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Mechanistic Information on $\mathrm{Cu^{II}}$ Metalation and Transmetalation of Chlorophylls

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Chlorophyll *a* and pheophytin *a* have been treated with various metal salts in solution. As a result, metallo derivatives of photosynthetic pigments were formed, or the chlorine ring was oxidized. These studies showed that both effects can be caused by $\mathrm{Cu^{II}}$ ions depending on the redox potential of its complexes formed in specific media. In this report the results of studies on metal insertion and exchange reactions are presented. According to the common use of the "acetate method" in the synthesis of metallotetrapyrroles, and the fact that acetates coordinated to $\mathrm{Cu^{II}}$ ions protect chlorophylls from oxidative degradation, $\mathrm{Cu^{II}}$ acetate monohydrate was selected as main reagent. The metalation and transmetalation processes were investigated with spectroscopic (UV/Vis absorption and emission) and kinetic (conventional and high-pressure) techniques. It turned out that differences in solvent properties can significantly affect not only the rates (metalation), but also the course (transmetalation) of the reaction. The most pronounced effect was found for the transmetalation reaction in acetonitrile, which was terminated at the very initial stage. As shown elsewhere, some special agents/factors, such as excess of acetate, are required to facilitate the dissociation of $\mathrm{Mg^{2+}}$ and to push the reaction forward towards formation of the $\mathrm{Cu^{II}}$ derivative of chlorophyll. The mechanisms of two other reactions are proposed on the basis of the determined activation parameters, which correspond to the general mechanisms for metalation and transmetalation of tetrapyrroles with sitting-atop and bimetallic intermediates, respectively. The bimetallic complex of chlorophyll in methanol is probably the most stable complex of this type observed ever. It confirms that spontaneous exchange of $\mathrm{Mg^{2+}}$ by $\mathrm{Cu^{II}}$ occurring in plants must result from specific conditions and/or involve contribution from other components of the photosystem.

Introduction

Metalloporphyrins play essential roles in many biological and synthetic systems. Most often they serve as catalytic centers in a variety of transfer reactions while their reduced derivatives, chlorins and bacteriochlorins, act as photoactive cofactors in photosynthesis.[1] In many instances, the central metal functions as a switch between various types of activities in which metalloporphyrins are involved. The central pocket in chlorophylls (Chls) and bacteriochlorophylls (BChls), main photosynthetic pigments, usually is occupied by the $\mathrm{Mg^{2+}}$ ion. The complexes of Mg are labile and its re-insertion is not straightforward; in vivo it requires specialized enzymes, Mg-chelatases, to catalyze this reaction,[2,3] whereas many divalent metal ions spontaneously bind to the Chl central pocket, forming very stable complexes.[4,5] The metal exchange reactions of Chls and BChls are of both theoretical and practical interest. For instance, a spontaneous insertion of transition metals, such as Cu and Ni (soil pollutants), may occur in vivo and lead to the irreversible impairment of photosynthetic activity in plants.[6,7] Recently, metallochlorophylls and metallobacteriochlorophylls came into focus for being interesting model pigments[8–11] and for their excellent photosensitizing properties.[12–16]

For decades already, metalation [Equation (1)] and transmetalation [Equation (2)] of porphyrins and Chls, shown in Equations (1) and (2), respectively:

$$\mathrm{H_2P + M^{2+} \rightleftharpoons MP + 2H^+} \tag{1}$$

$$M\mathrm{P} + \mathrm{M}^{2+} \rightleftharpoons \mathrm{MP} + M^{2+} \tag{2}$$

where $\mathrm{H_2P}$ is a free base and MP is a metallo complex, have been extensively studied.[17–20] The formation of metalloporphyrins from free base and metal ions [Equation (1)] occurs via multiple steps. A widely disputed key intermediate of metalation, the so called sitting-atop complex (SAT), involves a metal ion bound to two pyrrolenine nitrogens,

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while two protons still remain bound to the other two nitrogens. The SAT complex, postulated originally long ago by Fleischer and Wang,[21] only recently was spectroscopically identified by Funahashi et al.[22,23] A different type of intermediate, a bimetallic complex, in which the leaving and entering metal ions stick out on opposite sides of the macrocycle plane,[24] was suggested to appear in transmetalation reactions, see Equation (2). In case of Chls and BChls, structural features, in particular the isocyclic ring with its substituents and phytol side chain,[25] seem to play a role in these processes. These peripheral functionalities affect the electron density on the pyrollenine nitrogens,[8] make the entire macrocycle more rigid[25] and pose steric hindrance,[11] which are essential from the mechanistic point of view because the initial deformation of the macrocycle enables the release of protons and interactions with approaching metal ions.[26]

Although the interactions of tetrapyrrolic systems with metal ions are a subject of intensive studies, and the interest in their metalloderivatives lead to the development of many synthetic approaches, detailed mechanisms for both metal insertion and metal exchange in tetrapyrrolic systems are still not well established. Therefore, our previous study focused on the role of the acetate ion in facilitating metal insertion reactions.[27] In the present work we concentrated on the role of metal ion–solvent coordination in the metalation and transmetalation reactions of Chls. The reactions were investigated for a model system comprising of the transmetalation of chlorophyll *a* (Chla) and metalation of its free base pheophytin *a* (Pheo) with Cu^{II} in two solvents, methanol and acetonitrile (MeCN). The kinetics of the reactions was followed by absorption spectroscopy in combination with high pressure techniques and by steady state and time resolved fluorescence spectroscopy. The kinetics of metalation and the product of transmetalation are strongly controlled by solvent properties.

Results and Discussion

Choice of Model Reactions

Since long it is known that many divalent metal ions (e.g. Co^{II}, Ni^{II}, Zn^{II}, Cd^{II}, Hg^{II} and Pb^{II}) react spontaneously with Pheo or Chla in organic solvents, yielding respective metalloderivatives,[4,28] while some redox-active metal ions (e.g. Cu^{II} and Fe^{III}) may cause irreversible oxidation of the chlorin ring. From the mechanistic point of view, Cu^{II} is one of the most interesting cases because the course of its reactions with Chls, i.e. metal insertion vs. oxidation, can be controlled by selecting appropriate counterions. Therefore, in our model system we decided to investigate interactions of Pheo and Chla with various salts of Cu^{II}, viz. triflate (Tf), chloride, and acetate (Ac), in two organic solvents, viz. methanol and acetonitrile. The solvents were selected taking into account their inertness and the solubility of reactants. The course of the reactions with metal ions can be followed spectroscopically taking advantage of characteristic shifts of electronic transitions of Chla, especially of the Q_Y transition located near 660 nm.

The addition of Cu^{II} ions to a solution of Pheo caused considerable changes in the absorption spectrum of the pigment: a blue shift of the Q_Y band and shifts in the Soret band, with clean isosbestic points at 655 and 420 nm (see below), indicating the occurrence of the metalation reaction. Similar changes were observed during the reaction of Chla with Cu^{II}, but less pronounced than in the case of Pheo and their rates were slower (see further sections). As shown in Figures S1 and S2 in the Supporting Information, for the reactions of Cu^{II} with Chls, two other patterns of absorption changes were found: a gradual decrease of all absorption bands (Figure S1, Supporting Information), and a rapid decrease of the Q_Y band followed by its slower reformation (Figure S2, Supporting Information). The first two types of spectral changes are similar to those previously observed in reactions with other metals, whereas the third pattern seems to be a new type of reaction (manuscript in preparation).

Both, the metalation and transmetalation reactions, can be treated as ligand exchange processes. Therefore, the solvent and counterions should be considered as reactants that are able to compete with the tetrapyrrolic ligand for binding to the metal center. Ligand exchange involving a redox reactive metal species, such as Cu^{II}, can change its redox potential so considerably that metal insertion or substitution in the tetrapyrrolic core will be accompanied by electron transfer reactions. As a consequence, the tetrapyrrole π-electron system may undergo a reversible or irreversible oxidation. As indicated by the characteristic changes in the absorption spectra, the former process takes place e.g. when Chla reacts with $CuCl_2$ in MeOH (Figure S2) while the latter one leading to bleaching, is observed in the reaction of Pheo with $CuTf_2$ in $MeNO_2$ (Figure S1). Because bleaching of Pheo or Chla was never observed with $CuAc_2$, this salt was applied as the source of Cu ions in metalation of Pheo and transmetalation of Chla, but does cause side reactions in both MeOH and MeCN as the reaction medium.

Coordination Forms of Cu^{II} in MeOH and MeCN

In order to determine the coordinative forms of Cu^{II} at different Ac concentrations, spectroscopic titrations of $CuTf_2$ with tetrabutylammonium acetate (TBAA) were performed in MeOH and MeCN. In MeOH, the increasing Ac concentration favored the formation of dimeric complexes, whereas in MeCN monomers became the predominant species at $[Ac]/[Cu^{II}] > 3$.[27]

Additional information was obtained from the absorption spectra of $CuAc_2$ recorded at various concentrations, shown in Figure 1 as plots of the average extinction coefficient (ε) of Cu^{II} species coexisting at equilibrium as a function of total Cu^{II} concentration. In MeCN, ε changes within a quite narrow range, whereas in MeOH there is a clear increase in the average value of ε with increasing Cu^{II} concentration.

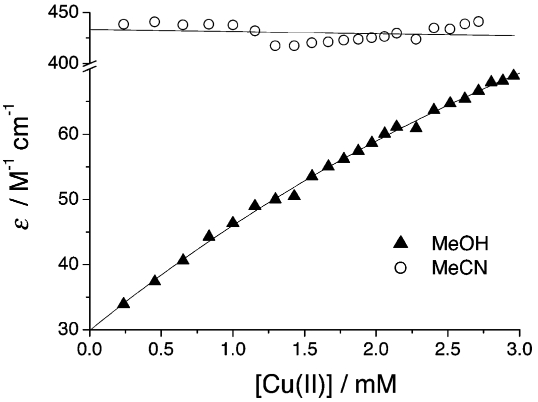

Figure 1. Dependence of the average extinction coefficients of Cu^{II} complexes formed in MeOH (▲) and MeCN (○) on the $CuAc_2$ concentration. Conditions: $T = 298$ K, $\lambda = 715$ nm.

Several $[CuAc_m(Solv)_n]^{2-m}$ complexes, where Solv denotes MeOH or MeCN, might possibly be present in solution of $CuAc_2$ and the following equilibria should be considered:

$$[Cu(Solv)_6]^{2+} + Ac^- \rightleftharpoons [Cu(Solv)_4Ac]^+ + 2Solv \qquad K_1 \qquad (3)$$

$$[Cu(Solv)_4Ac]^+ + Ac^- \rightleftharpoons [Cu(Solv)_2Ac_2] + 2Solv \qquad K_2 \qquad (4)$$

$$2[Cu(Solv)_2Ac_2] \rightleftharpoons [Cu_2Ac_4(Solv)_2] + 2Solv \qquad K_3 \qquad (5)$$

$$[Cu(Solv)_2Ac_2] + Ac^- \rightleftharpoons [CuAc_3]^- + 2Solv \qquad K_4 \qquad (6)$$

$$[Cu_2Ac_4(Solv)_2] + 2Ac^- \rightleftharpoons 2[CuAc_3]^- + 2Solv \qquad K_5 \qquad (7)$$

Each equilibrium involves a gradual binding of odd numbers of solvent molecules but Ac as chelate, not as monodentate ligand, should be considered, since only 1:1, 1:2 and 1:3 complexes were spectroscopically distinguished in these solvents.[27] Changes in the absorption spectra of $CuAc_2$ gave no evidence of any effect of dilution on equilibria (4) and (5) in MeCN. On the contrary, a strongly absorbing complex of Cu^{II} was observed in MeOH at high concentrations of $CuAc_2$. A slight blue-shift of the absorption maximum (not shown) points to dimer formation in reaction (5), although the coordination of a second Ac^- by $CuAc^+$ to form $CuAc_2$ in reaction (4) can also be expected to take place.

In both solvents, and in the absence of other potential ligands, Cu^{II} exists usually as $[Cu(Solv)_6]^{2+}$[29,30] but $CuAc_2$ forms dimeric species, both in the crystalline state[31] and in solution.[32–34] All four acetate (Ac) ligands involved in complexes of the type $[Cu_2Ac_4(Solv)_2]$, bridge the Cu^{II} ions making them inert towards metallotetrapyrrole formation.[35] Some activation of Cu^{II} ion can be expected in monomeric $[CuAc(Solv)_n]^+$ species[35,36] as the positive charge facilitates electrophilic attack on the pyrrolenine nitrogens, and the lone electron pairs on Ac oxygens can participate in hydrogen bonding, and hence, in deprotonation of the tetrapyrrole core. The concentration of this species is, however, controlled by equilibria (3) and (4) that are strongly shifted towards Ac coordination.[27]

Assuming $K_2 = 6\times10^{-3}$ M and $K_3 = 3\times10^{-4}$ M (on the basis of Grasdalen's work[37]) and taking into account that values of all other *Ks* are negligible in MeOH,[37] the concentrations of the co-existing Cu^{II} species were calculated for different total Cu^{II} concentrations. In 0.25 mM $CuAc_2$, the 1:1 complex predominates and $\Sigma[\text{monomer}] \approx 10[\text{dimer}]$, and when the Cu^{II} concentration is tenfold higher, $[\text{dimer}] > [\text{monomer}] \approx 2[CuAc^+]$. These estimations show that the equilibria and, consequently, the concentrations of the reactive species change drastically within a relatively narrow range of $CuAc_2$ concentrations. As reported by Grasdalen,[37] the extinction coefficients of $[CuAc(MeOH)_4]^+$, $[CuAc_2(MeOH)_2]$ and $[Cu_2Ac_4(MeOH)_2]$ in MeOH at 700 nm were estimated as ca. 19, 37 and 180 $M^{-1}cm^{-1}$, respectively. On this basis, the theoretical values of absorbance of $CuAc_2$ solutions in MeOH could be compared with the experimental ones. Thus, A_{700} measured at the lowest and highest concentrations of Cu^{II} used were 0.081 and 0.19, very close to the calculated values, 0.079 and 0.18, respectively.

In addition to the concentration effect, a significant influence of temperature on the Cu^{II} speciation in both solvents was found. As shown in Figure 2A, the absorbance at 715 nm in MeOH decreased with increasing temperature, with the effect being stronger at the higher concentration.

A similar trend was found for MeCN (not shown). The absorbance dependence on $CuAc_2$ concentration determined at various temperatures showed different trends in

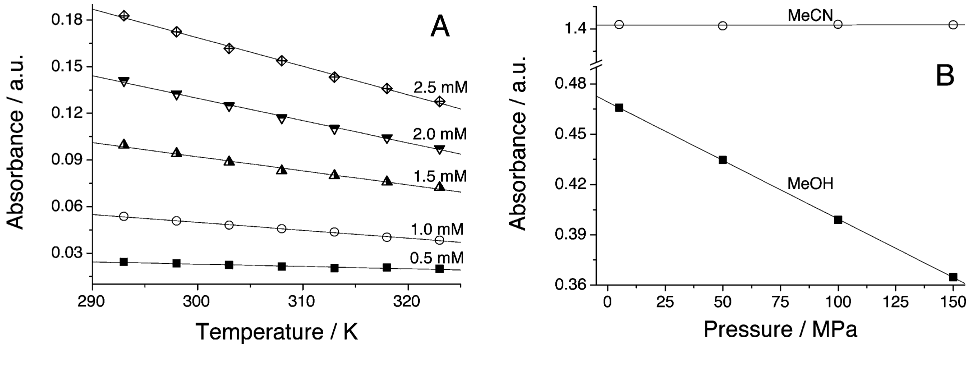

Figure 2. The influence of temperature (**A**) and pressure (**B**) on the speciation of $CuAc_2$ in MeOH (A and B) and MeCN (B). Conditions: A: $\lambda = 715$ nm. B: $\lambda_{MeOH} = 715$ nm, $\lambda_{MeCN} = 675$ nm, $[Cu^{II}] = 5$ mM, $T = 298$ K.

MeOH (Figure S3A) and in MeCN (Figure S3B). Whereas the absorbance measured at 675 nm in MeCN increased linearly, the relationship between A_{700} and [$CuAc_2$] in MeOH was apparently more complex, i.e. sigmoidal. The same model could be applied for the interpretation of the concentration dependence in MeCN, however, the curvature in MeOH is much more pronounced. Nevertheless, in both solvents the course of the relationship was steeper at lower temperatures.

A comparison of plots in Figure 1 and Figure S3B (Supporting Information) shows an agreement between the linear relationship of absorbance vs. total [Cu^{II}] and the independence of the average extinction coefficient on the total [Cu^{II}] in MeCN. This agreement is not as straightforward in MeOH, where the sigmoidal plot of absorbance vs. total [Cu^{II}] confirms a change in the Cu^{II} speciation. The drop of absorption at elevated temperatures can be attributed to the formation of monomeric species. Thus the monomer–dimer equilibria seem to be slightly more temperature-dependent in MeOH than in MeCN.

Upon increasing pressure up to 150 MPa, the decrease in the absorption of Cu^{II} complexes in MeOH was observed, largely reversible on decreasing the pressure. No such changes were seen in MeCN under analogous conditions. Thus, in contrast to the expected pressure-induced increase in dimer formation, no shift in equilibrium (5) occurred in MeOH and MeCN. Since the maxima of the absorption bands were not affected (not shown), it follows that increasing pressure favors dissociation of Ac in MeOH and does not influence the Cu^{II} speciation in MeCN.

The coordinative equilibria of $CuAc_2$ introduce additional difficulties in the kinetic studies of interactions of Cu^{II} with Chls and thus only apparent rate constants and activation parameters could be directly determined. The complete thermodynamic description of the Cu^{II}-Ac-Solv systems enabled to asses the concentrations of the reactive species and thus to estimate actual values of k_{obs}, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$. Present studies show that the rate of Cu–Chla formation depends strongly on the coordinative properties of the solvent. In MeOH, the formation of non-reactive dimeric species is favored by increased concentration of $CuAc_2$ and disfavored by temperature and pressure. The equilibria between Cu^{II} species in MeCN are less sensitive to these factors.

Metalation of Pheo

Spectroscopic Studies

The coordination of Cu^{II} ion by Pheo was followed by absorption and emission UV/Vis spectroscopy. Figure 3 (A) shows changes in the absorbance of Pheo solution which accompany the reaction with $CuAc_2$ in MeOH. Due to an overlap of the d-d transitions in Cu^{II} complexes with the Q_Y band of Pheo, a $CuAc_2$ solution (at least 100-fold excess over [Pheo]) was used as a reference. An immediate decrease in the absorbance at above 550 nm was observed immediately after mixing of the reactants.

In order to explain this effect, a simple experiment was performed in which both the sample and the reference cells were filled with 5 mM $CuAc_2$ solution in MeOH in the one compartment of the cell and neat solvent in the other compartment, such that the overall absorbance was zero over the whole spectral range. Then the contents of the cell in the sample beam were mixed, whereas the reference cell was kept intact. An instantaneous decrease in absorption between 550 and 900 nm was observed (inset 1 in Figure 3, A). This finding was analogous to that found during the spectroscopic investigation of Cu^{II} speciation in MeOH (Figure 1). Hence the lowering of the baseline in the long wavelength range of the spectra recorded during the reaction with Pheo must result from the rapid solvolysis of the $CuAc_2$ complex on dilution. Since the maximum of the absorption of the Cu^{II} complex remained at its earlier position, viz. ca. 700 nm, a shift in equilibrium (2) seems to be more justified than for equilibrium (1). The observed absorption changes may also be related to solvent coordination and Cu^{II}–Ac dechelation as the stability of a four-member ring is relatively low compared to that of larger ring systems. Thus, the succeeding dissociation of Ac^- could occur as a much slower step.

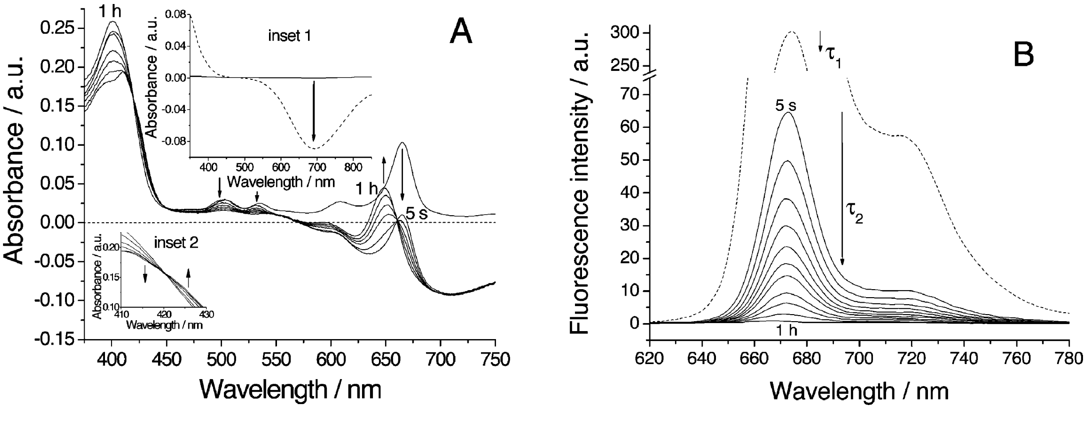

Figure 3. Absorbance (A) and fluorescence (B) changes accompanying the reaction of Pheo with $CuAc_2$ in MeOH. Conditions: [Pheo] = 2.5 μM, [Cu^{II}] = 2.5 mM, T = 298 K, λ_{exc} = 415 nm (B), t = 1 h. (A) inset 1: changes in the absorbance of the $CuAc_2$ solution after twofold dilution in a tandem cuvette; inset 2: isosbestic point at 420 nm.

The rapid decrease in the absorption in the long-wavelength range was followed by a slower step in which the pigment Q_Y band shifted from 666 to 648 nm while bands between 500 and 550 nm disappeared. At 298 K the reaction was complete within one hour. All these spectral changes point to the formation of a Cu^{II} derivative of Chla.

In the emission spectra that correspond to Cu–Chla formation in MeOH (Figure 3, B), kinetically two reaction steps could be distinguished. The first one was complete within the time of mixing (several seconds) and led to an 80% decrease in the initial fluorescence intensity. The subsequent slower step led to a complete quenching of the emission in the time scale comparable with that of changes in the absorbance observed in the incorporation of Cu^{II} into the tetrapyrrolic ring. Hence, it was assigned to the formation of coordinative bonds with Cu^{II} ion, whereas the fast preceding step indicates some weaker initial interaction between the reactants.

The baseline shift preceding the reaction with Pheo in MeCN was hardly observed (Figure S4A) and the subsequent changes in the absorption spectrum were analogous to that found in MeOH. Similarly, the Pheo emission in MeCN was gradually quenched in the presence of $CuAc_2$ and the fast initial decrease was even more efficient than in MeOH (Figure S4B).

Lack of the rapid absorption decrease at the stage of mixing the reactants in MeCN confirms that the K_2 and K_3 values are in MeCN significantly larger than in MeOH. Consequently, the concentration of the reactive Cu^{II} species was proportional to the total Cu^{II} concentration over the whole concentration range applied in the kinetic studies. Despite that the Cu^{II} dimer does not undergo dilution-induced solvolysis in MeCN, its initial interaction with Pheo, presumably of electrostatic nature, seems to be quite strong and manifest itself in efficient energy transfer from the macrocycle to the metal ion. A subsequent complex-formation occurs in a single step as indicated by the isosbestic points at 660 and 418 nm (Figure S4A).

Kinetic Studies

The kinetics of the metalation process was studied under pseudo-first-order conditions with an excess of $CuAc_2$. Absorption changes were monitored at 640 nm and the obtained kinetic traces were fitted to a single exponential function, resulting in values for the observed first-order rate constants, k_{obs}. Plots of k_{obs} vs. $[Cu^{II}]$ as a function of temperature are shown in Figure 4.

The linear concentration dependences in MeOH (Figure 4, A) revealed distinct intercepts. Since the overall absorbance changes that accompany the reaction did not depend on the Cu^{II} concentration, the intercepts must be interpreted in terms of a parallel reaction rather than a back reaction. Thus the rate law is given by Equation (8).

$$k_{\text{obs}} = k_1[\text{Cu}^{\text{II}}] + k_2 \tag{8}$$

The rate constants for the parallel reactions k_1 and k_2 are $0.72 \pm 0.05\ \text{M}^{-1}\text{s}^{-1}$ and $(6.7 \pm 0.8) \times 10^{-4}\ \text{s}^{-1}$ at 298 K, respectively.

A different type of concentration dependence was found in MeCN (Figure 4, B). A kinetic saturation was reached at high $[Cu^{II}]$ and the isothermal series of points could be fitted satisfactorily with a non-linear function of the type.

$$k_{\text{obs}} = \frac{k_1 K[\text{Cu}^{\text{II}}]}{1 + K[\text{Cu}^{\text{II}}]} + k_2 \tag{9}$$

In this model, k_1 and k_2 are the rate constants of two parallel reaction pathways involving different Cu^{II} species, while K represents the constant for the pre-equilibrium, preceding the rate-limiting step. At 298 K, the values of k_1, k_2 and K were calculated to be $5\ (\pm 1) \times 10^{-4}\ \text{s}^{-1}$, $7\ (\pm 2) \times 10^{-5}\ \text{s}^{-1}$ and $1204 \pm 218\ \text{M}^{-1}$, respectively.

Whereas the kinetics of Cu^{II} binding by Pheo in MeOH is described by the linear equation, the reaction in MeCN obeys the Tanaka's type of law proposed for porphyrin metalation.[38] The difference lies in the second term including k_2, which in the case of Tanaka's work was second order in metal ion concentration. In that model, one metal ion formed the intermediate described as the sitting-atop (SAT) complex, whereas the second one dropped into the deformed porphyrin nucleus from the opposite side.[39]. The origin of the difference in the rate laws found for the reactions in MeOH and MeCN is not straightforward. A possible explanation is that the seemingly linear relationship between k_{obs} and $[Cu^{II}]$ in MeOH only reaches saturation at very high Cu^{II} concentrations. This presumption follows from some earlier work on porphyrin metalation in which an apparently first order[19,40] or half order[41] dependence on the metal ion concentration turned out to be more complicated over a much wider concentration range.[38,42] Unfortunately, the kinetic studies on the metalation of Pheo were limited by the solubility of $CuAc_2$ in MeOH.

The K value determined in MeCN applies to the fast pre-equilibrium which precedes the actual coordination of Pheo by Cu^{II}. Regardless of the rate-determining step, which could be either a SAT complex-formation or its collapse to the final product,[43] K must be attributed to some physical interaction, e.g. a formation of an outer-sphere complex between the tetrapyrrole free base and the metal ion coordinated by Ac and solvent molecules. This step, recognized as an initial step in reactions of metal insertion into porphyrins, entails distortion of the tetrapyrrolic ring.[26,44] Pasternack and co-workers found kinetic evidence for such a pre-equilibrium in their studies on the reaction of Cu^{II} chloride with tetraphenylporphyrin in DMSO.[45] They found positive values for $\Delta H°$ and $\Delta S°$. In the case of Pheo, the increase in K with decreasing temperature illustrates the exothermic nature of the pre-equilibrium step, but since $\Delta H°$ and $\Delta S°$ are both negative, viz. $-89 \pm 7\ \text{kJ mol}^{-1}$ and $-242 \pm 22\ \text{J mol}^{-1}\text{K}^{-1}$, respectively, the outer-sphere complexation is driven by the enthalpy factor. The differences in the thermodynamic parameters on comparing TPP and Pheo reveal the importance of the structural differences between porphyrins and Chls. In the case of Pheo, such fast interaction between the tetrapyrrolic ring and Cu^{II} was confirmed spectroscopically in both solvents, since immediate

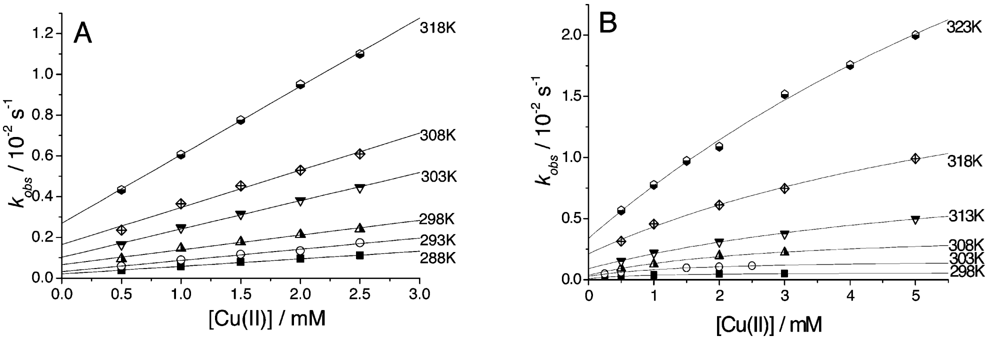

Figure 4. Dependence of k_{obs} on total Cu^{II} concentration in MeOH (A) and MeCN (B) at various temperatures. Conditions: [Pheo] = 2.5×10^{-6} M, λ = 640 nm.

fluorescence quenching was observed on mixing the reactants. This effect was slightly more pronounced in MeCN than in MeOH, although the correlation with the concentration of reactive Cu^{II} species in solution does not seem to be convincing in this case.

Thermal activation parameters for Pheo metalation in MeOH and MeCN were derived from the temperature dependence of k_1 and k_2 according to the Eyring equation. The effect of pressure on the Cu^{II} binding by Pheo in MeOH and MeCN was also studied. In this case, the rates of Cu–Chla formation were determined at various pressures within the pressure range up to 170 MPa. All kinetic, thermodynamic and activation parameters are summarized in Table 1.

In MeOH, $\Delta H^{\ddagger}$ for the Cu-dependent pathway was found to be 55 ± 3 kJ mol^{-1} and $\Delta S^{\ddagger}$ to be -61 ± 5 J mol^{-1} K^{-1}. The $\Delta H^{\ddagger}$ value in MeCN is almost three times higher, 143 ± 3 kJ mol^{-1}, and $\Delta S^{\ddagger}$ has a positive value, +184 J mol^{-1} K^{-1}. In the case of the Cu-independent pathway, the activation parameters were somewhat larger in MeOH, viz. $\Delta H^{\ddagger}$ = 65 ± 5 kJ mol^{-1} and $\Delta S^{\ddagger}$ = -91 ± 15 J mol^{-1} K^{-1}. More significant differences were found in MeCN, where $\Delta H^{\ddagger}$ for k_2 was smaller than for k_1, viz. 119 ± 8 kJ mol^{-1}, and $\Delta S^{\ddagger}$ was less than half of the value for the Cu-dependent pathway, viz. $+76\pm26$ J mol^{-1} K^{-1}.

$\Delta H^{\ddagger}$ determined for the Cu-dependent pathway in MeOH was quite similar to that reported by Pasternack et al.[45] as well as Turay and Hambright.[46] It was slightly higher for Pheo, probably due to a higher rigidity of the macrocycle, induced by the isocyclic ring. $\Delta S^{\ddagger}$ determined for metalation of TPP in DMSO and TMpyP in water were both negative and therefore similar to that obtained in the study on Pheo in MeOH.

The reaction of Pheo with $CuAc_2$ was previously investigated by Berezin and Smirnova, who pointed to the role of solvent in the kinetics of this process.[47–51] In alcoholic media it was linked to the stability of the Cu^{II} solvation shell, although no Ac–Solv equilibria were discussed in detail.[49] Moreover, no evidence for parallel reactions was presented. The values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were, however, quite comparable with the results presented in this report. Some studies were performed in nitrogen-containing media.[47] Although the authors did not use MeCN, some interesting conclusions concerning the present studies could be drawn from this work, especially since they found a decrease in the reaction rate with increasing concentration of total Cu^{II}. This finding was ascribed to some rearrangements of the coordination sphere of the Cu^{II} ion.

Table 1. Rate, activation and thermodynamic parameters for the formation of Cu–Chla.

Pheo + $CuAc_2$/MeOH	
k_1 (298 K) /M^{-1} s^{-1}	0.72 ± 0.05
$\Delta H^{\ddagger}$ /kJ mol^{-1}	55 ± 3
$\Delta S^{\ddagger}$ /J mol^{-1} K^{-1}	-61 ± 5
$\Delta V^{\ddagger}$ /cm^3 mol^{-1}	$+28\pm3$
k_2 (298 K) /s^{-1}	$6.7\ (\pm\ 0.8)\times10^{-4}$
$\Delta H^{\ddagger}$ /kJ mol^{-1}	65 ± 5
$\Delta S^{\ddagger}$ /J mol^{-1} K^{-1}	-91 ± 15
$\Delta V^{\ddagger}$ /cm^3 mol^{-1}	$+7\pm1$
Pheo + $CuAc_2$/MeOH	
K (298 K) /M	1204 ± 218
ΔH° /kJ mol^{-1}	-89 ± 7
ΔS° /J mol^{-1} K^{-1}	-242 ± 22
k_1 (298 K) /M^{-1} s^{-1}	$5\ (\pm1)\times10^{-4}$
$\Delta H^{\ddagger}$ /kJ mol^{-1}	143 ± 3
$\Delta S^{\ddagger}$ /J mol^{-1} K^{-1}	$+184\pm9$
$\Delta V^{\ddagger}$ /cm^3 mol^{-1}	$+16\pm1$
k_2 (298 K) /s^{-1}	$7\ (\pm2)\times10^{-5}$
$\Delta H^{\ddagger}$ /kJ mol^{-1}	119 ± 8
$\Delta S^{\ddagger}$ /J mol^{-1} K^{-1}	$+76\pm26$
$\Delta V^{\ddagger}$ /cm^3 mol^{-1}	0 ± 0
Chla + $CuAc_2$/MeOH	
k_1 (298 K) /M^{-1} s^{-1}	0.46 ± 0.02
$\Delta H^{\ddagger}$ /kJ mol^{-1}	54 ± 6
$\Delta S^{\ddagger}$ /J mol^{-1} K^{-1}	-72 ± 19
k_2 (298 K) /s^{-1}	$7.8\ (\pm0.3)\times10^{-4}$
$\Delta H^{\ddagger}$ /kJ mol^{-1}	49 ± 3
$\Delta S^{\ddagger}$ /J mol^{-1} K^{-1}	-139 ± 11

The values of the activation volumes in MeOH are positive, viz. $+28\pm3$ and $+7\pm1$ cm^3 mol^{-1} for the Cu-dependent and Cu-independent pathways, respectively, and correlate with the corresponding $\Delta S^{\ddagger}$ values. A positive but much smaller $\Delta V^{\ddagger}$ was found for k_1 in MeCN, whereas the parallel process turned out to be kinetically independent of pressure. Previously Tanaka reported activation volumes for the incorporation of various metal ions (but not Cu^{II}) into *N*-

methyltetraphenylporphyrin.[38] They were all positive but considerably smaller than both values of $\Delta V^{\ddagger}$ for the formation of Cu^{II} derivatives of Chla.

Transmetalation of Chla

Spectroscopic Studies

Absorption spectra recorded during the reaction of Chla with CuAc$_2$ in MeOH are shown in Figure 5.

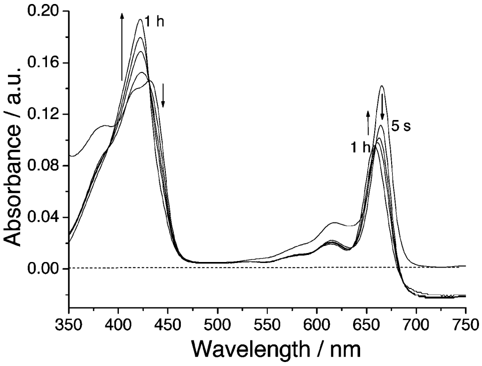

Figure 5. Absorption changes accompanying the reaction of Chla with CuAc$_2$ in MeOH. Conditions: [Chla] = 2.5 μM, [CuAc$_2$] = 2.5 mM, T = 298 K, t = 1 h.

Analogously to the spectroscopic studies on Pheo metalation, the CuAc$_2$ solution was used as reference. The apparent shift in baseline in the long wavelength range appeared immediately after mixing of the reactants inside the tandem cuvette. Under experimental conditions analogous to those applied in the studies on the metalation reactions, the blue shift of the Q$_Y$ band of Chla was approximately half as large as that of Pheo. Simultaneously, the split B bands of Chla merged into one and increased considerably in intensity.

A similar experiment was repeated at 333 K (not shown). The observed absorption changes were still very slow but proceeded further. The final spectrum recorded after 24 h showed the same position of the Q$_Y$ band as in the case of Pheo metalation. The stronger blue shift of the Q$_Y$ band, compared to this occurring at 298 K, was however accompanied by a meaningful decrease in absorption.

Changes in the absorption spectrum occurring during Chla reaction with CuAc$_2$ in MeCN at 298 K were hardly observable (Figure 6). A slight decrease in the Q$_Y$ band and a deformation of the Soret band were seen in the spectra recorded over several hours at 298 K.

Similarly to the metalation reaction, the fast initial decrease of absorption in the long wavelength range seems to be related to ligand exchange on the Cu^{II} center, viz. Ac for MeOH. The product of the reaction of Chla and CuAc$_2$ can not be, however, recognized as Cu–Chla. Instead, some intermediate of this reaction must be stabilized under the applied conditions as the final position of the Q$_Y$ band is 658 nm at 298 K. Further evidence was derived from the analysis of the shape of this band. It was compared with the shapes of Q$_Y$ bands of Chla, Cu–Chla and their mixtures recorded in MeOH (Figure 7).

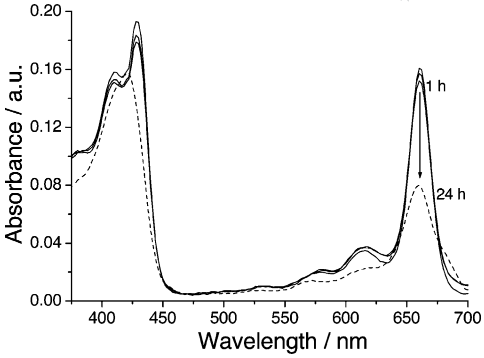

Figure 6. Absorption changes accompanying the reaction of Chla with CuAc$_2$ in MeCN. Conditions: [Chla] = 2.5 μM, [CuAc$_2$] = 5 mM, T = 298 K, t = 1 h. Dashed line represents the spectrum recorded after 24 h.

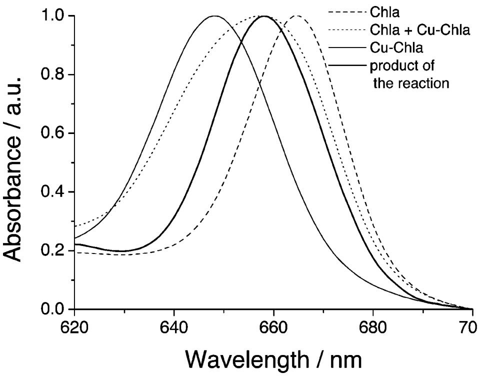

Figure 7. Comparison of the shapes of Q$_Y$ bands of Chla (dashed line), Cu–Chla (thin solid line), 1:1 mixture of Chla and Cu–Chla (dotted line) with the band of the product of Chla transmetalation with CuAc$_2$ (thick solid line). All spectra recorded in MeOH.

Depending on the [Chla]/[Cu–Chla] ratio, the position of the band center varied between 665 and 648 nm. However, the value of FWHM for the one at 658 nm (dotted line) occurred to be much larger than for the stable product of Chla transmetalation with CuAc$_2$ (thick solid line).

As indicated by the spectral changes recorded at 333 K, Cu–Chla can be obtained from Chla and CuAc$_2$ by increasing temperature. Unfortunately, a concomitant thermal decomposition of Chla prevents from conducting systematic kinetic measurements under such conditions. Therefore, further detailed analysis was focused on the first, relatively fast step of the formation of the stable intermediate.

The spectral changes that occurred in the presence of CuAc$_2$ (dotted line in Figure 6) should not be attributed only to thermal decomposition of Chla, which is stable in MeCN at 298 K (not shown). Presumably they should also be interpreted as a result of Chla oxidation by solvated Cu^{II} ions.

Emission spectra provided further information on the transmetalation of Chla by CuAc$_2$. Initially an immediate decrease in the fluorescence intensity, analogous to that observed during metalation of Pheo, was observed in both

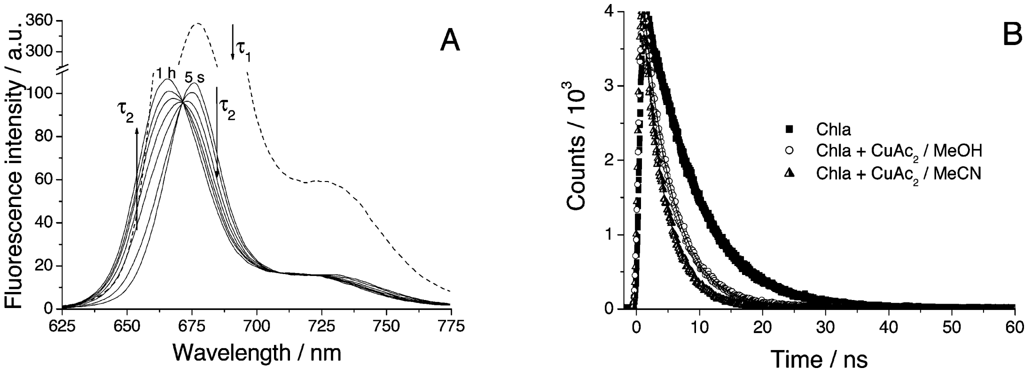

Figure 8. A. Emission spectra recorded during the reaction of Chla and CuAc$_2$ in MeOH. Conditions: [Chla] = 2.5 μM, [Cu^{II}] = 2.5 mM, T = 25 °C, t = 1 h, λ_{exc} = 415 nm. 8B: Decay of the emission of Chla and its stable intermediates in the reaction with CuAc$_2$ in MeOH and MeCN, λ_{exc} = 415 nm.

MeOH (Figure 8, A) and MeCN (not shown). Whereas a very slow subsequent quenching step occurred in MeCN, different changes were found in MeOH as a new emission band was formed with a maximum at 665 nm (Figure 8, A, that partially decayed during the subsequent extremely slow step. The time-resolved emission measurements provided additional information on the nature of the stable reaction intermediates.

Decay of the Chla emission in MeOH and MeCN (Figure 8, B) could be fitted well with a single component (95% contribution). The τ values are listed in Table 2.

Table 2. Life-times of Chl fluorescence.

Fluorofore	τ /ns
Chla[a]	8.0 ± 0.1
Chla–CuAc$_2$/MeOH[b]	4.2 ± 0.1
Chla–CuAc$_2$/MeCN[b]	3.4 ± 0.1

[a] In MeOH. [b] Reaction mixture 2 h after initiation.

The first step of Cu^{II}-induced emission changes in both MeOH and MeCN, was analogous to that found during the reactions of Pheo. It points at weak interactions between fluorophore, viz. Chla, and quencher, viz. Cu^{II} that were somewhat strengthened over several hours (MeCN), or an intermediate species could be formed (MeOH), in which valence electrons of incoming metal ion and the pigment π-electron system are still not coupled strongly. The time-resolved emission experiment showed that nearly whole population of Chla was engaged in this species.

On the basis of absorption and emission changes, one can assign the stable intermediate of the reaction of Chla and CuAc$_2$ in MeOH to the bimetallic complex in which outgoing Mg^{2+} and incoming Cu^{II} stick out on the opposite sides of the macrocycle's plane, postulated in mechanism of porphyrins transmetalation.[42] In order to obtain an independent evidence for its existence, solutions of Chla, CuAc$_2$ and the equilibrated mixture of Chla and CuAc$_2$ (approximately 2 hours after mixing the reagents) were exposed to electrospray ionization and the mass analysis was performed in the range of 200–2000 m/z (Figure S5, Supporting Information). The formation of a bimetallic complex could be indicated by the peak at 971/973 m/z coming from a deprotonaned Cu-13^2-hydroxy-Chla molecular ion, which was not observed in the spectrum of the equilibrated solution in MeCN and only hardly observable in the spectrum of Chla in MeOH, perhaps due to some traces of Cu^{II} in solvent. This interpretation, however, is not unambiguous because it can be also interpreted as 13^2-hydroxy-Chla solvated by two MeOH molecules. Insufficient stability of bimetallic tetrapyrrolic complexes under conditions of electrospray ionization may account for these observation. Moreover, some other peaks of very low intensities could also indicate the existence of bimetallic complex [e.g. at 1047 m/z assigned to (MeOH)Chla+CuAc].

Information obtained from absorption and emission measurements is insufficient for characterization of the Cu^{II}–Chla complex formed in MeCN. Although Cu^{II} efficiently quenches Chla emission, the metallic center remains loosely attached to the macrocycle, as the interaction does not meaningfully affect the absorption spectrum. The influence of Cu^{II} on the π-electron system of Chla in MeOH must then be considerably stronger. Regardless of solvent, further transformation into a typical Cu–Chla complex is hardly possible. A very slow decrease in the absorbance at the Q$_Y$ bands of the intermediates displayed a zero-order character, which rather corresponds to charge transfer from Cu^{II} to Chla than to binding of Cu^{II} to the macrocycle. As shown previously,[27] the fast transmetalation of Chla by CuAc$_2$ occurs in MeCN in the presence of excess Ac and is complete within minutes.

Kinetic Studies

The kinetic traces monitored at 655 nm showed a double-exponential decay, of which only the first one could be evaluated due to the interference of the subsequent decomposition of Chla (see above). The linear dependence of the observed rate constant on the Cu^{II} concentration (Figure 9) showed distinct intercepts, which indicate a considerable contribution of a Cu^{II}-independent pathway.

Hence, the rate law for this reaction is analogous to that determined for Pheo metalation in MeOH:

$$k_{obs} = k_1[\mathrm{Cu^{II}}] + k_2 \qquad (10)$$

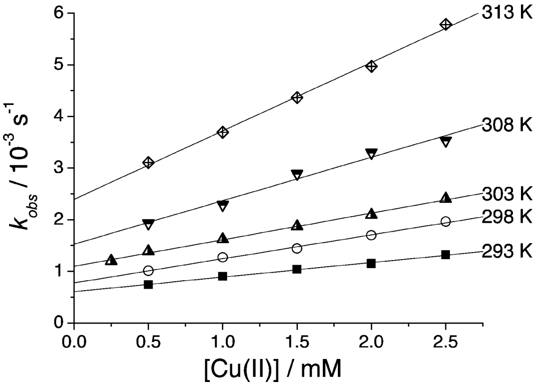

Figure 9. Dependence of the observed rate constant on the Cu^{II} concentration at different temperatures (transmetalation in MeOH). Conditions: [Chla] = 2.5×10^{-6} M, ambient pressure.

At 298 K the values of k_1 and k_2 are 0.46 ± 0.02 $M^{-1}\,s^{-1}$ and $(7.8\pm0.3)\times10^{-4}$ s^{-1}, respectively. As in the case of Pheo, the absorption changes that accompany the reaction of Chla showed no dependence on the Cu^{II} concentration. A similar experiment performed in the presence of $MgAc_2$ indicated no influence of the concentration of Mg^{2+} on the reaction rate. Therefore, one can assume that the values of k_1 and k_2 correspond to parallel reactions, and hence the kinetics of Chla transmetalation and Pheo metalation follow the same rate law, (8) and (10). The values of the rate constants, k_1 and k_2, are quite similar to those obtained for the reaction of Pheo with $CuAc_2$. In this way the role of solvent properties is manifested in both metalation and transmetalation processes.

Studies on the influence of temperature and pressure were performed in order to determine the activation parameters. The values of $\Delta H^‡$ and $\Delta S^‡$, calculated using the Eyring equation, were 54 ± 6 kJ mol^{-1} and -72 ± 19 J mol^{-1} K^{-1} for k_1, and 49 ± 3 kJ mol^{-1} and -139 ± 11 J mol^{-1} K^{-1} for k_2, respectively. The influence of pressure on the reaction showed an opposite effect when the Cu^{II} concentration was changed from 0.25 to 2.5 mM (Figure 10) and thus $\Delta V^‡$ could not be assessed.

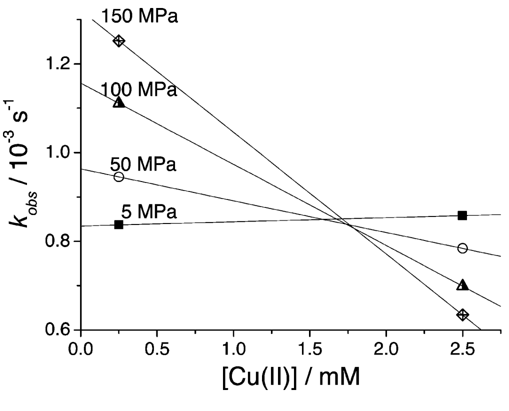

Figure 10. Chla transmetalation in MeOH: dependence of k_{obs} on Cu^{II} concentration at various pressures. Conditions: [Chla] = 2.5 μM, T = 298 K.

In both the Cu^{II}-dependent and Cu^{II}-independent reactions, $\Delta H^‡$ was again similar to the value determined for the metalation of Pheo. It confirms the role of solvent properties that determine the speciation of Cu^{II}. The necessity to consider the concentrations of the various reactive Cu^{II} species was manifested even stronger under elevated pressure. This effect can be explained by strong dependence of equilibria (4) and (5) on pressure.

The metal exchange reactions are typical for metalloporphyrins with large central ions and were usually studied for complexes of Cd^{II}, Hg^{II}, Pb^{II} or Cu^{I}.[24,46,52–54] The ionic radius of these metals renders them unfit for the tetrapyrrolic core. As a consequence, the activation enthalpies for the metal exchange reactions in metalloporphyrins are usually somewhat smaller than determined for Chl transmetalation. Moreover the values of $\Delta S^‡$ varied from slightly positive to large negative with the nature of the tetrapyrrolic ligand. Hence, there is no point in direct comparison of the activation parameters estimated for differently substituted porphyrins and Chla, especially as the in-plane position of Mg^{2+} prevents the latter from activating distortion.

Mechanistic Considerations

The difficulties with the interpretation of the apparent activation parameters determined for the metalation and transmetalation reactions result from the shift in equilibria (4) and (5) that accompany changes in concentration, temperature and pressure. Since K_2 and K_3 were determined only in MeOH at 298 K,[37] the real values of $\Delta V^‡$, $\Delta H^‡$ and particularly $\Delta S^‡$ can be merely assessed. The [Cu^{II}] values shown in Figures 4 (A, B) and 9 represent the total Cu^{II} concentration, i.e. a sum of contributions from all reactive species. According to previous studies,[27,35,36] the [$Cu_2Ac_4Solv_2$] dimer is inert in the metalation. Because the concentration of the fully solvated Cu^{II} is negligible in $CuAc_2$ solutions, only [$CuAc(Solv)_4$]$^+$ and [$CuAc_2Solv_2$] should be considered to react with Pheo. In MeOH, the contribution from both reactive Cu^{II} species changes with the total [Cu^{II}] and complicates matters significantly. For this reason, the concentrations of [$CuAc(Solv)_4$]$^+$ and [$CuAc_2Solv_2$] were estimated for various total [Cu^{II}] at 298 K using Grasdalen's data[37] and used to plot the k_{obs} dependences (Figure 11).

They show linear relationships between the k_{obs} and the concentrations of both [$CuAc(MeOH)_4$]$^+$ and [$CuAc_2(MeOH)_2$], allowing for two interpretations of these dependencies. If the parallel reactions involve two different Cu^{II} species, then the 1:1 and 1:2 complexes would be engaged in the Cu-dependent and Cu-independent pathways. Analogously to the metalation of porphyrins,[35] the 1:1 complex would be the most reactive Cu^{II} species. The electrostatic interaction with tetrapyrrole leading to outer-sphere complexation is favored by the positive charge, whereas the formation of Cu–N bonds is favored by the number of labile solvent ligands. Therefore, the dissociation of Ac from the complex with Cu^{II}, necessary for the formation of Cu–Chla, must be the slowest step of the reaction involving [CuAc-

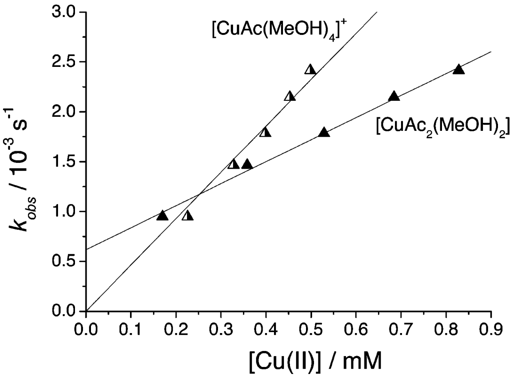

Figure 11. The dependence of k_{obs} on the concentration of the Cu^{II} species in MeOH at 298 K.

$(MeOH)_4]^+$, and hence this pathway is kinetically independent of the Cu^{II} concentration. The reaction involving the 1:2 complex seems to be much slower due to the number of Ac ligands.

Several reports proved the existence of SAT complexes in similar reactions,[55,56] and even if some question the existence of such type of intermediate,[57] it still is most appealing structure of intermediate common to the metalation pathways, predicted also by theoretical calculations.[58,59] Therefore this model should also be applicable to considerations for the metalation of Pheo, although the assignment of the SAT intermediate is ambiguous as no clear evidence for its formation was found.

Formation of SAT complexes requires the dissociation of at least two ligands from the initial metal complex. The geometry of the discussed Cu^{II} complexes is determined by Ac and the release of one Ac from $[CuAc_2(MeOH)_2]$ is necessary for the formation of a SAT complex. Dissociation of the second one would probably be faster, especially as the remaining Cu–Ac bonds are weakened by interaction with the tetrapyrrolic ligand. The above considerations seem to justify participation of the 1:2 complex in the Cu-dependent process. Such an interpretation somewhat questions the assumption that no back reaction occurs in the studied system. The dependence of k_{obs} on the concentration of the 1:2 complex reveals a distinct intercept, which can again be attributed to the reverse cleavage of the intermediate or a competitive reaction of Pheo with $[CuAc_2(MeOH)_2]$. Although the absorbance changes can be insufficient as evidence for a reverse or parallel reaction in the system containing the mixture of different metal species (Figure 4, A), the foregoing interpretation may be less convincing than the one based on the assumption that only single Cu^{II} species reacts with Pheo. Even though the concentration of the 1:2 complex seems higher than that of the 1:1 complex, the latter is favored by charge and comparative lability of ligands. Since it is still in excess over Pheo, it can be expected to arrange with the macrocycle in an outer-sphere complex of the SAT type, prior to possible similar arrangement involving the 1:2 species. The kinetics of the subsequent steps would be determined by the breakage of Cu–Ac or H–Pheo bonds. The latter can be excluded as being important only in the environment of very low basicity.[23] Moreover, deprotonation of the core nitrogens is assisted by Ac. Thus, the rate-determining step would depend on the structure and geometry of the intermediate. One should consider some kind of a metal–macrocycle combination in which protons or ligands such as Ac prevent the metal from moving into the coordinative plane. Proton dissociation may gain importance in less basic acetonitrile solutions but the presence of Ac causes it to be not as significant as the other reaction steps.

The participation of two metal ions in the metalation of one Pheo molecule is questionable. Unlike in the studies by Tanaka,[38] no evidence for the squared concentration dependence was found for the reaction of Pheo. This can be justified by the possible steric hindrance caused by e.g. the phytyl chain.[11,25]

A significant contribution of non-reactive dimers in the total concentration of $CuAc_2$ suggests that the real second-order rate constants k_1' must be much higher than the apparent k_1 values. For instance, at 298 K in MeOH, k_1 and k_1' are $0.72\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$ and $2.3\ \mathrm{M}^{-1}$, respectively. Consequently, the intercepts of the Eyring plots will be more distinct than in the case of the total $[Cu^{II}]$ and thus $\Delta S^{\ddagger}$ will differ significantly from the corresponding apparent parameters. In addition, an increase in temperature favors formation of Cu^{II} monomers, making the Eyring dependence somewhat steeper than in the case of the total $[Cu^{II}]$. Surprisingly, elevation of pressure causes equilibria (4) and (5) to shift to the left. The reason for this effect is unclear because it could be forced by either decomposition of $[Cu_2Ac_4MeOH_2]$ or dissociation of Ac from $[CuAc_2(MeOH)_2]$. The latter seems to be more probable especially as charged species are being formed.

Modified activation parameters for the reactions of Pheo/Chl in MeOH were estimated assuming that the absorbance changes result from changes in the contribution from Cu^{II} species, analogously to those caused by dilution. Temperature and pressure induced changes in the Cu^{II} absorbance were compared with the dilution curve obtained from the theoretical dependence of absorbance on the total $[Cu^{II}]$ determined from the K_2 and K_3 values. In this way, the actual total Cu^{II} concentration could be substituted by the apparent concentrations at 298 K and ambient pressure. Using the calculated relationship between the concentration of $[CuAc_2(MeOH)_2]$ and total $[Cu^{II}]$ as reference, the apparent concentration of monomeric species was estimated under all conditions. The modified activation parameters, listed in Table 3, were then determined from the appropriate equations.

The rate constants k_1 and k_2 for the Cu-dependent and Cu-independent pathways, respectively, can be considered as reasonably reliable since they relate to standard conditions. Obviously, the ratio of k_1'/k_1 is proportional to the ratio of total and monomeric 1:2 Cu^{II} concentrations. At the same time, the values of k_2 remained almost unaffected during the recalculation of $[Cu^{II}]$. As predicted from the temperature influence on the absorbance of the mixture of Cu^{II} complexes, $\Delta H^{\ddagger}{}'$ for k_1' are somewhat larger than in

Table 3. Rate and activation parameters for the reactions of Pheo and Chla with $CuAc_2$ in MeOH estimated for real concentrations of $[CuAc_2MeOH_2]$.

Parameter/unit	Pheo	Chla
k_1' (298 K) /$M^{-1}\,s^{-1}$	2.3 ± 0.1	1.45 ± 0.09
$\Delta H^{\ddagger\prime}$ /kJ mol^{-1}	63 ± 5	61 ± 6
$\Delta S^{\ddagger\prime}$ /J $mol^{-1}\,K^{-1}$	-25 ± 16	-36 ± 21
$\Delta V^{\ddagger\prime}$ /$cm^3\,mol^{-1}$	$+25 \pm 3$	–
k_2' (298 K) /s^{-1}	$5.8\ (\pm 0.8) \times 10^{-4}$	$7.3\ (\pm 0.5) \times 10^{-4}$
$\Delta H^{\ddagger\prime}$ /kJ mol^{-1}	60 ± 8	48 ± 4
$\Delta S^{\ddagger\prime}$ /J $mol^{-1}\,K^{-1}$	-107 ± 26	-144 ± 12
$\Delta V^{\ddagger\prime}$ /$cm^3\,mol^{-1}$	$+6 \pm 1$	–

the case of the experimental apparent $\Delta H^{\ddagger}$. The same parameters calculated for k_2' are quite similar (within error limits) to the corresponding values determined for k_2.

Conversion of $[Cu^{II}]$ mainly affects the values of $\Delta S^{\ddagger}$. The intercepts in the Eyring plots increase with increasing second-order rate constants and thus the values of $\Delta S^{\ddagger\prime}$ for the Cu-dependent pathway were much less negative than the corresponding $\Delta S^{\ddagger}$. On the contrary, $\Delta S^{\ddagger}$ for the parallel reactions became somewhat smaller.

The effect of pressure on the Cu^{II} speciation was not very significant so it could not affect the volume of activation derived from the pressure dependence based on the total $[Cu^{II}]$. Moreover, dimerisation at higher $[Cu^{II}]$ was balanced to some extent by the negative influence of elevated pressure. As a result, the values of $\Delta V^{\ddagger\prime}$ for the metalation were only slightly higher than the corresponding values for $\Delta V^{\ddagger}$. The problem with the interpretation of the effect of pressure on the rate transmetalation could not be solved by the conversion of the total $[Cu^{II}]$. The slopes in Figure 10 became even steeper in terms of the calculated concentrations of reactive $[CuAc_2(MeOH)_2]$ species.

The high average ε and the position of maxima in the absorption spectra of $CuAc_2$ solutions in MeCN indicate that equilibria (4)–(5) are strongly shifted to the right. This was further confirmed by the finding that Cu^{II} speciation was not affected by either dilution or pressure. The temperature-induced decrease in the absorbance, ca. 0.002 a.u./K, was comparable with that observed in MeOH. Because the equilibrium constants for $CuAc_2$ in MeCN are unknown, it is impossible to estimate the theoretical real activation parameters. Nevertheless, one can find some noticeable differences in the two solvents, and discuss them on the level of apparent experimental data. The acceleration of reaction of Pheo with Cu^{II} in MeOH by three orders of magnitude can be justified by the difference in solvent interaction either with the tetrapyrrole or with Cu^{II} ions. The latter case seems to be more probable, especially for metal insertion into the free base. Hence, the difference in the rates of the Cu-dependent reactions should be attributed to the ability of Ac to bridge Cu^{II} ions. As shown above, the conversion of the total $[Cu^{II}]$ mainly results in changes in $\Delta S^{\ddagger}$. Therefore, the large difference between the $\Delta H^{\ddagger}$ values for k_1 in MeOH and MeCN cannot be explained in the same way as the differences in the second-order rate constants. Similarly, the large positive values of $\Delta S^{\ddagger}$ are expected to become even larger if the contribution of the dimer in the overall Cu^{II} speciation is taken into account.

By comparing the activation parameters determined for both the metalation and transmetalation in MeOH, it is possible to link their values with solvent properties rather than with the process itself. Although the metal exchange requires a different energy input than a simple coordination of free base, the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for k_1 are almost identical. This indicates that the rate-determining step must involve the same type of substrate transformation. The same parameters for the Cu-independent reaction were slightly more divergent but still quite similar. Therefore, the rate-determining step in both the Pheo metalation and Chla transmetalation processes seems not to involve proton (Pheo) or magnesium (Chl) dissociation. Negative and presumably relatively small values of $\Delta S^{\ddagger}$, if related to the actual concentration of the reactive Cu^{II} species, point to an associative interchange mechanism and in this way confirm the similarity of the rate-determining steps in metalation and transmetalation. On the other hand, $\Delta V^{\ddagger}$ of metalation suggests a dissociative rather than an associative type of mechanism. For the reasons outlined above, it seems to be impossible to verify the mechanism of Chla transmetalation with the value of $\Delta V^{\ddagger}$. Nevertheless, it seems reasonable to assume that a relatively slow initial interaction between Cu^{II} ion and the tetrapyrrolic ligand precedes the subsequent proton dissociation. At the same time, the release of Mg ions was not confirmed in the spectroscopic studies and the reaction of Chla seems to terminate at the stable bimetallic intermediate.

Besides the apparent rate constants, the kinetics of the metalation in MeCN differed from the analogous process in MeOH in terms of the rate law. The saturation of the dependence of k_{obs} on total $[Cu^{II}]$ points to a fast pre-equilibrium, which can be ascribed to the outer-sphere complex-formation. The thermodynamic parameters show that such interaction is driven by the enthalpy factor which can be connected to charge distribution in Pheo and the Cu^{II} complex. Unlike in MeOH, subsequent bond formation turned out to be faster than the release of protons as indicated by the positive values of both $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$. It can be justified by the different abilities of the solvents to bind protons and to assist their dissociation from the free base core. Also the ability of MeOH to form hydrogen bonds can be of great importance in the case of Ac bound to Cu^{II} in the SAT-type intermediate. Such interactions weaken the coordinative bonds and accelerate formation of the final product.

Conclusions

The Cu^{II} derivative of Chla can be easily obtained in the reaction of Pheo with $CuAc_2$ in both MeOH and MeCN. However, the kinetic investigation met serious difficulties that resulted from the influence of the concentration, temperature and pressure on the Cu^{II} speciation. The apparent activation parameters point to the crucial role of solvent properties on the mechanism of Pheo metalation. Due to the abilities of MeOH to form hydrogen bonds or, alternatively, exchange protons, it can assist the dissociation of Ac from the Cu^{II} complex.

Under analogous conditions, Cu–Chla could not be obtained via transmetalation of Chla. In contrast to the rapid substitution of large metal ions such as Hg^{II}, Cd^{II} or Cu^{I},[24,54,60,61] Mg^{2+} fits into the cavity of the tetrapyrrole and the catalytic effect connected with the macrocycle deformation cannot be observed. As a result, a stable bimetallic complex, relevant to SAT, is formed. Its decay requires application of some chelating agents that can extract Mg^{2+} and thus enable Cu^{II} to move into the macrocyclic plane.[27]

Experimental Section

Pigment Preparation: Chla was extracted from cells of *Spirulina plantesis* by the method described elsewhere[62] and purified using an HPLC system with a semi-preparative column Varian RP C–18 (250 × 4.6, flow 1 mL min^{-1}) and MeOH as an eluent.[63] Pheo 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. Cu–Chla was obtained by the direct insertion of Cu^{2+} into Pheo in MeOH (at 298 K, reaction controlled spectrophotometrically). Pure pigments were stored at –40 °C under argon. All experiments were performed in dim light with freshly prepared solutions.

Solvents and Reagents: All solvents and salts used in the experiments were of analytical grade. MeOH, HAc, acetone, and chloroform were purchased from POCh, Poland. MeCN was obtained from Acros Organics (Belgium) and nitromethane ($MeNO_2$) from Fluka (Germany). $Cu(CF_3SO_3)_2$, $Fe(ClO_4)_3 \cdot 6H_2O$ and $Cu(ClO_4)_2 \cdot 6H_2O$ were obtained from Aldrich (Germany), $ZnCl_2$ and $Ni(ClO_4)_2 \cdot 6H_2O$ from Fluka, $Co(ClO_4)_2 \cdot 6H_2O$ from Strem Chemicals (Germany), $CuCl_2 \cdot 2H_2O$, $CuAc_2 \cdot H_2O$, $ZnAc_2$, $HgCl_2$, $CdCl_2 \cdot 2.5H_2O$ and $PbCl_2$ from POCh.

Equipment: Absorption UV/Vis spectra were recorded on 2101PC (Shimadzu, Kyoto, Japan) and Cary 5G (Varian, Palo Alto, CA, USA) spectrophotometers equipped with electronic temperature controllers. A custom-built high-pressure probe[64] combined with the Shimadzu 2101PC spectrometer was used in the high-pressure experiments. Fluorescence spectra were recorded using a LS55 spectrofluorimeter (Perkin–Elmer, Wellesley, MA, USA) equipped with a LTD6G thermostat (Grant Instruments Ltd., Cambridge, UK). In order to determine changes in the Chla fluorescence life time occurring in the transmetalation reactions, time-resolved emission measurements were performed using a FLS920P spectrofluorimeter (Edinburgh Instruments Ltd., Livingston, UK) equipped with a nanosecond-pulsed flashlamp.

Mass spectra were recorded on an electrospray ionisation spectrometer Finnigan MAT 95S. The spectra were recorded within the range of 200–2000 *m/z*.

Reactions of Chls with Metal Ions: The reactions of Pheo and Chla with selected metal ions were investigated with UV/Vis absorption spectroscopy. Metals were selected so that they represented s- (Li, Mg), p- (Pb^{II}) and d-block elements (Co^{II}, Ni^{II}, Cu^{II}, Hg^{II}, Fe^{III}, Cd) with consequent differences in coordinative properties. In order to minimize the contribution from the counterion to the ability of metal ions to bind to Chls, perchlorate, nitrate or trifluoromethanesulfonate metal salts were used as reagents. Reactions were performed in alcohol (ethanol or methanol), and some in acetone. To maintain appropriate conditions for spectroscopic measurements before and after mixing of reagents (i.e. the combination of concentration and optical path length), the measurements were performed using a 0.88 cm thick tandem cuvette. Identical volumes of the solutions of metal (5 mM) and Chl (≈ 2.5 µM) were placed on the opposite sides of the tandem cuvette and then mixed to initiate the reaction. Absorption spectra were recorded at 298 K until the reaction reached completion.

Preliminary studies on the metalation of Pheo and transmetalation of Chla with various Cu^{II} salts ($CuTf_2$, $CuCl_2$ and $CuAc_2$) were carried out in various solvents, i.e. nitromethane ($MeNO_2$), MeOH and MeCN. Experimental conditions such as concentration of reactants and temperature were analogous to those applied in the reactions with the other metals. The only difference was the use of Cu^{II} salt solutions as reference samples. In the cases of the reactions chosen for detailed kinetic studies (Pheo and Chla with $CuAc_2$ in MeOH and MeCN), emission spectra were recorded as well. Solutions of reactants were mixed in 10-mm-wide four-window cell and Chl was excited at 415 nm. Emission spectra were recorded in the range of 600–800 nm with the slits set at 15 and 2.5 nm for excitation and emission, respectively. The fluorescence lifetimes were determined for the stable products of Chla transmetalation with $CuAc_2$ in MeOH and MeCN. The reactions were followed to the end of the observable absorption changes. Then the solutions were excited at 415 nm and the decay of emission was measured at 675 nm at 298 K using the single photon counting system.

In order to identity the product of Chla transmetalation in MeOH, the solutions of Chla and Cu–Chla were placed in the tandem cell and the absorption spectra were recorded at various [Chla]/[Cu–Chla] ratios. The shapes of the Q_Y bands obtained for Chla/Cu–Chla equilibria were compared with the shape of the Q_Y band of the transmetalation product.

Kinetic studies were performed on three reactions: metalation of Pheo with $CuAc_2$ in MeOH and in MeCN, and transmetalation of Chla with $CuAc_2$ in MeOH. The concentration of tetrapyrrole was kept constant as 2.5 µM and the concentration of Cu^{II} was varied from 0.25 to 2.5 mM in MeOH, and from 0.5 to 5 mM in MeCN. In all measurements a 0.88 cm thick tandem cuvette was used. The concentration dependences were investigated under ambient pressure at temperatures between 288 and 323 K with steps of 5 K. Thus the second-order rate constants were determined. The activation enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) were calculated from the Eyring equation. In order to determine the activation volume ($\Delta V^{\ddagger}$), identical volumes of the reactant solutions were mixed and filled into the quartz pillbox, which was then placed inside the thermostatted high-pressure cell.[64] The pressure was gradually increased up to 175 MPa and the absorption spectra were recorded at 298 K (or 288 K in the case of the reaction of Chla) within the range of 350–800 nm.

$CuAc_2$ Speciation in MeOH and MeCN: The coordinative forms of Cu^{II} in MeOH and in MeCN were investigated with UV/Vis absorption spectroscopy. The solution of $CuAc_2$ (5 mM, 2 mL) was titrated in a conventional 1 cm quartz cuvette with 10 µL doses of concentrated (0.2 M) TBAA solution using a microsyringe (Hamilton, USA). Absorption spectra of the equilibrated solutions were recorded after each addition of TBAA. In addition, the influence of the [$CuAc_2$]/[Solv] ratio on Cu^{II} speciation was investigated by comparison of the absorbance measured at various $CuAc_2$ concentrations (0.25–3.0 mM) with the Lambert–Beer law at 298 K. The influence of temperature on the Cu^{II} speciation was studied for 0.5–2.5 mM (MeOH) and 1.0–5.0 mM (MeCN) solutions of CuAc$_2$ in the temperature range from 293 to 323 K in steps of 5 K. The pressure dependence of the Cu^{II} speciation in MeOH and MeCN was investigated at 298 K using a standard absorption spectrophotometer combined with the above-mentioned custom-built high-pressure probe. The pressure was adjusted to 5, 50, 100 and 150 MPa and then gradually released to ambient. Absorption spectra of the equilibrated solutions were recorded between 400 and 900 nm after each change in pressure.

Supporting Information (see also the footnote on the first page of this article): Mechanistic information on Cu(II) metalation and transmetalation of chlorophylls (2 pages, 5 figures).

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