namely, the oxidant concentration, solvent or counterion. The selection of the appropriate reaction pathway appears to depend on various factors, which are described in detail in this report. The analysis of the studied systems allows precise control over the reactivity of metal ions via selection of the potential ligands and adjustment of the concentration of reactants to the redox potential of the corresponding species present in solution. Precise description of the mechanisms in particular types of redox processes involving the photosynthetic pigments, will be presented in a forthcoming report.

Experimental

Chlorophylls.

Chla was isolated from the cells of cyanobacterium *Arthrospira maxima* obtained from the Culture Collection of Autotrophic Organisms in Trebon (Czech Republic). The pigment was extracted in methanol according to Iriyama[14] and purified by column chromatography on DEAE-Sepharose (Sigma, Germany),[15] and then by isocratic reversed-phase high-performance liquid chromatography (HPLC) (Varian RP-C18, 250×10 mm, flow rate: 4 mL/min) using methanol as the eluent. The separations were run on an HPLC system consisting of a ProStar 230 pump (Varian, USA) equipped with a TIDAS diode array detector (J&M, Germany) for online monitoring of absorption spectra. Pheoa was obtained by a short treatment of pure Chla with glacial HAc. It was stirred for 10 min at 323 K and evaporated under argon. Dissolving the residue in acetone and evaporation were repeated until HAc was completely removed. Pheo was purified on silica gel using chloroform as eluent. Pure pigments were stored at −20 °C under argon. All experiments were performed in dim light with freshly prepared solutions.

Solvents and reagents.

All chemicals used in the experiments were of at least analytical grade. Copper(II) trifluoromethanesulfonate (CuTf$_2$), copper(II) acetate monohydrate, Cu(AcO)$_2$, and tetrabutylammonium acetate

(Bu$_4$NAcO) were purchased from Aldrich (Sigma-Aldrich, Germany), and tetraethylammonium chloride (Et$_4$NCl) from Merck (Germany). Cu^{II} chloride dehydrate and solvents, i.e., methanol (MeOH), acetonitrile (MeCN) and nitromethane (MeNO$_2$), were obtained from POCh (Poland). 2,6-Lutidine, electrochemical standards and electrolytes (ferrocene, tetrabuthylammonium hexafluorophosphate) were purchased from Sigma-Aldrich Co. (Germany).

Spectroscopic measurements.

Lambda 35 and Lambda 950 (Perkin Elmer, USA) spectrophotometers were used for the measurement of UV-Vis absorption spectra. The temperature was fixed and stabilized during the experiments with an attached PTP-6 Peltier module. Spectroscopic titrations were carried out in 1 cm quartz cuvettes, whereas 0.88 cm quartz tandem cuvettes were used in the spectroscopic investigations of the reactions of Chla.

For the titrations small volumes (i.e., 10-25 μl) of titrant at a concentration of 0.2 - 0.5 M were added successively to 2 ml of the titrated solution (usually 5 mM). The content of the cuvette was mixed by hand and the spectrum was recorded in the range covering all relevant absorption bands of the investigated compounds (from 250 to 1100 nm).

The course of the reactions of Phea and Chla with Cu(II) salts was investigated spectrophotometrically within the range 350 to 1100 nm. Solutions of the dye (2.5 μM) in either MeOH, MeCN or MeNO$_2$, were mixed in the tandem cuvette with solutions of the Cu(II) salts of various concentration (in excess over the concentration of Chl to maintain pseudo-first-order conditions). The latter were prepared directly from commercially available preparations or from Cu(II) trifluoromethanesulfonate previously mixed with the salt of a suitable anion (chloride, acetate). Similarly, changes of the Chl's emission occurring as the result of the reaction with the Cu(II) salt, were recorded with the excitation wavelengths set, when possible, at the absorption isosbestic points. All experiments were performed at 298 K under ambient pressure.

Electrochemical measurements.

Cyclic voltammetry measurements were performed on an electrochemical analyzer BAS CV50W (Bioanalytical Systems, USA). A disk glassy carbon electrode was used as working electrode, and a platinum electrode and Ag/AgCl cell as auxiliary and reference electrodes, respectively. A 0.1 M solution of (Bu$_4$N)PF$_6$ was a basic electrolyte in suitable solvents (MeNO$_2$, MeCN, MeOH). Ferrocene was used to calibrate the reference electrode in non-aqueous solvents. Electrochemical titrations were carried out under analogous conditions to those secured in the spectroscopic titrations. Small doses of concentrated solutions of (Bu$_4$N)AcO and (Et$_4$N)Cl were mixed with diluted solutions of CuTfO$_2$ and the cyclic voltammograms were recorded immediately. The measurements were performed within the potential range from -1.2 to +0.9 V with scan rates between 25 and 100 mV/s.

Theoretical calculations.

The reported theoretical results were obtained within Density Functional Theory (DFT), as implemented in Turbomole v. 6.4,[16] with gradient-corrected Becke-Perdew functionals.[17-21] Resolution of Identity (RI) approach[22, 23] was used for computing the electronic Coulomb interactions. The chosen methodology has already proved

suitable for determination of the geometry of magnesium porphyrins, chlorophylls and bacteriochlorophylls.[24-27] The basis set of def2-TZVP quality[28] was employed for all atoms. The solvation was accounted for by COSMO model[29] with default radii for the elements (H = 1.30, C = 2.00, N = 1.83, O = 1.72) and 2.00 Å for copper. Three ε values, viz, 38.8 for acetonitrile, 32.7 for methanol, and 35.9 for nitromethane, were used in order to take into account the type of solvent used.

Results and discussion

Spectroscopic tracing of the types of reactions in Chl – Cu(II) systems. The main bands in the absorption spectra of metallochlorophylls, analogously to metalloporphyrins, result from the electronic transitions between π and π* orbitals of the macrocycle. Classic work by Gouterman,[30, 31] as well as more recent results from Sundholm's theoretical calculations,[32-34] explain among others, the effect of metal binding on the spectral changes in the UV-Vis range. Incorporation of the metal ion into the tetrapyrrolic cavity – which is usually a spontaneous process *in vitro*, and in the case of certain metal ions also *in vivo*[5, 7] – proceeds under standard conditions within a timescale ranging from a few minutes (formation of Sn-Pheo*a* in MeCN[35]) to several years (formation of Pt-Pheo*a* in MeOH[25]). This is accompanied by well-defined changes in the absorption spectrum, the most characteristic of which are: (i) hipsochromic shift of the Q_Y band from its original position, which is located, depending on the solvent, between 660 and 670 nm, and (ii) reduction of the number of low-intensity bands between 500 and 550 nm. The spectra of most metallochlorophylls have been thoroughly assigned.[11, 25, 36] Since the UV-Vis spectra of Chls reflect mainly the electronic state of the macrocycle, they are also useful in investigations of other types of interactions with the metal ion, involving electron transfer processes. This was confirmed in studies on major Chls' catabolites that provided *inter alia* detailed spectroscopic characteristics of the products of both one-electron oxidation processes,[37-40] as well as decomposition of the macrocyclic system.[41, 42]

The reactions of both Pheo*a* and Chl*a* with selected Cu(II) salts were performed in organic solvents, viz. MeNO₂, MeOH and MeCN, thus providing non-coordinating, coordinating protic and coordinating aprotic media, respectively. Selection of CuTf₂, CuCl₂ and CuAcO₂ was dictated by the need to consider the role of counter ions in the metal – tetrapyrrole interaction. Reactions were carried out at 25 °C under pseudo-first-order conditions with a fixed concentration of Chl (2.5 μM) and a large excess of Cu²⁺ that varied in the range of 2.5 - 50 mM. Three different patterns of spectral changes could be distinguished on changing the composition of the reaction system, i.e. solvent, counter ion or metal concentration. Figure 2 shows examples of these types of spectral changes observed in the reactions of Chl*a* and Pheo*a*.

The reactions that follow pattern **I** are shown in Fig. 2A and 2D. The most important feature of the spectral changes related to this pattern is a hipsochromic shift of the Q_Y band. This change is observed for both Chl*a* and Pheo*a* and clearly indicates formation of a complex with the entering metal ion. Changes in the other bands are quite incomparable. Disappearance of the bands of the free base in the range 500 to 550 nm, due to the symmetry of the compound,

cannot be observed in the reactions of its Mg derivative. All the features observed in Fig. 2D indicate formation of a typical Cu-Pheo*a* complex with a single central ion, Cu²⁺, placed in the cavity and the plane of the macrocycle. Spectral changes depicted in Fig. 2A correspond to stabilization of the intermediate, recognized as a type of binuclear complex, [Cu-Pheo-Mg]²⁺ (compare our previous report[12]). Its breakdown and release of Mg²⁺ requires application of specific conditions, such as the presence of excess acetate, acting as a specific "pulling" ligand.[43]
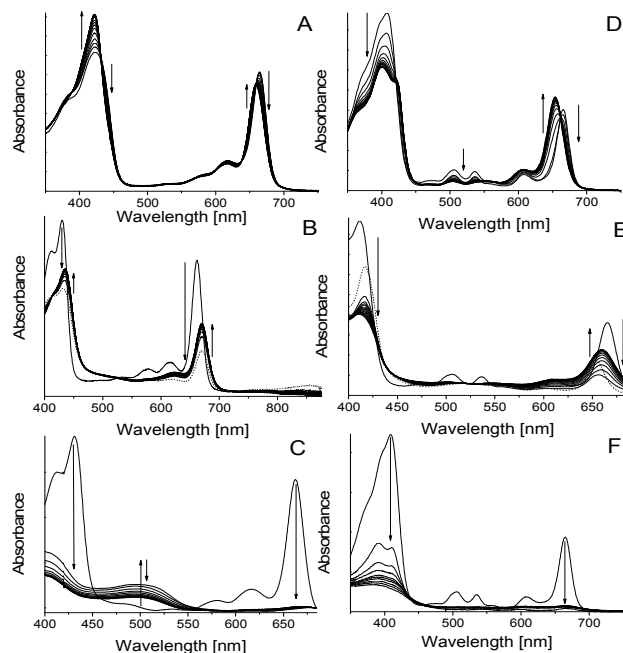

Figure 2. Different patterns of absorption changes accompanying the reactions of Chl*a* (A-C) and Pheo*a* (D-F) with CuTf₂ in organic solvents. Pattern **I** observed in the reaction of Chl*a* and Pheo*a* is shown in A and D, pattern **II** in B and E, and pattern **III** in C and F (see detailed description in the text). In the case of Chl*a* the reactions were carried out at [CuTf₂] = 50 μM in MeOH (A); 25 μM in MeCN (B); and 500 μM in MeNO₂ (C). In the case of Pheo*a*, [CuTf₂] = 500 μM in MeOH (D), 250 μM in MeNO₂ and 500 μM in MeCN. [Chl*a*] = [Pheo*a*] = 2.5 μM, T = 298 K, overall reaction time = 2 h.

Pattern **II** for the reactions of Chl*a* and Pheo*a* is represented by Fig. 2B and 2E, respectively. Regardless of the occupancy of the tetrapyrrole's cavity, the initial immediate intensity decrease in the Q_Y band is followed by its slower reconstruction. Since the reconstructed band is not identical to that of the starting compound, the apparent reversibility of the process is not comprehensive and versatile. This is particularly noticeable in the case of Pheo*a*, where the permanent disappearance of two bands in the range 500 to 550 nm points to the sustainable growth of symmetry within the central cavity (see Fig. 2E). An additional band can be found in the near IR range (>850 nm) within the mixing stage, i.e. simultaneously with the disappearance of the Q_Y band. The subsequent slower disappearance of the band (a few minutes) accompanies the increase in the basic Chl absorption band. Although there is a lack of publications concerning electron transfer processes in Chl–metal systems, the changes related to the initial step of the discussed

reaction type are analogous to those observed during the electrochemical oxidation of Chl*a*.[39, 40] Therefore, they should be attributed to the formation of the cation radical of the pigment. Decay of this reactive species, and/or possible formation of the (new) metallo derivative, form the subject of our further studies.

The final high-lighted pattern, **III**, refers to the disappearance of all characteristic bands of the pigment. Despite the differences in sequences of changes observed for Chl*a* and Pheo*a*, they undoubtedly lead to the destruction of the macrocyclic structure, in which a closed system of delocalized electrons is characterized by the distribution of energy levels giving intense and narrow B (Soret) and Q bands.[32, 33] The broad and ovoid band formed in place of the Soret band corresponds to the spectrum of 1-formyl-19-oxobilane[44] (Fig. 3), the main catabolite of chlorophyll.[13, 41, 45-47]
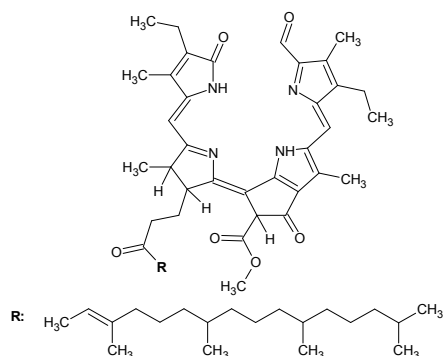

Figure 3. Structure of 1-formyl-19-oxobilane.

Such linear dihydrotetrapyrrole is formed by addition of two oxygen atoms and thus should be regarded as a product of further (i.e. 4 electron) oxidation. Obviously, such a transformation is a completely irreversible process, which is in agreement with the spectroscopic observations. Detailed studies on the metal-catalyzed mechanism of Chl's ring-opening will be part of our future study.

In order to estimate the boundary conditions for switching between the different reaction pathways, the reactions with defined Cu(II) salts, viz. CuTf$_2$, CuCl$_2$ and Cu(AcO)$_2$, were conducted within a wide range of concentrations. The upper and lower limits of the Cu(II) salt concentrations used in the experiments were determined by the solubility of the salt in a particular solvent (the highest [Cu^{2+}] used in our experiments was 50 mM for CuTf$_2$ in MeOH and MeCN, and for CuCl$_2$ in MeOH) and a reasonable reaction progress within 24 h. The latter restriction was due to a progressive metal-independent decomposition of the pigments in oxygenated solutions. Table 1 summarizes the ratios of [Cu(II)]/[Chl] at which the reaction course changes from metalation/transmetalation (**I**) to reversible (**II**) or irreversible (**III**) oxidation of the macrocyclic ligand.

The above table clearly shows that switching between the possible reaction courses in the Chl–metal system is controlled to a considerable extent by the interactions with other components. Obviously, the ability to undergo redox processes is controlled by the electron donation and acceptance potentials, as well as the ratio of the reagents' concentrations. Therefore, it is also clear that any change in the coordination sphere must play a significant role.

To the best of our knowledge, chloride and acetate complexes of Cu(II) in organic solvents, despite the simplicity of their composition, are relatively poorly characterized. Meanwhile, speciation of Cu(II) stands for diversity of effects observed in the reactions with Chls, such as solvent-dependent acetate-induced activation of the pigment in the course of transmetalation.[43]

Table 1. Diversity of the reaction pathways occurring at different [Cu(II)]/[Chl] ratios at 298 K.

Solvent	Salt	Pheo*a*	Chl*a*
MeNO$_2$	CuTf$_2$	I (< 20); II (20 – 100); III (100)	I (< 200); II (> 200)
	CuCl$_2$	I	II (< 2000); III (> 2000)
	CuAcO$_2$	I	I
MeOH	CuTf$_2$	I	I (< 50); II (> 50)
	CuCl$_2$	I	II
	CuAcO$_2$	I	I
MeCN	CuTf$_2$	I (< 20); III (> 20)	I (< 50); II (> 50)
	CuCl$_2$	I (< 20); III (> 20)	II (< 500); III (> 500)
	CuAcO$_2$	I	I

As shown by the data in Table 1, the stoichiometric presence of AcO$^-$ vs Cu^{2+} fully protects both Pheo*a* and Chl*a* against oxidative degradation. The most distinct tendency to both one-electron oxidation and tetrapyrrolic ring-opening is exhibited by weakly coordinated complexes containing solvent ligands or, optionally, triflate ions.[48] In turn, chloride complexes, within a broad concentration range, are involved in reversible electron transfer processes involving Chl*a* and simple metalation of Pheo*a*.

To gain more insight into the role of coordinating counter ions, the reactions of Pheo*a* and Chl*a* with CuTf$_2$ in the three organic solvents were carried out in the presence of various amounts of (Et$_4$N)Cl and (Bu$_4$N)AcO. The results of the spectroscopic observations are summarized in Table 2. Reactions were performed under pseudo-first-order conditions with a large excess of metal salt. Starting from CuTf$_2$, which oxidizes Chls in each studied solvent, the electronic and macrocyclic structure of dihydrotetrapyrrole is preserved as the concentration of either Cl$^-$ or, even more, AcO$^-$ is increased.

Table 2. Distribution of the reaction patterns within the range of coordinating anion's concentration, [L], at 298 K, [Cu]/[Chl] = 2000.

Chl	[Cu]/[L]	MeNO$_2$		MeOH		MeCN	
		Cl$^-$	AcO$^-$	Cl$^-$	AcO$^-$	Cl$^-$	AcO$^-$
Pheo*a*	1:0	III	III	III	III	III	III
	1:1	III	I	I	I	III	III
	1:2	III	I	I	I	III	III
	1:3	III	I	I	I	I/III	I
	1:5	III	I	I	I	I/III	I
Chl*a*	1:0	III	III	II	II	III	II
	1:1	III	II	II	II	III	II
	1:2	III	II	II	I	III	II
	1:3	II	II	II	I	II	I
	1:5	II	II	II	I	II	I

The reactions running in the presence of a double excess of the coordinating anion are significantly slowed down with further increase in its concentration (especially chloride). One can find Pheo*a* to be resistant to single electron donation and more prone to

multi-electron decomposition, whereas Ch*la* exhibits quite opposite tendencies. These differences can be accounted for in terms of the redox properties of Chls (see below). Moreover, the observed changes in Cu(II) reactivity towards Chls must be related, besides concentration, to the changes in ligand composition. More information on the Cu(II) speciation in chosen organic solvents was obtained from spectroscopic titrations of CuTf$_2$, as well as from electrochemical analyses.

Coordinative forms of Cu^{2+} in organic solvents.

The above-mentioned differences in the reaction courses and formed products, for obvious reasons, must be due to differences in the coordination forms of Cu(II) present in the studied systems. To shed more light on this issue, the speciation of Cu(II) was investigated in MeOH, MeCN and MeNO$_2$ using UV-Vis spectroscopy. The solutions of CuTf$_2$ were titrated directly in a quartz cuvette with small amounts of concentrated (Et$_4$N)Cl and (Bu$_4$N)AcO solutions. The absorption spectra were recorded after each addition within the range that enabled us to track the changes in intensity and energy of the d-d transitions. The CT transitions as being too intense – except for some cases – were omitted from consideration. Figure 4 presents an example of the changes observed in the Cu(II)–anion–solvent systems.
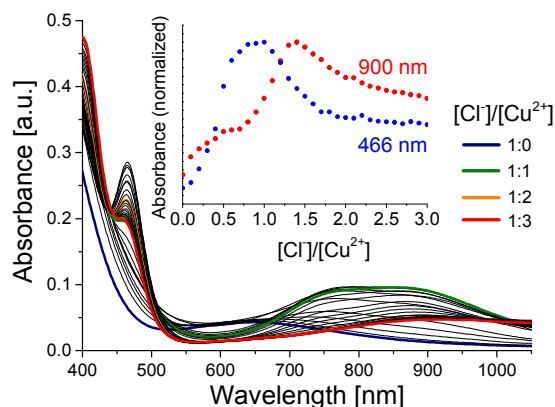

Figure 4. Changes in the composition of [Cu$_m$(MeNO$_2$)$_{6-n}$Cl$_n$]$^{2m-n}$ complexes. Absorbance changes that occur during the titration of CuTf$_2$ with (Et$_4$N)Cl in MeNO$_2$. Inset: the titration curves recorded at 466 (●) and 900 nm (●), T = 298 K.
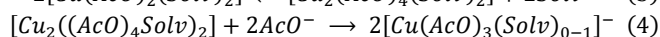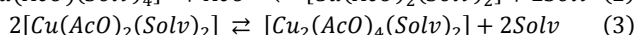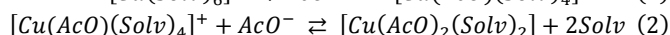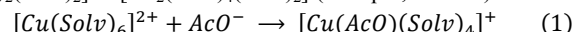
On increasing the concentration of both Cl$^-$ and AcO$^-$, the intensity of the broad band in the low energy visible and near IR ranges, increases in each solvent. Such a significant increase in the probability of d-d transitions in the Cu(II) complex must involve a change from an almost perfect octahedral geometry to a distorted one, probably caused by the Jahn-Teller effect typical for d^9 ions. This could suggest that Cu(II) solvate complexes reveal a reduced deformation probably due to the uniformity of the ligand field, but also due to weak metal–ligand interactions in which the J-T effect could be less important.

A detailed description of the speciation of Cu(II)-acetate complexes in MeOH and MeCN was given in our earlier reports.[12, 43] The results presented there, although consistent with the formation of non-reactive binuclear species bridged by up to four acetate ions,

indicated a different behaviour of these complexes in both solvents driven by the excess AcO$^-$, leading to the differentiation of their susceptibility to ligand substitution.[43] Regardless of solvent, an increase in temperature causes a considerable decrease in the intensity of the band related to the d-d transition, thus pointing to the decay of [Cu$_2$(AcO)$_4$(Solv)$_2$] to [Cu(Ac)$_2$(Solv)$_4$] species. A quite similar effect can be obtained by the application of high pressure. Spectra recorded at elevated pressure showed a 25 % decrease in absorbance of Cu(AcO)$_2$ in MeOH solution as the pressure was increased to 150 MPa using a high pressure optical cell.[12]

The titration of CuTf$_2$ with (Bu$_4$N)AcO in MeNO$_2$ provided information about the influence of the AcO$^-$ concentration on the change of the prevailing forms of Cu(II) complexes in this solvent. The band at 785 nm, which is present in the solution of CuTf$_2$, undergoes a considerable increase in its intensity when the AcO$^-$ concentration is increased up to 0.5 equivalents (Fig. S1 in ESI). At the same time, the energy of the observed transition increases, which manifests itself in a shift in the band maximum to 707 nm. Subsequently, a ca. 30 % decrease in absorbance is accompanied by a bathochromic shift reaching 727 nm at the concentration ratio [AcO$^-$]/[Cu(II)] = 2.5. Then another increase in the absorbance is observed together with a strong hipsochromic shift (up to 636 nm) at a concentration ratio of 3:1. Simultaneously, there is a rapid increase in the absorbance over the high-energy range of the spectrum.

Comparison of our results with Grasdalen's work on the speciation equilibria in the Cu(II)–AcO$^-$–MeOH system, allows to determine the formation of binuclear complexes of the type [Cu$_2$(AcO)$_4$(Solv)$_2$] in all the solvents tested, including MeNO$_2$. They are distinguishable by the molar absorption coefficient, which according to Grasdalen increases from 19 through 37 to 180 M^{-1} cm^{-1} with a change in complex composition from [Cu(AcO)(Solv)$_4$]$^+$ via [Cu(AcO)$_2$(Solv)$_2$] to [Cu$_2$(AcO)$_4$(Solv)$_2$] (cf. eq. 1, 2 and 3).

$$[Cu(Solv)_6]^{2+} + AcO^- \longrightarrow [Cu(AcO)(Solv)_4]^+ \quad (1)$$
$$[Cu(AcO)(Solv)_4]^+ + AcO^- \rightleftarrows [Cu(AcO)_2(Solv)_2] + 2Solv \quad (2)$$
$$2[Cu(AcO)_2(Solv)_2] \rightleftarrows [Cu_2(AcO)_4(Solv)_2] + 2Solv \quad (3)$$
$$[Cu_2((AcO)_4Solv)_2] + 2AcO^- \longrightarrow 2[Cu(AcO)_3(Solv)_{0-1}]^- \quad (4)$$

Such a clear increase in the absorbance in the low energy range in MeNO$_2$ for [AcO$^-$]/[Cu(II)] = 1 - 1.5 may be related to the formation of some type of bridged species, as shown by the subsequent decrease in the intensity of the band, which probably cannot be assigned to the incorporation of another acetate ion into the complex with a single central ion. Comparison with the results obtained in MeOH and MeCN leads to the hypothesis that the actual binuclear form is created only after exceeding the [AcO$^-$]/[Cu(II)] ratio of 2.5, which is further indicated by a significant increase in the energy of the d-d transitions.

Theoretical calculations enabled the determination of the stable structures of binuclear complexes formed in each of the studied solvents with a [Cu(II)]/[AcO$^-$] ratio equal to 0.5 (see Fig. 5), as well as for the complexes which may be present in an excess of acetate in MeCN (Fig. 6). In all the studied [Cu$_2$(AcO)$_4$(Solv)$_2$] systems, solvent molecules occupy axial positions at the elongation of the Cu-Cu axis. The bond lengths between copper and solvent molecules indicate a very tight binding between Cu^{2+} and MeCN, as indicated by the shortest bond, whereas the loosest one occurs in methanol. On the contrary, a comparison of the Cu-Cu distances in the dimer

geometries reveals that the core of the complex is the most contracted in MeOH, whereas the least in MeCN (2.54 vs. 2.58 Å). Such an arrangement may indicate that the binuclear species formed in MeCN would undergo facile scission upon binding of additional acetate ions. This hypothesis is further supported by the elongation of the Cu-Cu distance in binuclear species caused by the substitution of MeCN molecules by AcO⁻ ions: 2.58 < 2.69 < 2.77 Å on going from $[Cu_2(AcO)_4(MeCN)_2]$ through $[Cu_2(AcO)_5(MeCN)]^-$ to $[Cu_2(AcO)_6]^{2-}$ (see Fig. 6).
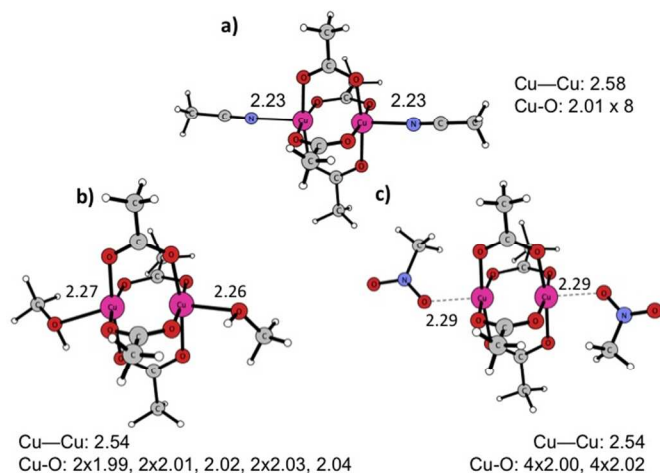

Figure 5. Geometric structures of binuclear $[Cu_2(AcO)_4(Solv)_2]$ species, where Solv = MeCN (a), MeOH (b) and MeNO₂ (c).
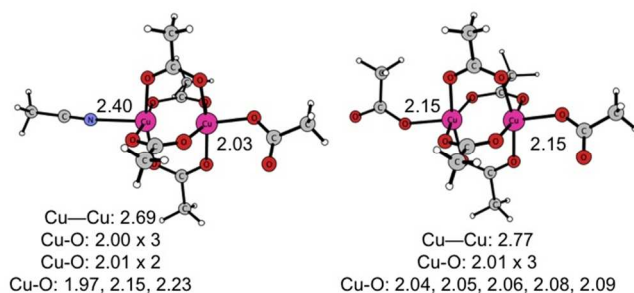

Figure 6. Geometric structures of binuclear copper species formed in MeCN in an excess of acetate ions: $[Cu_2(AcO)_5MeCN]^-$ (left) and $[Cu_2(AcO)_6]^{2-}$ (right).

Another substantial diversity within the tested solvents, concerns the stability of the binuclear species in the presence of an excess AcO⁻. In contrast to MeOH, one can find the complexes in MeNO₂ and MeCN to be susceptible to decomposition that is forced by the binding of additional acetate ligands (Eq. 4). A shift of the lower AcO⁻ concentration limit in MeNO₂ to the [AcO⁻]/[Cu(II)] ratio of 3, may be somewhat surprising when the smaller donor number (DN) of this solvent (2.7) compared to MeCN (14.1) is taken into account.[49-51] The much higher DN for MeOH (19.0) can in some way help to account for the higher stability of the binuclear complexes in this solvent.

Regardless of the solvent, the binuclear complexes are therefore the predominant species present in Cu(AcO)₂ solutions. The contribution of this form, increases further with increasing

concentration of Cu(AcO)₂ (compare Fig. S1 and Fig 4 in reference 43), which admittedly does not alter the course of the reaction with Chls but may significantly affect the results of the kinetic analysis.[12]

Analogously, the speciation of CuCl₂ was investigated in MeOH, MeCN and MeNO₂ using UV-Vis spectroscopy. The solutions of CuTf₂ were treated with small volumes of concentrated (Et₄N)Cl. As a consequence of increasing [Cl⁻], changes were observed in two regions of the spectrum. The expected increase in absorbance in the red and near-infrared region was observed in each solvent, however, the maximum absorbance is far from that reached by the acetate complexes under similar conditions. On comparing the solvents, chloride and acetate complexes of Cu(II) achieve the molar absorption coefficients of 3.2 and 55 (MeNO₂), 5.5 and 70 (MeOH), as well as 6 and 450 M⁻¹ cm⁻¹ (MeCN), respectively. Further increase in the concentration did not entail any significant changes in the absorption spectra and therefore such ε values, especially for (Et₄N)Cl titrated solutions, should be assigned to Cl-saturated compounds (presumably $[CuCl_2Solv_2]$), whereas in fully solvated species it does not exceed 1 M⁻¹ cm⁻¹. More pronounced changes are observed in the UV−Vis region up to 500 nm, since changes in the local environment of Cu(II) should be stronger reflected in CT than d-d transitions.

Whereas in MeOH and MeNO₂ a gradual increase in the absorbance is observed as the concentration of Cl⁻ is increased, changes in the high-energy region of the spectrum in MeCN are significantly more complex (Fig. 7). This complexity is probably a consequence of the properties of the solvent itself since it also appears in the case of the acetate complexes.
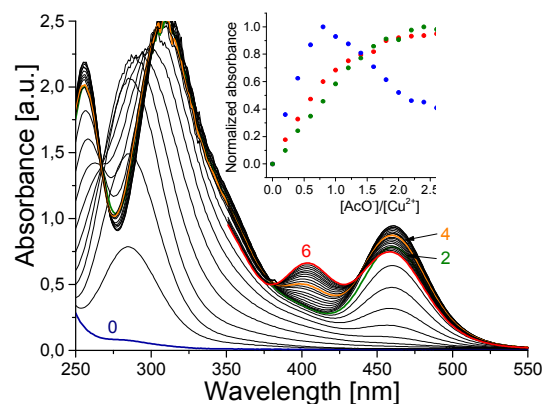

Figure 7. Absorbance changes that accompany the increase in [Cl⁻] in the solutions of 0.01 M CuTf₂ in MeCN at 298 K. Inset: titration curves in the red and NIR range were monitored at 806 (MeCN), 900 (MeNO₂) and 890 nm (MeOH). Coloured numbers on the graph indicate spectra recorded for specific [AcO⁻]/[Cu²⁺] ratios.

In contrast to other solvents, changes in the CT transitions concern a single band: quite weak in MeNO₂ where the maximum ε at 469 nm reaches 75 M⁻¹ cm⁻¹, and much stronger in MeOH, well beyond the measurement possibilities. The difference between ε values for CT and d-d transitions makes it impossible to analyze changes in the respective bands at the same level of Cu(II) concentration. For this reason we have omitted the analysis of changes in the band, taking also into account the fact that a decrease

in Cu(II) concentration will entail a change in [Cl⁻]/[Solv] and thus affect the speciation. The simplest case, viz. MeNO₂, shows an identical course of changes in both the absorption bands in the UV-Vis-NIR range. A plateau is reached at a ratio of 2.

Regardless of the inability to track changes in the UV range, it can be expected with high probability that a similar situation occurs in MeOH, and the only difference is the intensity and energy of the CT bands. A better insight can be obtained with respect to the structural analysis of chloride complexes in aqueous solution. X-ray studies reported by D'Angelo et al. led to the determination of the order of filling in the spatial position of the octahedral Cu(II) aqua complex by entering chloride ions.[52] According to this work, the experimental coordination number (CN) for Cl⁻ in aqueous solutions of CuCl₂ is only 0.4. Introduction of the second charged ligand requires application of a considerable excess and the average CN = 2 is achieved at the concentration ratio of 1:30. While the first Cl⁻ ligand is introduced in the equatorial position as indicated by the analysis of bond lengths, the second one substitutes a water molecule in the axial position.

Table 3. Geometric parameters of different $[CuCl(Solv)_5]^+$ and $[CuCl_2(Solv)_4]$ species obtained from DFT calculations.

Complex	CN (Cu²⁺)	No. S*	R_Cu-Solv [Å]	R_Cu-Cl⁻ [Å]
$[CuCl(MeCN)_5]^+$	5	1	1.989; 1.989; 2.051; 2.217	2.282
$[CuCl(MeOH)_5]^+$	3**	3	1.960; 1.961	2.559
$[CuCl(MeNO_2)_5]^+$	5 (6***)	1(0***)	2.051; 2.068; 2.095; 2.356	2.205
cis-$[CuCl_2(MeCN)_4]$	4 (distorted tetrahedron)	2	2.013; 2.017	2.221; 2.225
trans-$[CuCl_2(MeCN)_4]$	4 (distorted square)	2	1.956; 1.956	2.258; 2.258
cis-$[CuCl_2(MeOH)_4]$	4 (distorted square)	2	2.029; 2.137	2.185; 2.252
trans-$[CuCl_2(MeOH)_4]$	4 (distorted square)	2	2.035; 2.036	2.253; 2.253
cis-$[CuCl_2(MeNO_2)_4]$	4 (distorted square)	2	2.099; 2.138	2.182; 2.199
trans-$[CuCl_2(MeNO_2)_4]$	4 (square)	2	2.078; 2.093	2.202; 2.229

* number of solvent molecules in the 2nd coordination sphere;
** MeOH prefer to make hydrogen bonds between one and the other rather than to coordinate Cu(II) ion;
*** 5th ligand is placed in the sixth coordination position, but the Cu-Solv distance is 2.829 Å

In the case of organic solvents of weaker coordination ability, it is expected to shift the formation of electrically neutral complex species to lower values of [Cu(II)]/[L]. Such predictions are supported by the decreasing polarity of the neutral ligands, and with it the dielectric constant, whereby the solvent molecules must make a smaller contribution to the increasing enthalpy of binding of the anionic ligands due to the stronger shielding effect of the central ion and electrostatic repulsion between the polarized molecules. This may provide some explanation for saturation of the absorbance of Cu(II) complexes that is observed with increasing [Cl⁻] exceeding a double excess relative to the [Cu(II)]. Moreover, less polar solvents stabilize the charged particles to a lesser extent, which further facilitates the inclusion of the second chloride ion into the coordination sphere.

Seeking a clarification for the relative complexity observed in MeCN, particularly related to the chloride complexes, but also discernible in the case of acetate complexes, could be due to the cleavage of electronic levels in systems revealing the J-T effect. Since MeCN ligands are exceptionally weakly coordinated to Cu(II), as suggested by the results of DFT calculations, the octahedral

distortion involving elongation of the axial bonds is especially pronounced (compare data in Table 3). Such large J-T effects should result in stronger abolition of the degeneration of e_g and t_{2g} orbitals, thus allowing the appearance of an additional d-d transition. In contrast, the geometry of mixed H₂O/solvent complexes in MeOH and particularly in MeNO₂, are nearly octahedral (Table 3) and lead to the simplification of the absorption spectrum.

Given that the structures of Cl/MeCN complexes of Cu(II) have not been fully characterized, the possibility of greater isomeric diversity in this solvent should also be considered. The rigor of a *cis* isomerism in aqueous solution is certainly weakened when changing the solvent. Such an approach in turn applied to acetates, would provide an additional argument for different effects of the excess AcO⁻ on the stability of binuclear structures. The chloride ions, although known for their clustering properties and the specific efficiency in inner sphere electron transfer processes, do not form exceptional thermodynamically stable bridged complexes with Cu(II). Although there is evidence for the existence of such combinations in the solid phase, there is lack of both literature and experimental proof on the subject in solution, suggesting the monomeric complexes as predominant species in such solutions. Small changes in the ε values upon dilution (Fig. S2 in ESI) and increasing temperature (Fig. S3 in ESI), rather indicate dissociation of Cl⁻ from the neutral Cu(II) complexes than cleavage of the –Cl– bridges. Reports of the coexistence of cationic and anionic forms, namely $[CuCl(Solv)_{n-1}]^+$ and $[CuCl_3(Solv)_{n-3}]^-$, in certain organic solvents such as DMF, could not be sufficiently confirmed in the present study.

Based on the theoretical calculations for $[CuCl_2(Solv)_2]$ complexes, we attempted to rationalize the observed differences in UV-VIS spectra observed in the studied solvents. The geometric structures of the selected coordinative forms of copper ions with chloride ions in the studied solvents are proposed on the basis of theoretical modelling (see Table 3 and Fig. S4 in ESI). It appears that the coordination number of copper is reduced upon ligation of the anionic ligand.

The results of our theoretical modelling show that the immediate surroundings of the Cu(II) ion may vary in MeCN between distorted tetrahedron and distorted square for *cis*- and *trans*-systems, respectively (see Table 3). Such a change may give rise to the complication of the UV-Vis spectra, not observed for the analogous systems in MeOH and MeNO₂, where for both *cis*- and *trans*-complexes the immediate surroundings of the central metal is the same (distorted square for MeOH and square for MeNO₂). This explanation may seem unsatisfactory when comparing the Gibbs free energy difference between the *cis*- and *trans*-isomers in MeCN, which equals -27.4 kJ/mol, more than that found for MeOH (-21.2 kJ/mol) and MeNO₂ (2.0 kJ/mol). However, knowing that MeCN is a labile ligand, it may be assumed that rapid isomerisation of the complex, results in the co-existence of the cis- and trans-isomers in solution and, consequently, copper ions in different geometrical environments. Such an isomerisation may occur also in other solvents, nevertheless, not changing the geometry surrounding the metal ion.

Boundary conditions for electron transfer processes. As shown above, the redox processes in transition metal ion -

(metallo)dihydrotetrapyrrole systems are affected by the choice of solvent, and most of all the coordinating counter ion. Both, acetate that prevents oxidation of the macrocycle, as well as chloride that favours reversible one-electron processes, fulfil their functions in this system not only through impact on the reduction potential of Cu(II), but also through their individual characteristics. However, it is the redox potential of the reactive species present in the solution that determines the limiting concentrations required to switch the reaction pathways.

In order to determine the redox potentials of the main coordination forms of Cu(II) constituting the studied systems, electrochemical titrations were performed on $CuTf_2$ in each solvent using the approach applied in the spectroscopic determination of the speciation. Small amounts of concentrated $(Et_4N)Cl$ and $(Bu_4N)AcO$ were added to the titrated solutions of Cu(II) solvate complexes and the CVs were recorded. The same technique was also applied to determine the redox potentials of the pairs consisting of the tetrapyrrolic compounds, namely Pheo*a*, Chl*a* and Cu-Pheo*a*, and their respective cation radicals. Sample voltammograms recorded in MeCN are shown in Fig. 8.
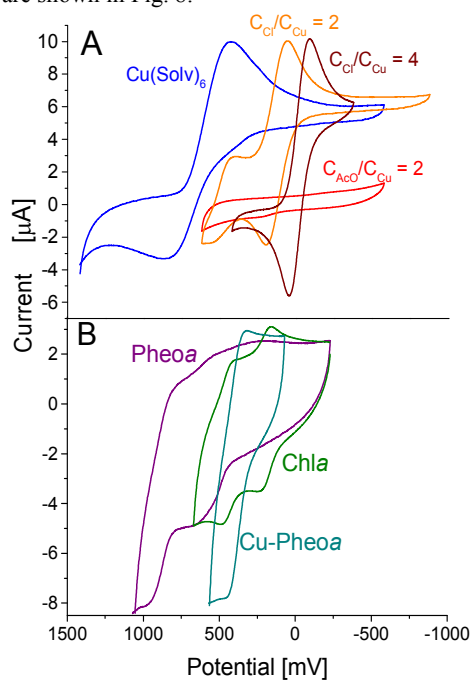

Figure 8. A comparison of the cyclic voltammograms of Cu^{2+} species (A) with Chls (B) in MeCN – the original plots recorded versus Ag/AgCl electrode.

Comparison of the voltammograms for Chls and various Cu(II) species indicates the possibility of the occurrence of electron transfer between the incoming metal and the tetrapyrrolic ligand. Detailed analysis of the results of the electrochemical measurements (see Table 4) provides a basis for predicting changes in the pathways of the Chls reactions with Cu(II). The comparison of the redox potentials of both photosynthetic pigments and various coordination forms of Cu(II) appearing in organic solvents in the presence of potential anionic ligands, allows to determine the limits for reactant concentrations at which a ligand substitution type of reaction is expected to change into an electron transfer process.

The redox behaviour of photosynthetic pigments have been extensively studied and described for a variety of simple and complex systems.[38-40, 53-55] The basic context of this type of research was usually the primary photosynthetic electron transfer. The results provided a detailed characterization of both cationic[39, 55] and anionic[54, 55] radicals of Chls, the products of one-electron transfer that are strictly related to the biological systems. Moreover, these studies have established a linear relationship between the redox potential of the π-electron system and the electronegativity of the central metal ion.[36] Such dependence is also known for metalloporphyrins, thus indicating the generality of this effect in relation to tetrapyrrole-derived compounds.[56]

Our results are in good agreement with the above observations. First of all, the Fuhrhop's electropotential series[56] is maintained in each of the tested solvents (Fig. 8B, Table 4). The difference between the half-wave potential of Chl*a* and its free base varies between different solvents, which to some extent may be elucidated by solvation and other solvent-dependent intramolecular interactions. Location of Cu-Pheo*a* in the middle of the scale provides a simple explanation for the loss of photosynthetic properties of plants growing on soils contaminated by this metal.[7]

Table 4. Observed half-wave potentials of Chls and Cu(II) complexes, related to NHE by comparison with the $FC^+|FC$ couple.

Redox pair		MeOH	MeCN	MeNO$_2$
Pheo*a*$^+$|Pheo*a*		+1.275	+0.139	+1.370
Chl*a*$^+$|Chl*a*		+0.710	+0.724	+1.175
Cu-Pheo*a*$^+$|Cu-Pheo*a*		+0.980	+0.969	+1.190
	Cu(Solv)$_6$	+0.410	+1.169	+1.280
	[Cl$^-$]/[Cu^{2+}] = 2	+0.392	+0.695	+0.812
Cu^{2+}|Cu$^+$	[Cl$^-$]/[Cu^{2+}] = 4	+0.404	+0.678	+0.791
	[AcO$^-$]/[Cu^{2+}] = 2	-0.053	—	+0.369
	[AcO$^-$]/[Cu^{2+}] = 4	-0.049	—	—

Differences in the solvation of the individual pigments are also reflected in the shapes of the oxidation and reduction bands. Regardless of the subtle changes at the transition between the solvents, it should be noted that the shape of the voltammogram of Pheo*a* indicates practically irreversibility of the one-electron oxidation. This pattern is essentially preserved in all tested solvents, thus remaining in conformity with the conclusions drawn from the observations of the reactions with Cu(II). In the case of Chl*a*, both oxidation and reduction signals point to full reversibility of the electron transfer process. It is somewhat weaker in MeCN (Fig. 8B) and also reflected in the course of the reactions with Cu(II) for low [L]/[Cu] values.

This and not another redox characteristic of photosynthetic pigments, probably stands in a logical relationship to the structure and activity of the "Z scheme" in the light-dependent phase of photosynthesis. Assuming that excited Chl*a*, while maintaining an appropriate scale, retains the ability for reversible oxidation, its photo-induced ionization meets all the criteria required for the

initiator of the electron transfer chain, starting from the oxygen evolving complex. Meanwhile Pheo*a*, being the first acceptor in PSII, must actually provide only the reversibility of the reduction process. It can, therefore, be concluded that while maintaining the same range of radiation absorbed, the choice of the Mg complex as the key element of the antenna system is dictated by both twice the efficiency of the solar energy conversion, expressed by ε, and the expected difficulties in returning to the ground electronic configuration of the Pheo*a*$^+$ form.

The change in solvent and, in particular, the use of a salt with a coordinating anion, have as expected a huge effect on the oxidative properties of Cu(II) (compare Fig. 8A). This effect is clearly pronounced in the case of acetate complexes. Increasing the ratio of [AcO$^-$]/[Cu(II)] from 0 to 2 entails reduction of the half-wave potential of the Cu(II) center by more than 0.45 V in MeOH and twice as much in MeNO$_2$.

While increasing the concentration of the coordinating anion, the shape of the voltammograms recorded in MeOH indicates the presence of at least two coordinative forms of Cu(II), as well as adoption of partially irreversible characteristics. On turning to an excess AcO$^-$, the signal is simplified which is understandable given the fact that under these conditions almost exclusively two-centre forms are present in solution. The difference between the anode and cathode potential may indicate the partial irreversibility of the redox couple, or results from the coupling of two metal centres.

The increase in AcO$^-$ concentration in MeNO$_2$ is accompanied by flattening of the recorded signal, which practically disappears in MeCN (Fig. S5 in ESI). Lack of evidence for electron donation or acceptance by the Cu(II) center observed over a wide range of potential, whatever it is caused by, indicates the disappearance of the danger of pigment oxidation in the presence of these ions and preservation of the non-redox pathways of both metalation and transmetalation of Chls.

The reduction of the half-wave potential of the Cu^{2+}|Cu$^+$ couple occurs also in the presence of chloride ions (Table 4). This effect is smaller, particularly for a ratio of [Cl$^-$]/[Cu(II)] = 2, i.e. for the coordination forms present in solutions of CuCl$_2$. One can observe the anodic peak to disappear and the amount of signalling components to be reduced with increasing concentration of Cl$^-$. This finding is in agreement with studies on Cu(II) speciation. Whereas this effect is very pronounced in MeCN and MeNO$_2$, it is barely noticeable in MeOH (compare Table 4).

In order to estimate the boundary conditions for electron transfer processes between Chls and Cu(II) complexes, the basic Nernst equation (5) was used under standard temperature and pressure conditions (298 K and 1 bar, respectively).

$$\Delta G^o = -F\left(E_{Cu} - E_{Chla} + \frac{RT}{nF}ln\frac{[Cu]}{[Chla]}\right) \quad (5)$$

Hence, for a one-electron transfer processes:

$$\frac{[Cu]}{[Chla]} = exp\left(\frac{F}{RT}(E_{Cu} - E_{Chla})\right) \quad (6)$$

The concentration ratios calculated for ΔG = 0, listed in Table 5, should be regarded as the apparent values, working on the assumption that 100% of Cu(II) is present in its reactive form. This assumption is quite consistent with reality, but wrong for CuAcO$_2$ solutions in which, within the tested range of concentration, non-reactive binuclear complexes are the predominant species. At higher Cu(II) concentration, and thus larger [Cu(II)]/[Chl], clustering is maintained and, therefore, the values given in Table 5 are underestimated.

Table 5. Minimum theoretical excess of [Cu(II)] on pigment necessary for electron transfer process. Data calculated on the basis of the redox potentials determined for Chl*a* and Pheo*a* as well as for CuTf$_2$ in the presence of different concentrations of Et$_4$NCl or Bu$_4$NAcO.

Chl	Cu/L	MeNO$_2$	MeCN	MeOH
Chl*a*	CuTf$_2$	1.67×10^{-2}	2.97×10^{-8}	1.19×10^{5}
	[Cl$^-$]/[Cu^{2+}]=2	1.38×10^{6}	3.09×10^{0}	2.39×10^{5}
	[Cl$^-$]/[Cu^{2+}]=4	3.13×10^{6}	6.00×10^{0}	1.50×10^{5}
	[AcO$^-$]/[Cu^{2+}]=2	4.30×10^{13}	∞	8.06×10^{12}
	[AcO$^-$]/[Cu^{2+}]=4	∞	∞	6.90×10^{12}
Pheo*a*	CuTf$_2$	3.33×10^{1}	3.11×10^{-1}	4.28×10^{14}
	[Cl$^-$]/[Cu^{2+}]=2	2.75×10^{9}	3.24×10^{7}	8.64×10^{14}
	[Cl$^-$]/[Cu^{2+}]=4	6.22×10^{9}	6.28×10^{7}	5.41×10^{14}
	[AcO$^-$]/[Cu^{2+}]=2	8.56×10^{16}	∞	2.91×10^{22}
	[AcO$^-$]/[Cu^{2+}]=4	∞	∞	2.49×10^{22}

Theoretically estimated concentration ranges of Cu(II), which favour oxidation of the dihydrotetrapyrrolic ring, are in general agreement with the experimental observations of the reaction courses. Under pseudo-first-order conditions, a relatively small excess of metal must induce the removal of an electron from the π-electronic system of the macrocycle. According to the results of the electrochemical measurements, the reaction of Pheo*a* in MeOH should be the exception. However, this was not confirmed in a direct experiment. A particularly low activation threshold for the redox process in MeCN appears to be due to poor coordination of Cu(II) by solvent molecules, as concluded from DFT calculations.

The lower oxidation potential of Chl*a* obviously makes it more sensitive towards a metal ion. Oxidation is observed in a wide range of concentrations of the chloride complex, while the AcO$^-$ ions virtually secure selection of the non-redox pathway. In the case of Pheo*a*, even coordination of Cl$^-$ induces an analogous effect, with the exception of MeNO$_2$ solutions. The difference in the redox potentials of the Mg complex and the free base does not account for the difference in the mechanism of electron transfer and its subsequent consequences.

Conclusions

Copper(II) ions are among the major metallic inhibitors of photosynthesis, both due to its abundance and reactivity towards tetrapyrrolic compounds. The availability of different reaction pathways in chorophyll - Cu(II) systems in the presence of atmospheric oxygen, draws attention to specific components of the cellular environment, providing protection of the pigment molecules from oxidative degradation of the macrocycle. The presence of carboxylate groups in the coordination sphere of the metal ion is one way of significantly reducing its redox potential and thus the probability of chlorophyll degradation. In vitro, this effect can be explained first of all by the change in speciation, leading to an increase in the concentration of polynuclear forms of Cu(II)

complexes. In such systems, the solvent plays an important role, since its solvation ability toward metal ions can significantly affect the speciation equilibria, inter alia, by stabilization or destabilization of the bridged forms.

Distinct differences between Chl*a* and Pheo*a* observed as a consequence of the occurrence of conditions that facilitate electron transfer, can be predicted by the different characteristics of the shapes of the oxidation bands in the electrochemical experiments. Reversible formation of the cation radical of Chl*a* and degradation of the macrocyclic structure in Pheo*a*, are probably the result of some additional factors that require thorough investigation. A more in-depth insight into the mechanisms of these two different processes will be presented in a forthcoming report.

Acknowledgements

Notes and references

[a] Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland. E-mail: orzel@chemia.uj.edu.pl

[b] Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek 8, 30-239 Kraków, Poland.

[c] Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland.

[d] Inorganic Chemistry, Department of Chemistry and Pharmacy, University of Erlangen-Nürnberg, Egerlandstrasse 1, 91058 Erlangen, Germany. E-mail: rudi.vaneldik@fau.de

1. S. J. Baum, B. F. Burnham and R. A. Plane, *P. Natl. Acad. Sci. USA*, 1964, 52, 1439-1442.
2. T. Masuda, *Photosynth. Res.*, 2008, 96, 121-143.
3. J. D. Reid and C. N. Hunter, *J. Biol. Chem.*, 2004, 279, 26893–26899.
4. C. J. Walker and R. D. Willows, *Biochem. J.*, 1997, 327, 321-333.
5. H. Küpper, F. Küpper and M. Spiller, *Photosynth. Res.*, 1998, 58, 123–133.
6. H. Küpper, F. Küpper and M. Spiller, *J. Exp. Bot.*, 1996, 47, 259-266.
7. H. Küpper, I. Setlik, M. Spiller, F. Küpper and O. Prasil, *J. Phycol.*, 2002, 38, 429–441.
8. A. S. Brandis, Y. Salomon and A. Scherz, in *Chlorophylls and Bacteriochlorophylls. Biochemistry, Biophysics, Functions and Applications.*, eds. B. Grimm, R. J. Porra, W. Rudiger and H. Scheer, Springer, Dordrecht, 2006, ch. 32, pp. 462-483.
9. T. J. Dougherty, *Photochem. Photobiol.*, 1993, 58, 895-900.
10. V. Rosenbach-Belkin, L. Chen, L. Fiedor, Y. Salomon and A. Scherz, in *Photodynamic Tumor Therapy. 2nd and 3rd Generation Photosensitizers*, ed. J. G. Moser, Harwood Academic Publishers, Amsterdam, 1998, pp. 117-125.
11. G. Hartwich, L. Fiedor, I. Simonin, E. Cmiel, W. Schafer, D. Noy, A. Scherz and H. Scheer, *J. Am. Chem. Soc.*, 1998, 120, 3675-3683.
12. Ł. Orzeł, R. van Eldik, L. Fiedor and G. Stochel, *Eur. J. Inorg. Chem.*, 2009, 2393–2406.
13. W. Rüdiger, *Photochem.*, 1997, 46, 1151-1167.
14. K. Iriyama, N. Ogura and A. Takamiya, *J. Biochem.*, 1974, 76, 901-904.
15. T. Omata and N. Murata, *Plant Cell Physiol.*, 1983, 24, 1093-1100.
16. a. d. o. U. o. K. a. TURBOMOLE V6.4 2012, -. Forschungszentrum Karlsruhe GmbH, s. a. f. TURBOMOLE GmbH and www.turbomole.com.
17. A. D. Becke, *Phys. Rev. A*, 1988, 38, 3098-3100.
18. P. A. M. Dirac, *Proc. R. Soc. Lond. A*, 1929, 123, 714-733.
19. J. P. Perdew, *Phys. Rev. B*, 1986, 33, 8822-8824.
20. J. C. Slater, *Physical Review*, 1951, 81, 385-390.
21. S. H. Vosko, L. Wilk and M. Nusair, *Canadian Journal of Physics*, 1980, 58, 1200-1211.
22. K. Eichkorn, O. Treutler, H. Oehm, M. Haeser and R. Ahlrichs, *Chem. Phys. Lett.*, 1995, 240, 283-290.
23. K. Eichkorn, F. Weigend, O. Treutler and R. Ahlrichs, *Theor. Chem. Acc.*, 1997, 97, 119-124.
24. J. Heimdal, K. P. Jansen and A. Devarajan, *J. Biol. Inorg. Chem.*, 2007, 12, 49-61.
25. Ł. Orzeł, A. Kania, D. Rutkowska-Żbik, A. Susz, G. Stochel and L. Fiedor, *Inorg. Chem.*, 2010, 49, 7362-7371.
26. D. Rutkowska-Żbik and T. Korona, *J. Chem. Theo. Comp.*, 2012, 8, 2972-2982.
27. D. Rutkowska-Żbik, M. Witko and L. Fiedor, *J. Mol. Model.*, 2013, 19, 4661–4667.
28. A. Schaefer, C. Huber and R. Ahlrichs, *J. Chem. Phys.*, 1994, 100, 5829-5835.
29. A. Klamt and G. Schüürmann, *J. Chem. Soc., Perkin Trans. 2*, 1993, 799-805
30. M. Gouterman, *J. Mol. Spectrosc.*, 1961, 6, 138-163.
31. M. Gouterman, G. H. Wagniere and L. C. Snyder, *J. Mol. Spectrosc.*, 1963, 11, 108-127.
32. D. Sundholm, *Chem. Phys. Lett.*, 1999, 302, 480–484.
33. D. Sundholm, *Chem. Phys. Lett.*, 2000, 317, 545-552.
34. D. Sundholm, *Chem. Phys. Lett.*, 2000, 317, 392-399.
35. Ł. Orzeł, J. Waś, A. Kania, D. Rutkowska-Żbik, L. Fiedor, M. Witko and G. Stochel, *submitted to Inorg. Chem.*, 2014.
36. D. Noy, L. Fiedor, G. Hartwich, H. Scheer and A. Scherz, *J. Am. Chem. Soc.*, 1998, 120, 3684-3693.
37. T. Watanabe and K. Honda, *J. Am. Chem. Soc.*, 1980, 102, 370-372.
38. T. Watanabe and M. Kobayashi, in *Chlorophylls*, ed. H. Scheer, CRC Press, Boca Raton, 1991, vol. 1.
39. D. C. Borg, J. Fajer, R. H. Felton and D. Dolphin, *P. Natl. Acad. Sci. USA*, 1970, 67, 813.
40. M. S. Davis, A. Forman and J. Fajer, *P. Natl. Acad. Sci. USA*, 1979, 76, 4170-4174.
41. D. A. Lightner, T. A. Wooldridge, S. L. Rodgers and R. D. Norris, *Experientia*, 1980, 36, 380-382.
42. K. S. Lee and L. M. Gartner, *Pediatr. Res.*, 1976, 10, 782-788.

43. Ł. Orzeł, L. Fiedor, A. Kania, M. Wolak, R. van Eldik and G. Stochel, *Chem. Eur. J.*, 2008, 14, 9419 – 9430.

44. M. Ulrich, S. Moser, T. Mueller and B. Kräutler, *Chem. Eur. J.*, 2011, 17, 2330-2334.

45. B. Kräutler, B. Jaun, K. Bortlik, M. Schellenberg and P. Matile, *Angew. Chem.*, 1991, 30, 1315-1318.

46. B. Kräutler, B. Jaun, P. Matile, K. Bortlik and M. Schellenberg, *Angew. Chem. Int. Ed.*, 1991, 30, 1315-1318.

47. J. Berghold, K. Breuker, M. Oberhuber, S. Hörtensteiner and B. Kräutler, *Photosynt. Res.*, 2002, 74, 109-119.

48. D. Pocic, J.-M. Planeix, N. Kyritsakas, A. Jouaiti and M. W. Hosseini, *Cryst. Eng. Comm.*, 2005, 7, 624–628.

49. V. Gutmann, *The donor-acceptor approach to molecular interactions*, Plenum Press, New York-London, 1978.

50. F. L. Riddle Jr. and F. M. Fowkes, *J. Am. Chem. Soc.*, 1990, 112, 3259-3264.

51. G. Wypych, ed., *Handbook of Solvents*, ChemTec Publishing-William Andrew Inc., Toronto - New York, 2001.

52. P. D'Angelo, E. Bottari, M. R. Festa, H.-F. Nolting and N. V. Pavel, *J. Chem. Phys.*, 1997, 107, 2807-2812.

53. J. P. Chauvet, R. Viovy, R. Santus and E. J. Land, *J. Phys. Chem.*, 1981, 85, 3449-3456.

54. J. Fajer, D. C. Borg, A. Forman, D. Dolphin and R. H. Felton, *J. Am. Chem. Soc.*, 1973, 95, 2739-2741.

55. S. Sinnecker, W. Koch and W. Lubitz, *J. Phys. Chem. B*, 2002, 106, 5281-5288.

56. J.-H. Fuhrhop, K. M. Kadish and D. G. Davis, *J. Am. Chem. Soc.*, 1973, 95, 5140-5147.

Journal Name

ARTICLE

Fine tuning of copper(II) – chlorophyll interactions in organic media. Metalation versus oxidation of the macrocycle

Łukasz Orzeł,* Bartłomiej Szmyd, Dorota Rutkowska-Żbik, Leszek Fiedor, Rudi van Eldik* and Grażyna Stochel
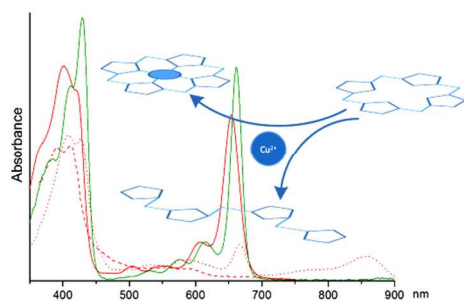
A variety of pathways and products of chlorophyll *a* reactions with Cu(II) salts were found in organic solvents. With this model system we show how the reactivity of metal ions can be tuned by slight changes in the composition of the medium.