Metal-Substituted Bacteriochlorophylls. 1. Preparation and Influence of Metal and Coordination on Spectra

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Abstract: In contrast to porphyrins and chlorins, the direct metalation of bacteriochlorins is difficult. Nevertheless, Cu$^{2+}$ and Zn$^{2+}$ can be introduced into bacteriopeophytin in acetic acid, whereas Cd$^{2+}$ can be inserted in dimethylformamide. The former reactions depend on the substituents of the isocyclic ring: they are facilitated if enolization of the $\beta$-ketoester system is inhibited. Starting with [CD]-bacteriochlorophyll-a or its 13$^2$-hydroxy derivative, a series of metallo-bacteriochlorins with central divalent ions Pd$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, and Mn$^{2+}$ have been obtained by transmetalation. Like in the parent Mg complex, the four principal optical transitions are well-separated in these complexes, and their responses to changes in the central metal and its coordination state can be followed in detail. The energies of the $Q_x$ and $B_y$ transitions are almost independent of the central metal, whereas the $Q_y$ and $B_x$ transition energies change significantly, depending on the central metal as well as the presence of additional axial ligands. If the complexes are grouped by their coordination number, empirical linear correlations exist between these shifts and the ratio $\chi_M^P/r_M$, where $\chi_M^P$ is Pauling’s electronegativity value and $r_M$ is the ionic radius of the metal. A similar correlation was found for those $^1$H NMR signals influenced mainly by the ring current and for the redox potentials. This observation was in contrast with the linear relationships with $\chi_M^P$ alone, found for metal-substituted porphyrins. The spectral variations influenced by the central metal and its state of ligation are attributed, within the framework of the four-orbital model, to the electrostatic interaction of the electron densities in the four orbitals with the effective charge of the central metal ions, which is most pronounced for the $a_2u$ orbital (HOMO-1). Ligation studies have revealed that addition of the first axial ligand decreases the effective charge of the central metal by approximately 50% and addition of the second axial ligand by another 20% with respect to the absence of axial ligands. The singlet-triplet splitting deduced from fluorescence and phosphorescence measurements is similar for [Pd]-, [Cu]-, [Zn]-, and [Mg]-BChl (4550 ± 100 cm$^{-1}$).

Introduction

The Mg-containing (bacterio)chlorophylls (BChl) and their free bases, the (bacterio)pheophytins (BPhe), are essential to photosynthesis. They act as light-harvesting or as redox pigments enabling light-induced charge separation within the reaction center.$^1$ The pigments are also potentially useful photosensitizers, e.g., in photodynamic tumor therapy.$^2$–$^7$ Complexes of cyclic tetrapyroles with metals other than Mg were studied in the chlorin$^8$–$^9$ (17,18-dihydroporphyrin) and especially in the porphyrin$^{10}$–$^{13}$ series to better understand their spectroscopic and redox properties. These complexes show a linear relationship between the electronegativity ($\chi_P^p$; Pauling values) of the central metal and the redox potential.$^9$ Interpretation of the optical spectra has been less straightforward.$^{10}$–$^{12}$ mostly because of insufficient experimental information. The electronic

References

transitions are pairwise degenerate in metallo-porphyrins \((E_Q \approx E_0, E_B \approx E_0)\). In metallochlorins, the visible bands \((Q_1, Q_4)\) are well-separated, but \(Q_2\) is often hardly discernible, and the \(B\) bands are again (nearly) degenerate.

Compared to the chlorins (chlorophylls), bacteriochlorins \((7,8,17,18\text{-tetrahydroporphyrins})\) and, in particular, bacteriochlorophylls are of potential advantage because all of their four \(\pi-\pi^*\) transitions predicted by the four-orbital model are well separated.\(^{4,15}\) However, limited information has been available about bacteriochlorins with central metals other than Mg.\(^ {4,16,25}\)

We have found that direct metalation methods for chlorins\(^ {23,26}\) were successful with only a few metals when applied to the free base analogues of bacteriochlorins, the bacteriopheophytins.\(^ {16a,17,25}\) However, Strell and Urumov’s principle of transmetalation\(^ {27}\) to indirectly prepare different metal-substituted chlorins could be adapted for preparing additional metal-substituted bacteriochlorophylls \([(M]-\text{BChl})\]. Starting from \([\text{Cd}]-\text{BChl}\) \((8a,\text{or its 13}-\text{OH derivative, 8b}), \text{Pd}^{2+}, \text{Co}^{2+}, \text{Ni}^{2+}, \text{Cu}^{2+}, \text{Zn}^{2+},\) and \(\text{Mn}^{2+}\) were incorporated into BPhes \((1a, b),\) to yield the pigments \(2a, b\) to \(6a, b\) and \(9a, b\) (Figure 1).

The redox chemistry,\(^ {19,22,25}\) EXAFS spectra,\(^ {23}\) and incorporation of some of these complexes into bacterial reaction centers has been independently reported.\(^ {17,18,20,21,24}\) Here we describe their preparation and spectral characterization, and we present a correlation between their physical properties and the characteristic parameters of the central metal. A quantitative analysis of the data based on electrostatic interactions between the metal and all the negative and positive (partial) charges on the macrocycle in the frontier orbitals is the subject of the following paper.\(^ {28}\)

**Results and Discussion**

**Preparation.** Besides the Cd necessary to form the precursor complex for the transmetalation, only Cu and Zn could be introduced into BPhes or BChls in reasonable yields by direct methods.\(^ {17,25}\) Although the metalation of BPhes with \(\text{Zn}^{2+}\) or \(\text{Cd}^{2+}\) by the acetate/dimethylformamide (DMF) method (Experimental Section) is almost independent of modifications at the macrocycle, the conditions for the acetic acid method (Experimental Section) used to achieve metalation with Cu (or Zn) strongly depend on the substituents at position C-13.\(^3\)

Low yields were observed for the metalation of BPhes \((\text{BChl})\) with \(\text{Cu}^{2+}\) in acetic acid when the reaction mixture was stirred for several hours at 20°C; at 100°C the reaction was completed within 15 min with a 90% yield. In contrast, incorporation of \(\text{Cu}^{2+}\) into \(\text{13}-\text{hydroxy-BPhe}\) to yield \(5b\) was already completed in 10 min at ambient temperature. Since \(\text{13}-\text{hydroxylation}\) inhibits enolization of the BPhes \(\beta\)-ketoester system of ring V, the reaction was also tested with \(\text{13}-\text{decarbomethoxy-BPhe}\), which lacks these chelating substituents. Again, metalation was as fast as for \(\text{13}-\text{hydroxy-BPhe}\). Incorporation of \(\text{Zn}^{2+}\) into the different BPhes derivatives by the acetic acid method resulted in a similar although less pronounced kinetic difference. Metalation of BPhes \((\text{BChl})\) by \(\text{Zn}^{2+}\) was completed after 120 min of reflux at 100°C, and that of the \(13\)-derivatives after 30 min.

We propose that the kinetic differences observed in applying the acetic acid method reflect the release rate of the central protons of BPhes prior to or simultaneous to metal incorporation (the rate-determining step).\(^ {29}\) However, the functioning of metalation in acetic acid is surprising but may be explained by a catalytic effect: acetic acid protonates the conjugated keto

![Figure 1](image-url)

**Compound**

<table>
<thead>
<tr>
<th>M</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>2H</td>
<td>Pd</td>
<td>Co</td>
<td>Ni</td>
<td>Cu</td>
<td>Zn</td>
<td>Mn</td>
<td>Cd</td>
<td>Mn</td>
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</tr>
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**Figure 1.** Structure formulas. a and b after the numbers indicate R = H and R = OH, respectively. Phy = phyl (C\(_{20}\)H\(_{39}\)).

**(29)** Besides this intuitive mechanism, also other, more complex mechanisms may account for the observed differences in the rate of metalation by the acetic acid method, but some of them can be ruled out. For instance, complexation of the metal ions to the isocyclic carbonyls that may act as a bidentate ligand can explain the low reactivity of BPhes. However, significant variations in the Q transition should accompany such complexation (ref 56). Since no such spectral changes were observed during the insertion of \(\text{Cu}^{2+}\) and \(\text{Zn}^{2+}\), this mechanism cannot be ruled out. Another explanation could be that the BPhes modified at C-13 are more amenable to the steric distortions required for the formation of an intermediate sitting atop (SAP) the complex (ref 30). However, the steric effects around the C-13 do not seem to play an important role, since \(\text{13}-\text{decarbomethoxy}\) and \(\text{13}-\text{hydroxy}\) derivatives exhibit similar reactivities in the metalation reaction. Furthermore, such distortions are also expected to affect the BPhes optical absorption (ref 10), in contrast to what has been observed in our experiments.
The absorption and fluorescence spectra of the 13\textsuperscript{2}-OH pigments (1b–9b) matched those of the respective 13\textsuperscript{3}-H parent compounds, except for a systematic blue shift of the Q\textsubscript{x} absorption by ~5 nm. The mass spectra were always shifted by 16 mass units to higher values. All wavelengths are in [nm].

Fluorescence in DE/petroleum ether/2-propanol (5:5:2; v/v/v) at 298 K (77 K). Electronegativity (\(\chi\)) and effective ionic radii (\(r^*\)) in 10 \(^{-6}\) m for 6-fold coordination (data in brackets use radii for 4-fold coordination) from ref 13. If data used in Figure 3 are attributed to different coordination numbers, the radii were estimated using a 5% increased overestimated (Kälin, Scheer, and Schraml, unpublished results).

Fluorescence in pyridine-\textsubscript{d}\textsubscript{6}, sharp signals (~ = extensive line broadening because of paramagnetic central metal). Not fluorescent (Spx fluorolog 221). Sharp \(1\ H\) NMR signals in C\textsubscript{6}H\textsubscript{5}CN. Pd analysis is error prone. It can be both grossly underestimated, and \(\epsilon\) accordingly overestimated, by incomplete removal of Pd from the macrocycle, or overestimated (~ understimated) by band overlap (Kalín, Scheer, and Schraml, unpublished results).

The [Cd] complexes are readily accessible by the acetate/DMF method and can be transmetalated with excellent yield to the other metal complexes under mild conditions (Experimental Section). The easy transmetalation using [Cd]-BChl as a precursor is unexpected and is probably due in part to the large ionic radius (\(r^*\)) of Cd\textsuperscript{2+} (95 pm) compared to Mg\textsuperscript{2+} (\(r^*\) = 72 pm). It is assumed that Cd does not fit into the “inner hole” spanned by the four nitrogens of the macrocycle and that it forms a sitting-atop complex. This is supported by the low stability of the Cd complex, which already demetalates at pH 6–7. Another factor is the solvent (acetone) in combination with the counterion (chloride) of the metal used for the reaction. During transmetalation, CdCl\textsubscript{2} and [M]-BChl are formed in equilibrium with the reactands, and the very low solubility of CdCl\textsubscript{2} in acetone causes its removal and shifts the equilibrium to the side of the end products.

All end products were characterized by absorption, fluorescence, \(1\ H\) NMR, and FAB-mass spectroscopy (FAB-MS) (Tables 1 and 2). The Mn\textsuperscript{3+} and Co\textsuperscript{2+} oxidation states, under...
at 77 and 298 K (Table 1) that can be detected ($\Phi_F > 10^{-3}$) can be attributed to the respective complexes by their fluorescence excitation spectra. The Stokes shifts of all fluorescent complexes were similar (260 ± 70 cm$^{-1}$) and almost metal- and temperature-independent.

At 298 K, phosphorescence was detectable for [Cu]-BChl at ~1230 nm and for [Pd]-BChl at 1170 nm. The difference between the fluorescence and phosphorescence energies of [Pd]-BChl corresponds to a singlet–triplet energy splitting of $\Delta E_{ST} = (4530 \pm 70)$ cm$^{-1}$. [Cu]-BChl did not show any detectable fluorescence. Assuming a Stokes shift similar to that of the other complexes, the hypothetical fluorescence was estimated at 787 nm, which gives $\Delta E_{ST} = (4580 \pm 70)$ cm$^{-1}$. Phosphorescence for both complexes was recorded in tolune, in which the complexes showed absorption maxima that are almost identical to those in DE or DE/petroleum ether/2-propanol. Both complexes should be four-coordinated in these solvents with no additional axial ligand as indicated by the correlation between absorption spectra and coordination discussed below.

The singlet–triplet splitting energies for four-coordinated [M]-BChl are of a size similar to those of natural BChl in its 5-fold ($\Delta E_{ST} = 4610$ cm$^{-1}$) or 6-fold coordination state ($\Delta E_{ST} = 4550$ cm$^{-1}$), as well as that of [Zn]-BChl in different solvents ($\Delta E_{ST} = 4550$–4600 cm$^{-1}$). In this series of metal-substituted BChls, the singlet–triplet splitting energies are then almost independent ($\Delta E_{ST} = 4500$–4600 cm$^{-1}$) of the central metal and of its state of ligation.

**UV/Vis Absorption.** The UV/vis absorption spectra of all complexes show four distinct bands labeled $B_1$, $B_2$, $Q_1$, and $Q_2$ in order of decreasing transition energy, and they resemble those of BChl and BPhe. These bands show a characteristic pairwise dependence when the central metal is changed: the $B_1$ and $Q_1$ transition energies are only slightly affected by changing the central metal or its coordination. In contrast, strong and distinct differences are seen between the $B_1$ and $Q_2$ bands. Replacing Mg by Ni, for example, results in blue shifts of 1750 and 1400 cm$^{-1}$ in the $B_1$ and $Q_2$ bands, respectively, whereas the $B_2$ and $Q_1$ bands are shifted by less than 150 cm$^{-1}$. Large shifts for $B_2$ and $Q_1$ and minor shifts for $B_1$ and $Q_2$ are also seen for the other metals (Table 1).

The large shifts of $Q_1$ and $B_2$ can be rationalized qualitatively by the four-orbital model. According to this model the UV/vis spectra result from transitions among the four orbitals: $a_{2u}$ (HOMO-1), $a_{1u}$ (HOMO), $e_g$ (LUMO), and $e_y$ (LUMO+1). In a zero-order approximation neglecting configuration interaction, $B_2$ is a transition from $a_{2u}$ to $e_y$, $B_1$ from $a_{1u}$ to $e_y$, $Q_1$ from $a_{2u}$ to $e_g$, and $Q_2$ from $a_{1u}$ to $a_{1u}$. The $a_{2u}$ orbital has high electron densities at the four nitrogens nearest to the metal in the “inner hole” of the porphyrin system, whereas the $e_y$ and $e_g$ orbitals have substantial electron densities on only two nitrogens and the $a_{1u}$ orbital has almost no electron density at the nitrogens. Therefore, a change in the effective positive charge at the BChl center is expected to modify the orbital energies, via electrostatic interactions with the $\pi$ electrons, by $\Delta E_{a_{2u}} \gg \Delta E_{e_g} \approx \Delta E_{e_y} \gg \Delta E_{a_{1u}}$ and the energy of the $Q_1$ and $B_2$ transitions will be modified to a much larger extent than that of $Q_2$ and $B_1$. Still, following this simple picture, $Q_1$ and $B_2$ are also expected to be somewhat modified, but in the opposite direction. This will be treated more elaborately considering configuration interaction among the individual single-electron promotions in the accompanying paper.

**Solvent Dependence of Optical Spectra.** Any effect of the central metal on the energy of the four orbitals is expected to be additionally modulated by axial ligation of the central metal in [M]-BChl, because ligation probably reduces the effective charge of the central metal and hence its electrostatic interaction with the $\pi$ electrons. In agreement with this, the spectra of BPhe, which cannot bind additional ligands because of the lack of a central metal, are nearly solvent-independent. Pronounced solvent shifts (1110 cm$^{-1}$) have, however, been reported for the $Q_1$ band of BChl and have been related to a change from 5- to 6-fold coordination, i.e., to the presence of one or two axial ligands. Changes in the transition energy of the $Q_1$ band were much smaller. The $B$ bands have not been investigated in previous studies, but here we observed a shift of 1270 cm$^{-1}$ for the $B_1$ band upon changing the solvent from DE to pyridine. The large shifts of the BChl’s $Q_1$ and $B_2$ transition energies and the absence of significant shifts in BPhe indicate that the spectral modifications reflect neither a change in the solvent refractive index nor differences in the polarity of the [M]-BChl excited states but rather changes in the metal coordination number ($n_k$).

The observations found for BChl are also relevant to the other [M]-BChls reported here, as long as the metal can acquire an $n_k$ larger than four. For example, the $Q_1$ and $B_2$ bands of [Cu]-BChl and [Co]-BChl are red-shifted by (1090 ± 50) cm$^{-1}$ and by (1450 ± 150) cm$^{-1}$, respectively, similar to the shifts observed for BChl itself. Although [Zn]-BChl, [Cd]-BChl, and [Mn]-BChl are less affected, the solvent-induced shifts of their electronic transition energies are significant ($\Delta E_{Q_1} = (525 ± 125)$ cm$^{-1}$, $\Delta E_{B_2} = 2500$ cm$^{-1}$). Figure 2) which probably reflects a change from $n_k = 4$ in DE to $n_k = 6$ in pyridine. This change is supported by its analogy to Ni-porphyrins and $^1$H NMR combined

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36 This labeling was derived for the $D_4h$-symmetric metal-substituted porphyrins but is applicable to chlorins and bacteriochlorins despite reduced symmetry.
with CD spectra (vide infra). The only metal complex with little solvent effect on the Qa and B transition (<300 cm−1) is [Pd]-BChl, due to the preferential 4-fold coordination of Pd in all solvents.

As for [Ni]- and [Pd]-BChl, EXAFS measurements indicated a 4-fold coordination for [Zn]-BChl in DE.23 It seems reasonable to assign a 4-fold coordination also to Cd-BChl in DE because of the chemical similarity of Cd and Zn. Tetracoordination for these complexes as well as for [Cu]- and [Co]-BChl in DE is also indicated by analogy to the [M]-porphyrins and -chlorins.13,26,41

The only M-BChl with established 5-fold coordination is for BChl (M = Mg) in DE. However, since pentacoordination of [Zn], [Cu], and [Co] in strong nucleophiles such as pyridine has been thoroughly documented23 and is exhibited in [Zn], [Cu]-, and [Co]-porphyrins dissolved in pyridine,41 we might expect that n = 5 for the respective [M]-BChls. The assignment of 5-fold coordination for [Zn]-BChl in pyridine is strengthened by the finding that it is also 5-fold coordinated in the bacterial reaction center.23 There, the Qa absorption band for this molecule is located at ~595 nm (16 800 cm−1), which is red-shifted by 500 cm−1 with respect to the same transition in pyridine (17 300 cm−1). This is similar to the environmentally induced red shift of (700 ± 200) cm−1 observed for BChl when its Qa transition energy for pentacoordination in the reaction center is compared with the Qa transition energy for pentacoordination in DE.21,44 Thus [Zn]-BChl should be pentacoordinated in pyridine. Sixfold coordination is indicated only for [Mg]- and [Ni]-BChl in pyridine (vide supra).

**Linear Correlation of Transition Energies with Scaled Metal Electronegativity χ_M/r_M.** In the porphyrin and chlorin series, the influence of the central metals on the redox properties of the [M]-porphyrins and [M]-chlorins have been linearly correlated to the central metals’ electronegativities (χ_M). It has been suggested that an increase in χ_M should cause a shift of the N-σ electrons toward the metal which would substantially lower the potential of the N-π and C-π electrons (inductive effect45). However, this approach failed to account for the experimental results in a rigorous manner.46

For [M]-BChls, a similar dependence of the redox properties22 and especially of the optical transition energies presented here failed to provide good linear relationships unless the complexes were classified into three groups according to their coordination number. Attempts to relate changes in the Qa and B transition energies of the complexes studied in each group with χ_M resulted in a moderate linear correlation (Table 3). The linearity within each group was improved, however, when χ_M was scaled by 1/r_M (where r_M is the ionic radius of the metal). This χ_M/r_M ratio had already been used by Buchler13 as a criterion for the binding strengths of a variety of metals to porphyrins ("stability factor").

Intuitively, the normalization of χ_M to the ionic radius r_M serves as a scaling factor. This could relate to the special situation in BChl: Since the macrocycle and hence its central

![Figure 3. Correlation of the B (top) and Qa transition energies (bottom) with χ_M/r_M of the BChl’s central metal (indicated in the figure). Coordination numbers n_c: (△) n_c = 4, (−) linear fit; (●) n_c = 5, (−−) linear fit; (■) n_c = 6, (−−−) linear fit (see text for details).](image)

**Table 3. Parameters for Linear Regression of the [M]-BChls’ B_transition and Qa Transition Energy E_M,T (Figure 2) at Central Metal Coordination Number n_c. According to eq 1 (E_M,T in cm−1, r_M in 10−14 m, and χ_M Given in charge/m). Correlation Coefficient χ_M^2 Given for the Fits of the Respective Absorption Maxima vs the Expression underneath in Parentheses. Indices of a and b Given in eq 1 Are Left Out Here For Clarity.**

<table>
<thead>
<tr>
<th>n_c</th>
<th>a ± Δa</th>
<th>b ± Δb</th>
<th>χ_M^2</th>
<th>χ_M^2</th>
<th>χ_M^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1648 ± 157</td>
<td>2447 ± 461</td>
<td>0.983</td>
<td>0.875</td>
<td>0.972</td>
</tr>
<tr>
<td>5</td>
<td>816 ± 80</td>
<td>25612 ± 206</td>
<td>0.991</td>
<td>0.880</td>
<td>0.985</td>
</tr>
<tr>
<td>6</td>
<td>531 ± 29</td>
<td>2578 ± 66</td>
<td>0.999</td>
<td>0.963</td>
<td>0.976</td>
</tr>
</tbody>
</table>

*Too few data points to justify linear regression; parameters are just shown for the sake of completeness.*
particular coordination number \( n_c \) and transition “T” but are independent of the incorporated metal.

In the accompanying manuscript\(^{28}\) this relationship will be explored following Mulliken and others\(^{3,47,48}\) who claimed that electronegativity (i) depends on the metal’s particular environment and valence state and (ii) represents an electrostatic potential induced by an effective positive charge \( Q_M \) at the metal atom center. We propose that by changing the metal coordination number \( n_c \) we modify the metal electronegativity. This effect is moderated by the particular \( \pi \) orbital because each orbital contains nitrogen centers that are coordinated with the metal. Further, a decrease in the metal ionic radius \( r_{M} \) decreases the orbital overlap of the metal with the nitrogens, and thereby the screening of the effective positive charge. In light of these considerations, the first term \( \delta_{A}^{x} \) in eq 1 represents the change in a particular transition energy due to a change in metal electronegativity within the BChl frame, while the second term \( \delta_{B}^{x} \) represents a reference transition energy. The physical meaning of \( \delta_{B}^{x} \) will be clarified in the subsequent paper\(^{28}\) where we shall also derive the effective positive charge for each of the substituting metals. The empirically derived variable \( \chi_{M}^{P}r_{M}^{i} \) is linearly related to the actual effective charge \( Q_M \) of the central metal.

This treatment also provides a rationale for scaling \( \chi_{M}^{P} \) in the frame of the “rigid inner hole” discussed above: As the metal’s ionic radius decreases, the overlap between the metal orbitals and those of the nitrogens decreases, and this is expected to decrease the screening of the metal’s effective charge (and to increase the ionic character of the bond). To roughly compensate for this effect with varying sizes of the central metal, it seems sufficient to scale \( \chi_{M}^{P} \) by \( 1/r_{M}^{i} \). Qualitatively, this scaling can also correct for metals being too large to fit into the inner hole of the porphyrin by decreasing the effective charge as compared to coplanar complexes. Here, only Cd\(^{2+}\) qualifies for such a sitting-atop complex, which does not allow for a quantitative evaluation.

A similar rationale may account for the need to group the [M]-BChls studied according to \( n_c \) before establishing the relationships with \( \chi_{M}^{P}r_{M}^{i} \). Each additional ligand increases the shielding of the metal’s nucleus (note that \( r_{M}^{i} \) increases with \( n_c \) and that this increase is accounted for in the empirical correlation). In these groups, the spectra of [Pd]-, [Ni]-, [Cu]- and [Zn]-BChl in DE can be used to define the correlation between these transitions and \( \chi_{M}^{P}r_{M}^{i} \) for tetracoordinated [M]-BChls. Note that the \( B_x \) and \( Q_x \) transition energies of [Cd]-BChl fit into this correlation very well (Figure 3). This observation supports the assignment of a 4-fold coordination to [Cd]-BChl in DE.\(^{50}\)

The \( Q_x \) and \( B_x \) transition energies of [Cu]-, [Co]-, [Zn]-, and [Cd]-BChl in pyridine show a different but linear correlation with \( \chi_{M}^{P}r_{M}^{i} \) (Figure 3). As discussed previously, these complexes are probably 5-fold coordinated in pyridine. Since the slopes of the linear correlations for 4- and 5-fold coordination are different, the comparatively small solvent shifts of the \( B_x \) and \( Q_x \) transitions for [Zn]- and [Cu]-BChl (vide supra) are due to their low \( \chi_{M}^{P}r_{M}^{i} \) values. Finally, the \( Q_x \) transition energy of [Mg]-BChl in pyridine has been shown to relate to its 6-fold coordination.\(^{38}\) [Ni]-BChl in pyridine should also exhibit 6-fold coordination, so that these and the corresponding \( B_x \) transitions can serve to define a third line that correlates 6-fold coordinated [M]-BChls with their \( \chi_{M}^{P}r_{M}^{i} \) values (Figure 3).

The linear correlations between the \( Q_x \) or \( B_x \) transition of [M]-BChls and \( \chi_{M}^{P}r_{M}^{i} \) (grouped by coordination numbers) are consistently better than those with \( \chi_{M}^{P} \) and, although to a lesser extent, with \( \chi_{M}^{P}r_{M}^{i} \) (Table 3). The largest deviations from linear correlation with \( \chi_{M}^{P}r_{M}^{i} \) are given for [Mn]-BChl in DE or pyridine and [Mg]-BChl in DE. The Mn “anomaly” may reflect the presence of oxygen-bridged [Mn]-BChl dimers in equilibrium with monomers,\(^{17}\) which may influence the two transitions differently. There is no evidence that [Mg]-BChl in DE forms such dimers. However, since it is known to exhibit 5-fold coordination (while lying on the line of 4-fold coordination), its \( \chi_{M}^{P}r_{M}^{i} \) value in the 5-fold coordination may be underestimated by approximately one-third. A very similar deviation is obtained for [M]-BChls in the \( B_{A,B} \) (= “monomeric”) binding sites of purple bacterial reaction centers, where a 5-fold coordination has been proven.\(^{23,51,52}\) The protein supposedly imposes a fixed geometry on the complex, although the 3-acetyl group of the nearby BPhes could be regarded as a potential additional candidate for ligation, or at least for influencing the central metal of the monomeric [M]-BChls. In this environment, [Mg]-BChl, [Zn]-BChl, and [Ni]-BChl show \( Q_x \) transitions at 600, 595, and 585 nm, respectively,\(^{17,18,24}\) The correlation of this transition with \( \chi_{M}^{P}r_{M}^{i} \) for [Zn]- and [Ni]-BChl shows a \( \delta_{B}^{x} \) value identical to the one obtained for the 5-fold coordinated [M]-BChls in solution (vide infra), but again the \( \chi_{M}^{P}r_{M}^{i} \) value for [Mg]-BChl is underestimated by approximately one-third. The obvious deviation of Mg, which is of central biological importance, needs to be further explored. Note that Mg is the only metal studied that lacks \( d \) orbitals, and therefore \( P / M / y \) electron donation from and \( d \pi \rightarrow e \sigma \) back-bonding to the axial ligands might be different.

The influence of electron donation from an additional axial ligand on \( \chi_{M}^{P} \) is illustrated by the decreasing slope with increasing coordination number (Table 3). Since the influence on \( r_{M}^{i} \) is accounted for, adding the first axial ligand reduces \( \chi_{M}^{P}r_{M}^{i} \) (and thus \( Q_M \)) by approximately 40% (\( Q_x \)) to 50% (\( B_x \)), whereas the second axial ligand induces only a reduction of 25% (\( Q_x \)) to 20% (\( B_x \)) relative to \( n_c = 4 \).\(^{53}\)

As shown in Figure 3 and Table 3, \( \delta_{A}^{x} \) is larger than \( \delta_{B}^{x} \). In a zero-order approximation neglecting configuration interaction, this indicates either a larger admixture of eg to the \( Q_x \) transition than of e\(_{g}\) to the \( B_x \) transition, or a stronger effect of the central metal on e\(_{g}\) than on e\(_{g}\). A semiquantitative estimate of the central metal's influence on the individual orbital energies is provided by electrochemical data of the [M]-BChls in THF.\(^{22}\)


\(^{(53)}\) A coordination number \( n_c \) of four is likely for most of the [M]-BChls examined electrochemically in tetrahydrofuran according to their Q\(_{c}\) absorption (ref 22).

\(^{(54)}\) There is, within the error of the correlation, no change in the constant, if \( n_c \) changes from five to six. The solvent for both coordination situations is pyridine. When \( n_c \) changes from four to five the constant experiences a weak relative increase (<5% for \( B_x \) or \( Q_x \)). The change from 4- to 5-fold coordination is accomplished by a solvent change from DE to pyridine for almost all [M]-BChls. However, such an increase is very sensitive to minor changes in the slope of the respective correlations. Thus it is better to judge the pure solvent effect on the constant from BPhes and [Pd]-BChl, which have no axial ligands in every solvent, but their \( Q_x \) and \( B_x \) transition energies are lowered by approximately 300 cm\(^{-1}\).
where according to the Q transitions, most of the complexes are tetra-coordinated.\(^{(54)}\) There, the redox potentials are also linearly correlated with \(\frac{\chi_M}{f_M^2}\). The potentials \(E_{1/2}\) vs Ag/AgCl for the oxidation and reduction exhibit a slope of \(-1300\) (HOMO, \(a_{2u}\)) and \(-1360\) (LUMO, \(e_y\)), respectively, in agreement with the minor variability of the Q transition in the series of examined [M]-BChls. Comparison with \(d_{en}^c\) for the \(B_y\) (\(2a_{2u} \rightarrow e_y\)) and \(Q_y\) (\(2a_{2u} \rightarrow e_y\)) transitions of \(\sim 150\) and \(\sim 1150\), respectively, for \(n_c = 4\), enables estimating the orbital shift per unity of \(\frac{\chi_M}{f_M^2}\) and yields \(\Delta E_{2a_{2u}} \approx -2500\) cm\(^{-1}\), \(\Delta E_{e_y} \approx -1360\) cm\(^{-1}\), \(\Delta E(a_{2u}) \approx -1300\) cm\(^{-1}\) and \(\Delta E_{e_y} \approx -850\) cm\(^{-1}\).

On the basis of this estimate, the difference between \(d_{en}^c\) and \(d_{en}^y\) can be attributed to a stronger effect of the central metal on \(e_y\) than on \(e_x\).

\(^{1}H\) NMR Measurements. No \(^{1}H\) NMR signals were obtained for the paramagnetic complexes [Co]-, [Cu]-, and [Mn]-BChl because of extensive line broadening. [Ni]-BChl showed broad lines in pyridine, indicating a paramagnetic, octahedral complex; however, it is diamagnetic in \(\text{CH}_3\text{CN}\) (sharp lines) and probably square-planar-coordinated. Although quadratic pyramidal [Ni]-complexes (\(n_c = 5\)) can exhibit paramagnetic high-spin as well as low-spin configurations, we disfavor 5-fold coordination in any of the solvents because of the shift in the Q transition (531 nm in \(\text{CH}_3\text{CN}\) and 598 nm in pyridine, Figure 2) and the dependence on \(n_c\) derived above. The assignment of \(n_c = 4\) in \(\text{CH}_3\text{CN}\) and \(n_c = 6\) in pyridine is supported by EXAFS measurements.\(^{(54)}\) The central metal of [Ni]-BChl in reaction centers is 5-fold-coordinated, showing a Q absorption at \(-858\) nm. [Ni]-BChl with \(n_c = 5\) in solution should then absorb around 560 nm, if the environmentally induced red shift is taken into account.

The \(^{1}H\) NMR signals of selected protons of the diamagnetic complexes in the [M]-BChl series are listed in Table 2. In general, the chemical shift decreases for a given proton of the pyridine-solvated [M]-BChls (M = Pd, Zn-, Mg-, and Cd) with decreasing \(\frac{\chi_M}{f_M^2}\) values.\(^{(55)}\) This agrees with the electron-withdrawing effect (\(-I\) effect) of the central metal that leads to deshielding of the protons and thus to a low-field shift. Of particular interest are the meso protons (5-, 10-, and 20-H), which are closest to the \(\pi\) system and show the largest ring-current shifts. The more distant protons such as 17-H, 18-H, 2-CH\(_3\), and 13\(^{-}\)-CO\(_2\)CH\(_3\) are influenced less, but the general direction is the same. This is qualitatively consistent with the electrostatic effect of the central metal on the ring current of the \(\pi\) system.

On the basis of this assignment, the increasing chemical shifts with a decreasing \(-I\) effect (\(\frac{\chi_M}{f_M^2}\)) value for the 13\(^{\text{pro}}\)-proton and the 12-CH\(_3\)-protons are unexpected. They may be rationalized as a result of the involvement of (partial) enolization for the [M]-BChls. Although significant populations of the enol form of the \(\beta\)-ketoester system of the isocyclic ring V (probably a 13\(^{-}\)-en-13\(^{\text{pro}}\)-ol form) have never been detected by \(^{1}H\) NMR measurements of [Mg]-BChl.\(^{(56,57)}\) The H exchange at 13\(^{-}\)-H as well as epimerization at this chiral position have been explained by keto-enoltautomerism.\(^{(58)}\) A reduced \(\pi\)-electron density could stabilize the transition state to the enol form by a reduced overlap of the developing (negatively charged) \(\pi\) orbital at position C-13\(^{\text{pro}}\) with the neighboring \(\pi\) orbitals. The relative contribution of the enol form and therefore the mean electron density at position 13\(^{\text{pro}}\) is increased. This would result in an increased screening (decreased chemical shift) for the proton at position C-13\(^{\text{pro}}\) with increasing \(\frac{\chi_M}{f_M^2}\) values. Vinylogous enolization, used for explaining the \(^{1}H/H^\text{I}\) exchange at 12-CH\(_3\) in BChl,\(^{(59)}\) would copy the behavior of the proton at C-13\(^{\text{pro}}\) to the protons 12-CH\(_3\) (and potentially even to 3-CO\(_2\)CH\(_3\)).

**Circular Dichroism.** The CD spectrum of [Ni]-BChl in pyridine has signs and intensities similar to those of BChl (Figure 4, bottom) and all the other [M]-BChls including the square-planar [Pd]-BChl (not shown). Such a CD signal is expected for a roughly planar macrocycle with octahedral Ni. In contrast, the CD signals for [Ni]-BChl in \(\text{CH}_3\text{CN}\) or DE are \(-10\)-fold stronger (Figure 4, upper trace) than those of all the other complexes in the series. This strong increase in CD intensity probably reflects \(S_2\) ruffling of the macrocycle typical for square-planar, low-spin [Ni]-iso-bacteriochlorin,\(^{(50)}\) which renders the macrocycle inherently disymmetric.

The change from planar to \(S_2\) ruffling is probably related to the small ionic radius of low-spin Ni\(^{2+}\) (\(-6\) pm, compared to \(\sim 70\) pm with high-spin) that shortens the [Ni]-N bond length. However, there is no concomitant change in the Q transition energy. Connick et al.\(^{(61)}\) observed a 525 cm\(^{-1}\) red shift in Q\(_{\text{I}}\) for a similar high-spin(low-spin transformation in a [Ni]-tris-
β-oxoporphyrin (formally a dihydro-bacteriochlorin) and a 306 cm⁻¹ blue shift in a [Ni]-bis-β-oxoporphyrin (formally an isobacteriochlorin), whereas Fajer et al.¹⁰ reported a much larger red shift of ~1350 cm⁻¹ for the Q₂ transition upon distortion of a Zn-porphyrin (with unchanged spin state). This may indicate that the high-spin to low-spin change alone causes a blue shift which is (almost) canceled in [Ni]-BChl by the concomitant distortion.

Conclusions

Metal-substituted bacteriochlorophylls provide a wealth of spectroscopic data, which, because of the band’s overlap, is not readily available from porphyrins or chlorins. The series of [M]-BChls was used to test the influence of the central metal as well as the presence of additional axial ligands on the energies of the four lowest electronic transitions, Bₘ, Bᵢ, Qₘ, Qᵢ, in the framework of the four-orbital model. Because of the fixed geometry of the macrocycle, much of the data gave satisfactory linear correlations with X(M)²μₐ rather than with Xₐ alone. There, rₘ serves as a scaling parameter and X(M)²μₐ accounts for the effective charge of the central metal in the fixed frame of the macrocycle. Yet, further scaling is needed to account for the effect of the metal valence and axial ligation. This is achieved by grouping [M]-BChls according to their (putative or experimental, when available) coordination number. For those X(M)²μₐ correlations using Xₐ values that do not account for different coordinations per se, axial ligands can be regarded as a factor that mainly reduces the effective charge and thus the electronegativity of the central metal. The ratio rₘ(X)²μₐ⁻¹ is therefore a useful heuristic parameter in relating spectral and redox properties in the conformationally restricted macrocyclic complexes. Alternatively, the ligand effect may be accounted for by using the concept of equalization of electronegativities.⁶² If successful, this approach may enable considering linear relationships as mentioned above without preclassification of the coordination or metal valence state. Thus, in view of the changing concepts of electronegativity, a more rigorous approach is desirable. Such a concept is outlined in the subsequent manuscript,²⁸ which is based solely on electrostatic interactions of the central metal with the frontier orbitals of the macrocycle.

Experimental Section

⁷a was isolated from Rhodobacter sphaeroides using standard methods.⁴¹,⁴³,⁶⁴ Hydroxylation at position C-13 to yield ⁷b was done by storage of ⁷a in methanol for 5 days in the dark and subsequent silica gel chromatography.⁵⁶,⁶⁶ C-1₃-decarboxamethylation was achieved by 12 h of reflux in pyridine.⁵⁶,⁶⁶ Demetilation of ⁷a (⁷b) was achieved by treatment with small amounts of glacial acetic acid to yield ⁷a (¹b).⁶⁷ Direct Metalations by Acetate/DMF. ⁸a (⁸b) was prepared by refluxing ¹a (¹b), (~70 µM) in dimethylformamide, with a 250-fold excess of anhydrous Zn(OAc)₂ and 50 mM sodium ascorbate for 120 (30) min at 100 °C. The acetic acid was then evaporated in a stream of N₂, and the Zn complex was extracted with diethyl ether and purified on a preparative ModCol HPLC column (250 × 25.4 mm) packed with Bakerbond Silica NP (particle size 10 µm; pore diameter 150 Å). ⁶a was eluted isocratically (10 mL/min) with a mixture of 2-propanol (5%), methanol (5%), and n-hexane (90%), v/v with a retention time of about 17 min, with ~75% yield of the purified compound. ⁶b was purified by column chromatography on silica gel, using the same solvent mixture as for HPLC, giving a yield of 90–95%. The Zn-derivative of 1₃-decarboxamethoxy-BPhe or 1₃-decarboxamethoxy-BChl can be obtained under the same conditions; reaction time is 30 min at 100 °C; isolation and purification is identical to that of ⁶b.

⁵a was prepared by refluxing ¹a or ⁷a, (~70 µM) in glacial acetic acid, with a 250-fold excess of anhydrous CuO and sodium ascorbate (50 mM) for 15 min at 100 °C. ⁵b is formed at ambient temperature by mixing ¹b or ⁷b, (~70 µM) in glacial acetic acid, with a 250-fold excess of anhydrous CuO and 50 mM sodium ascorbate. The Cu derivative of 1₃-decarboxamethoxy-BPhe or 1₃-decarboxamethoxy-BChl was obtained at conditions identical to those described for ⁵b. Despite using CuO, the Cu⁷⁺ complexes were always formed due to the presence of residual oxygen or disproportionation. Isolation and purification was done as described for the Zn⁷⁺ complexes prepared by the glacial acetic acid method, yielding ~75% (⁵a), ~90% (⁵b), and ~90% (Cu derivative of 1₃-decarboxamethoxy-BChl).

Transmetalation. Addition of metal chlorides to a solution of the Cd complexes ⁸a or ⁸b (~60 µM) in acetonitrile resulted in transmetalation to ²a-b or ⁹a-b. The reactions were carried out under strict Ar protection and ran to completion. The metal chlorides were added at a 10-fold molar excess (⁵a-b and ⁹a-b), 100-fold molar excess (³a-b), or to saturation (²a-b, ³a-b, and ⁹a-b). The reactions occurred practically instantaneously at 298 K, except for ²a-b and ³a-b (~30–40 min reflux) and were followed spectroscopically. Small amounts of C7–C8-oxidized products (λmax ~ 680 nm) were formed due to the presence of residual oxygen which can be suppressed by adding Na-ascorbate (saturated). Isolation and purification of the products was done as for ⁸a-b, yielding 90–95% of pure product. Absorption, emission, the molecular ion of the mass spectra, and the main signals of the H NMR spectra are listed in Tables 1 and 2. Full spectra are available as Supporting Information.

Instrumentation. UV/vis absorption spectra were recorded on a Perkin-Elmer Lambda 2 spectrophotometer. Fluorescence emission spectra were determined with a Spex Fluorolog 221 equipped with a 450-W xenon lamp and were normalized to the sensitivity of the photomultiplier tube and excitation energy. Maximum optical densities for fluorescence measurements were <0.1 cm⁻¹ and excitation was into the Q₁ absorption band of ¹a-b, ³a-b, and ⁹a-b. Circular dichroism spectra (CD) were recorded on a Dichrograph CD6 (Jobin Yvon). Fast atom bombardment mass spectra (FAB-MS) were recorded on a CH22SS 100 mass spectrometer (Varian MAT) or a Finnigan MAT 9000 with a Cs gun. Liquid–surface ionization was done in a matrix of m-hydroxybenzyl alcohol. H NMR spectra were recorded on a 360-MHz Bruker, model AM360. Standard solvent was pyridine-d₅, and chemical shifts are in ppm against tetramethylsilane as internal standard. Extinction coefficients were determined by ICP/IPCMS atom absorption spectra (AAS) of the central metals; before combustion, the solvent in samples of ¹a-b to ⁹a-b with quantified optical densities was first

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6a (⁶b) was prepared by refluxing ¹a (¹b), (~70 µM) in dimethylformamide, with a 1000-fold excess of anhydrous Zn(OAc)₂ for 60 (75) min at 110 °C (reflux at 163 °C decreases the reaction time to 5 min). The reaction was followed spectroscopically and run to completion. Isolation and purification of products was done as for ⁸a-b (yield ~80%). Metalation by the acetate/DMF method can be extended to other derivatives of BPhe, when reaction conditions are slightly varied. For instance, metalation of 3- vinyl-BPhe or 3- vinyl-1₃-hydroxy-BPhe with Zn(OAc)₂ proceeds under identical conditions within ~40 min at 120 °C.
evaporated in quartz glass tubes and the samples then treated with concentrated nitric acid to allow complete release of the metal.

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Supporting Information Available: Listings of experimental procedures and spectra of Table 2 (absorption, fluorescence, \(^1\)H NMR, FAB-MS), additional absorption spectra in pyridine, [CD] spectra, and electron density distribution for BChl (adapted from ref 37) (65 pages). See any current masthead page for ordering information and Web access instructions.

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