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EVIDENCE FOR 5- AND 6-COORDINATED MAGNESIUM IN BACTERIOCHLOROPHYLL *a* FROM VISIBLE ABSORPTION SPECTROSCOPY*

THOMAS A. EVANS** and JOSEPH J. KATZ

Chemistry Division, Argonne National Laboratory, Argonne, Ill. 60439 (U.S.A.)

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SUMMARY

The visible absorption spectrum of bacteriochlorophyll *a* (Bchl) in the 560–620 nm (yellow) region can be used to establish the coordination state of the central Mg atom. Five-coordinated Mg species absorb near 580 nm, whereas 6-coordinated Mg species are red-shifted to about 610 nm. Five-coordination is confirmed to be the principal coordination state of Mg in self-aggregated bacteriochlorophyll. The equilibrium constant for the reaction Bchl · Py + Py $\rightleftarrows$ Bchl · Py_2 has been determined from computer-assisted analyses of spectral data, where Py represents pyridine. The spectral criteria for the coordination state of Mg in bacteriochlorophyll advanced here are shown to be applicable to both in vitro and in vivo systems. Similar spectral behavior is exhibited by chlorophylls *a* and *b*, and a band at 633 nm is shown to be associated with the presence of 6-coordinated Mg in chlorophyll *a*.

INTRODUCTION

The coordination properties of the central magnesium atom in chlorophyll have for some time now been recognized to play a decisive role in chlorophyll function in photosynthesis [1, 2]. In vitro studies by infrared [3–5] and nuclear magnetic resonance spectroscopy [6–11] provide substantial support for the view that the chlorophyll central Mg atom with coordination number 4 is coordinatively unsaturated, and that as a consequence one or both of the Mg axial positions must be occupied by an electron donor group. The necessary electron donor may be another chlorophyll molecule (via the *keto* C=O function in ring V), an interaction that forms chlorophyll dimers and oligomers [12]. Alternatively, extraneous nucleophiles (e.g., the Lewis bases pyridine, acetone, tetrahydrofuran, etc.) can form chlorophyll · L_1 and chlorophyll · L_2 adducts in which the central Mg atom has the coordination number 5 or 6 [2]. Proton magnetic resonance studies [8] indicate 5 is the preferred

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** Resident Research Associate, 1974, under the Associated Colleges of the Midwest-Argonne Semester Program; permanent address: Chemistry Department, Denison University, Granville, Ohio, 43023 (U.S.A.).

coordination state for Mg in chlorophyll *a*, but under forcing conditions both axial positions can be occupied. The ready determination of the coordination state of the central Mg atom in equilibria $Chl \cdot L_1 + L \rightleftarrows Chl \cdot L_2$ has been a matter of some difficulty. We have now found that in bacteriochlorophyll systems spectral changes in the yellow region of the visible absorption spectrum (560–620 nm) provide a ready measure of the coordination states of the Mg in $5 \rightleftarrows 6$ equilibria.

Miller and Dorough [13] were the first to observe that the changes in the visible absorption spectrum of Mg tetraphenyl (MgTPC) in benzene on addition of pyridine (Py) can be interpreted in terms of 5- and 6-coordinated Mg. Miller and Dorough interpreted their spectral observations in terms of equilibria in which MgTPC is converted first to a monopyridinate and then to a dipyridinate.

$$MgTPC + Py \rightleftarrows MgTPC \cdot Py \qquad K_1 \cong 4000\ \text{lmol}^{-1} \tag{1}$$

$$MgTPC \cdot Py + Py \rightleftarrows MgTPC \cdot Py_2 \qquad K_2 = 0.82\ \text{lmol}^{-1} \tag{2}$$

Storm and Corwin [14] demonstrated by proton magnetic resonance that the site of pyridine coordination in Mg tetraphenylporphin is the Mg atom. Katz et al. [15] have shown that the central Mg atom is the site of coordination of alcohols with chlorophylls and that the equilibrium constant for the formation of the 5-coordinated monosolvate from the chlorophyll dimer or oligomer is about 100 times larger than that for the 6-coordinated disolvate.

Experimental results suggesting a relationship between the species bacteriochlorophyll $\cdot L_1$ and bacteriochlorophyll $\cdot L_2$ and the yellow region of the bacteriochlorophyll *a* spectrum were first reported by Freed and Sancier [16]. As the role of Mg in the coordination behavior of bacteriochlorophyll *a* had not been elucidated at that time, the implications of their experiments do not seem to have been fully recognized. Freed and Sancier examined the temperature dependence of the visible absorption spectrum of methylbacteriochlorophyllide (which has a visible absorption spectrum indistinguishable from bacteriochlorophyll) in the donor solvent n-propylether and observed a shift from 570 nm to $\approx$ 605 nm on cooling from 300 to 160 °K. This is readily explained, as shown below, as a result of the conversion of bacteriochlorophyll · monoetherate (5-coordinated Mg) at room temperature to a mixture of mono- and di-etherates at 228 °K, and nearly complete conversion to the dietherate (6-coordinated Mg) at 160 °K.

In this paper we show that the visible spectra in the yellow region of the spectrum can be used to determine the proportion of 5- and 6-coordinated Mg in both in vitro and in vivo bacteriochlorophyll systems. A computer-assisted analysis of the spectra provides quantitative data that can be used to calculate the equilibrium constant for the conversion of bacteriochlorophyll · monopyridinate to the dipyridinate. In the interpretation of our spectral data we have made much use of the discussions of the electronic transition spectroscopy of bacteriochlorophyll of Goedheer [17] and Olson [18].

EXPERIMENTAL

Materials

Chlorophyll *a*, chlorophyll *b* and bacteriochlorophyll *a* were prepared by the

methods of Strain and Svec [25]. Bacteriochlorophyll *a* was extracted from *Rhodopseudomonas palustris*, and thus contains phytol as the esterifying alcohol. Solvents were dried with neutral or basic alumina and were stored over 3 Å molecular sieves. All solvents were degassed on a vacuum line before use.

Solution preparation

All operations were carried out in a nitrogen atmosphere, either in a dry-box or in a glove-box (I_2R) attached to the cell compartment of the spectrophotometer, which was also continuously purged with dry N_2. All glassware, including absorption cells and syringes, was cleaned with dry CCl_4 and dried in an oven at 80 °C before use. Chlorophylls were dried by the procedure of Ballschmiter et al. [12] by codistillation with CCl_4 followed by drying in a high vacuum at 70 °C for at least one hour. Chlorophyll stock solutions approx. 10^{-3} M were diluted to obtain the final concentrations desired. Chlorophyll solutions in aggregating solvents were found to be stable for periods of one to two weeks when stored in a N_2 atmosphere and protected from light. Carbon tetrachloride solutions were an exception and appeared particularly susceptible to bleaching by light. In strongly basic solvents (amines) chlorophylls degraded after about a week. Titrations with disaggregating ligands were carried out directly in a 1 cm quartz cell (volume 4.5 ml) with addition of the titrant made with appropriately sized precision micropipets.

Instrumentation

Electron transition spectra were recorded on a Cary 14 spectrophotometer interfaced to the Argonne Chemistry Division's Xerox Sigma 5 computer. Spectra were measured in quartz cells with path lengths from 1 micron to 1 cm to accommodate chlorophyll concentrations from 10^{-2} to 10^{-5} M.

Digital computer deconvolution

Deconvolution of the spectral data was carried out on the Sigma 5 by the methods we have described in detail in our previous publications [19, 20]. The variable metric minimization procedure by Davidon [21] as modified by Chamot [22] was reprogrammed for the Sigma 5 by Dr A. Zielen of the Chemistry Division. The program handles a maximum of 10 absorption peaks and Gaussian line shapes were specified in all cases [20]. A baseline correction feature permits a constant, linear, or quadratic correction to the baseline.

RESULTS AND DISCUSSION

The three species of bacteriochlorophyll we will be primarily concerned with are bacteriochlorophyll *a* self-aggregates, bacteriochlorophyll *a* · monopyridinate, (Bchl · Py), and bacteriochlorophyll *a* · dipyridinate (Bchl · Py_2). In nonpolar solvents such as carbon tetrachloride, bacteriochlorophyll *a* occurs as aggregates formed by coordination interactions between the Mg atom of one bacteriochlorophyll *a* and donor functions (the *keto* C=O ring V and the acetyl C = O group in ring II) of another; self-aggregates, $(\text{bacteriochlorophyll})_n$, of considerable size can form, in which the value of n is a function of solvent, concentration, temperature, etc. Thus, the spectra of bacteriochlorophyll *a* in carbon tetrachloride shown in Fig. 1A are those

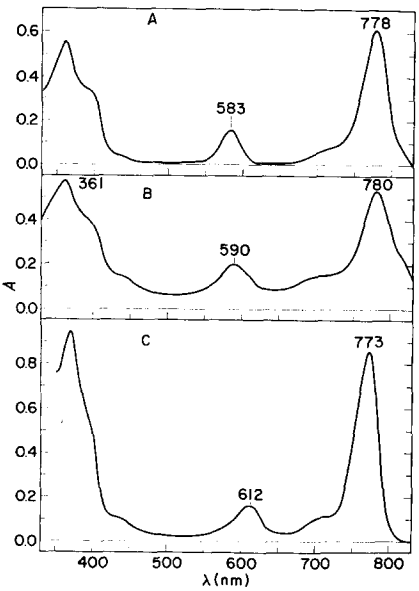

Fig. 1. Visible absorption spectra of bacteriochlorophyll *a*. (A) 10 μM in CCl_4; (B) 0.01 M in CCl_4; (C) 10 μM in *n*-butylamine.

of a dilute solution in which the bacteriochlorophyll *a* occurs as dimers and trimers, and a concentrated solution (Fig. 1B) in which large aggregates, $(\text{bacteriochlorophyll})_n$, occur, where $n > 10$ [12]. It is evident that the electronic transition spectra are not very sensitive to the size of the bacteriochlorophyll *a* aggregates. From the very small changes in the yellow region, we deduce the Mg to be in essentially the same coordination state in both small and large aggregates. Bacteriochlorophyll *a* dissolved in a very strong base such as n-butylamine or triethylamine on the other hand is completely disaggregated. The disaggregation can be related to a major change in the visible absorption spectrum in which the absorption peak near 580 nm is shifted to 612 nm (Fig. 1C). We interpret this spectral shift to result from the conversion of bacteriochlorophyll oligomers in which bacteriochlorophyll occurs with the Mg coordination number of 5, to a bacteriochlorophyll · $(n\text{-butylamine})_2$ species in which the Mg atom has the coordination number of 6. This interpretation follows along the lines of Miller and Dorough [13] previously cited.

Spectral evidence for 5- and 6-coordination in various bacteriochlorophyll a adducts

The effects of base on the yellow band has been explored by titrating bacteriochlorophyll aggregates with pyridine. Pyridine is a weaker base than *n*-butylamine, but is chemically more inert than a primary amine such as *n*-butylamine and as a weaker base is not as effective in generating 6-coordinated Mg species. The principal absorbance peak in the yellow region of the spectrum of aggregated bacteriochlorophyll is an asymmetric peak at 583 nm (Fig. 2A). Addition of a small amount of pyridine results in the formation of the nearly Gaussian peak in Fig. 2A. ^{1}Hmr studies have shown that a bacteriochlorophyll : ligand ratio of 1 : 10 is sufficient to disaggregate the bacteriochlorophyll to form a monomeric bacteriochlorophyll-ligand complex

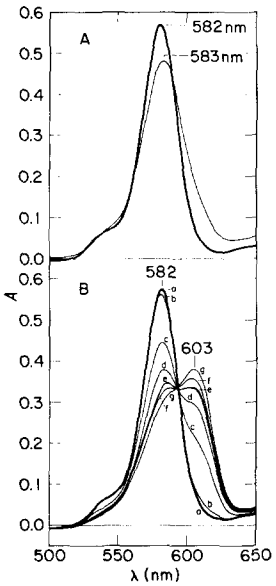

Fig. 2. Effect of pyridine on the visible absorption spectrum of bacteriochlorophyll *a* in the yellow region of the spectrum. (A) Thin line, [bacteriochlorophyll], 30 μM in toluene; thick line, spectrum of bacteriochlorophyll · monopyridinate, [bacteriochlorophyll], 30 μM; [pyridine], 1.1 mM solvent, toluene. (B) Effect of pyridine concentration on bacteriochlorophyll in toluene; a) [bacteriochlorophyll], 30 μM; b) [pyridine], 1.1 mM; c) [pyridine], 0.028 M; d) [pyridine], 0.055 M; e) [pyridine], 0.082 M; f) [pyridine], 0.11 M; g) [pyridine], 0.14 M.

[9]. The ligand ratio in Fig. 2A is 1 : 37, and thus the pyridine concentration in this solution is sufficient to result in complete transformation of bacteriochlorophyll oligomer to bacteriochlorophyll · monopyridinate. Additional pyridine causes the absorbance at 582 nm to decrease and results in the formation of a second pyridine-containing species absorbing at 603 nm (Fig. 2B). The isosbestic point in Fig. 2B clearly indicates a pyridine-dependent equilibrium between two bacteriochlorophyll species:

$$\text{Bchl} \cdot \text{Py} + \text{Py} \rightleftarrows \text{Bchl} \cdot \text{Py}_2 \qquad (3)$$

Similar spectral changes have been observed with n-butylamine and tetrahydrofuran, although the magnitude of the red shift associated with the formation of a 6-coordinated species is less pronounced for tetrahydrofuran, as might have been expected for a ligand considerably less basic than pyridine.

We suggest that the yellow region of the bacteriochlorophyll spectrum can be used as evidence for 5- and 6-coordinated Mg, since the absorbance of 6-coordinated species is consistently red shifted relative to 5-coordinated Mg complexes. Thus, the asymmetric absorbance of aggregated bacteriochlorophyll in Fig. 2A can be interpreted to indicate predominantly 5-coordinated Mg since it has its absorption maximum at 583 nm, with the red shoulder indicating the presence of a small amount of a 6-coordinated bacteriochlorophyll species.

Deconvolution of electron transition spectra of bacteriochlorophyll a

Computer deconvolution techniques were used to obtain a more quantitative assessment of the nature of the spectral changes in the various bacteriochlorophyll *a* systems. Use of the absorption maximum in the yellow region of the bacteriochlorophyll spectrum for calculating equilibria is complicated by the presence of at least four overlapping peaks. Thus, deconvolution procedures increase the information content of the spectra. The initial parameters in the deconvolution program are the peak position, peak height, and the width at half-height. Reasonable deconvolutions were obtained only when some of the input parameters were restricted and not allowed to change as the computer calculated a decomposition of the absorption envelope.

The values of parameters to be restricted were obtained from spectra in which the peak of interest was as well-defined as possible. Thus, a value for the peak position, and linewidth of the absorbance component characteristic of bacteriochlorophyll · monopyridinate was obtained from the spectrum in Fig. 2A. Similarly, values for peak position and linewidth for the peak at $\approx$ 570 nm and for the absorbance characteristic of bacteriochlorophyll · dipyridinate were obtained from the low temperature spectra in Fig. 3 in which bacteriochlorophyll · dipyridinate was the predominant species. Cooling increases the 610 nm absorbance and decreases the blue shoulder, indicating increased bacteriochlorophyll · dipyridinate formation at the expense of the monopyridinate. Under these circumstances a peak at 570 nm was observed whose presence was not obvious from the room temperature spectra. Thus, the low temperature spectra give a better idea of the intrinsic line shape of the bacteriochlorophyll · dipyridinate absorption peak. The λ_{max} values for the Gaussian components and their linewidths should be considered to have limits of ± 2 nm, although replicate determinations in completely independent experiments give values well

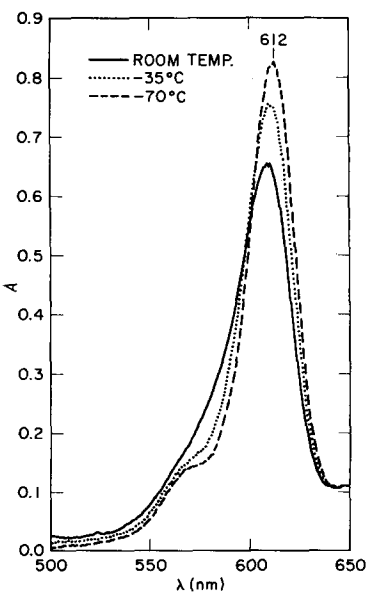

Fig. 3. Effect of temperature on bacteriochlorophyll · dipyridinate in toluene. [Bacteriochlorophyll], 22 mM; [pyridine], 0.21 M.

within these limits. The validity of a particular deconvolution was judged by the fit to the experimental curve and consistency with other deconvolutions to which it could be compared.

Usually peak heights at selected wavelengths are used to deduce the concentration of a species from spectrophotometric data. Where the absorption maxima of various species strongly overlap, as in the cases considered here, deconvolution into Gaussian components whose areas are then used as a measure of species concentration is a preferred analytical procedure.

A computer deconvolution of the spectrum of aggregated bacteriochlorophyll itself in toluene solution is shown in Fig. 4A, and the parameters of the Gaussian components obtained by deconvolution are listed in Table I. The component at 582 nm is assigned to 5-coordinated Mg, and the peak at 606 nm to 6-coordinated Mg. As bacteriochlorophyll contains both *keto* C=O and acetyl C=O functions that can act as donors to Mg, and an additional two ester C=O functions, it is obvious that self-aggregation of bacteriochlorophyll can take place in many different ways. By analogy with chlorophyll *a*, the *keto* C=O function is likely to be the strongest donor [10, 11]. ^{1}Hmr aggregation studies indicate that the acetyl C=O also is involved in self-aggregation [7, 9]. Thus, 5-coordination in a bacteriochlorophyll aggregate may imply keto C=O · · · Mg as the predominant interaction, whereas 6-coordination may indicate species formed by *keto* C=O · · · Mg · · · O=C acetyl interactions. Regardless of the exact details of the coordination mechanism, which still remains to be worked out, the spectra would appear to suggest clearly that aggregated bacteriochlorophyll at room temperature contains for the most part 5-coordinated Mg. The Gaussian at 568 nm we consider to be independent of the coordination equilibria, as its parameters hold fairly constant through the entire range of deconvolutions. The component at 543 nm is unaffected by conversion of bacteriochlorophyll oligomer to bacteriochlorophyll · monopyridinate, but decreases as the dipyridinate is formed. These changes can be noted in Figs 2A and 3.

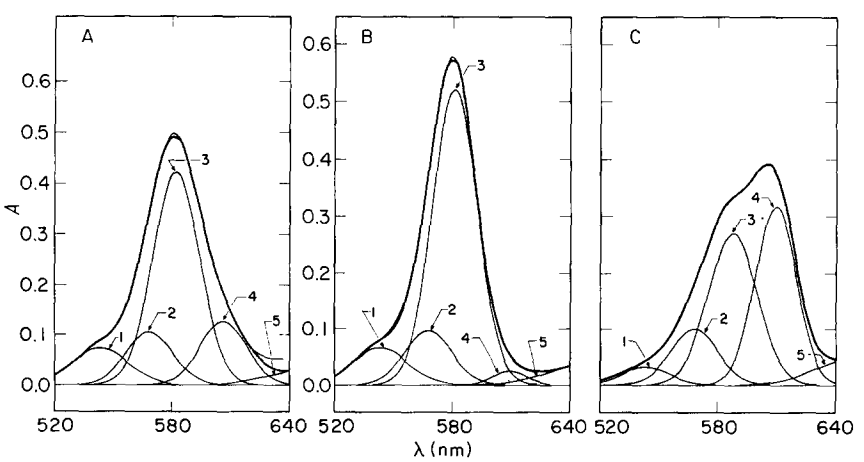

Fig. 4. Deconvolution of bacteriochlorophyll oligomer, bacteriochlorophyll · monopyridinate and a bacteriochlorophyll · monopyridinate-dipyridinate mixture. In all three deconvolutions, [bacteriochlorophyll] is 30 μM in toluene. (A) No pyridine added; (B) [pyridine], 28 μM; (C) [pyridine], 0.14 M. The Gaussian components are numbered from left to right for convenience.

TABLE I

GAUSSIAN COMPONENTS OF THE YELLOW REGION ABSORPTION BAND OF VARIOUS BACTERIOCHLOROPHYLL SPECIES[a]

([Bacteriochlorophyll] = 30 μM)

Species[b]	Pyridine	λ_1* (nm)	δ_1 (nm)	A_1 (nm)	λ_2* (nm)	δ_2* (nm)	A_2 (nm)	λ_3 (nm)	δ_3 (nm)	A_3 (nm)	λ_4 (nm)	δ_4 (nm)	A_4 (nm)	λ_5* (nm)	δ_5 (nm)	A_5 (nm)
$(Bchl)_n$	0	543	34	2.4	568	28	3.1	582	28	13.0	606	28	3.8	650	46	1.0
Bchl · Py	28 μM	543	31	2.10	568	28	2.9	581	26	15.0	610	19.5	0.5	650	46	1.4
Bchl · Py+ Bchl · Py_2	0.14 M	543	28	0.9	568	28	2.9	587	29	8.4	609	24	8.2	650	46	1.7

[a] Symbols: *, deconvolution parameters not allowed to vary in computer deconvolution; λ, wavelength maximum of Gaussian component; δ, Gaussian linewidth at half-height; A, area of Gaussian component.

[b] $(Bchl)_n$, bacteriochlorophyll oligomer; Bchl · Py, bacteriochlorophyll · monopyridinate; Bchl · Py_2, bacteriochlorophyll · dipyridinate.

Deconvolution results for the titration with pyridine of bacteriochlorophyll oligomers (Fig. 4A) to monopyridinate (Fig. 4B) and then to a mono- and di-pyridinate mixture (Fig. 4C) are shown in Fig. 4 and in Table I. The component at 582 nm in Fig. 4B is assigned to absorption by bacteriochlorophyll · monopyridinate. The small component at 610 nm probably arises from the presence of a small amount of dipyridinate. Addition of pyridine to bacteriochlorophyll · monopyridinate produces a mixture of mono- and dipyridinate (Fig. 4C). The deconvolution in Fig. 4C is characteristic of a mixture of the two species. The 568 nm peak remains the same throughout the titration. The 543 nm component has decreased. The areas of the 584 nm and 609 nm peaks can be used to estimate the concentrations of bacteriochlorophyll · mono- and di-pyridinate, respectively, and this is the data used to calculate an equilibrium constant for the formation of bacteriochlorophyll · dipyridinate from the monopyridinate.

Equilibrium constant for the formation of bacteriochlorophyll · dipyridinate

For the reaction

$$\text{Bchl} \cdot \text{Py} + \text{Py} \rightleftarrows \text{Bchl} \cdot \text{Py}_2 \tag{4}$$

the equilibrium constant is defined by the equation

$$\text{K} = \frac{[\text{Bchl} \cdot \text{Py}_2]}{[\text{Bchl} \cdot \text{Py}]\,[\text{Py}]} \tag{5}$$

and the fraction of dipyridinate complex formed is obtained experimentally according to Eqn. 6.

$$F_c = \frac{A_{\text{Bchl}\cdot\text{Py}} - A_m}{A_{\text{Bchl}\cdot\text{Py}}} = \frac{Y}{X} \tag{6}$$

where $A_{\text{Bchl}\cdot\text{Py}}$ is the area of the absorbance due to bacteriochlorophyll · pyridine when the bacteriochlorophyll is totally converted to monopyridinate, and A_m is the area of the bacteriochlorophyll · pyridinate peak for a solution which contains a mixture of the mono- and di-pyridinate forms. The total concentration of bacteriochlorophyll remained constant throughout the experiment. The equilibrium constant was obtained by a graphical solution of Eqn 7 [13].

$$\frac{1}{Y} = \frac{1}{K \cdot X \cdot [\text{Py}]} + \frac{1}{X} \tag{7}$$

A plot of $1/Y$ versus $1/[\text{Py}]$ was evaluated by the method of least squares and the equilibrium constant was obtained from the slope and intercept. Calculations from optical absorbance values taken from the original spectra gave results similar to those obtained from the deconvolution data.

Deconvolution data for a typical titration experiment are given in Table II. A value of $K = 11 \pm 2\ \text{lmol}^{-1}$ was obtained for the formation of bacteriochlorophyll · dipyridinate in toluene at room temperature. This can be compared to $K = 10\ \text{lmol}^{-1}$ for the formation of chlorophyll *b* · dipyridinate in *n*-propylbenzene obtained by Freed and Sancier [16] and Seely's value of $K = 16\ \text{lmol}^{-1}$ (in nitromethane) and $K = 34\ \text{lmol}^{-1}$ (acetonitrile) for chlorophyll *a* · (4-ethylpyridine)$_2$ formation [23].

TABLE II

DECONVOLUTION RESULTS FOR TITRATION OF BACTERIOCHLOROPHYLL MONOPYRIDINATE TO DIPYRIDINATE IN TOLUENE[a]

([Bacteriochlorophyll] = 40 μM)

Conc. Pyridine (M) $\times 10^2$	Peak height[b]	λ_1 (nm)	δ_1 (nm)	A_1 (nm)	Peak height[b]	λ_2 (nm)	δ_2 (nm)	A_2 (nm)	Peak height[b]	λ_3 (nm)	δ_3 (nm)	A_3 (nm)	Peak height[b]	λ_4 (nm)	δ_4 (nm)	A_4 (nm)
0.15	0.08	543*	26	2.3	0.14	568*	28*	4.2	0.72	582	26	20.6	0.03	610	15.6	0.05
1.5	0.07	543*	25	1.8	0.13	568*	28*	3.8	0.63	584	26	18.7	0.15	611	22	3.6
2.9	0.06	543*	25.4	1.6	0.13	568*	28*	3.9	0.55	584	27	16.5	0.24	610	23	6.1
4.3	0.05	534*	25	1.4	0.14	568*	28*	4.1	0.50	584	27	14.9	0.30	609	24	7.8
5.6	0.04	543*	23.4	1.2	0.14	568*	28*	4.1	0.47	585	27	13.9	0.34	610	24	8.9
7.0	0.04	543*	24	1.1	0.13	568*	28*	4.0	0.44	586	27	13.0	0.37	610	24	9.8

[a] Symbols: for definition of symbols, see footnote *a* of Table I.

[b] In absorbance units.

Evidence for 5- and 6-coordinated Mg in other chlorophylls

Differences in the red envelope of chlorophyll *a* also can be correlated with the coordination state of the central Mg atom. Addition of excess pyridine (10 mM) to a 10 μM chlorophyll solution in octane results in changes on the blue side of the red envelope. Conversion of chlorophyll *a* · monopyridinate to chlorophyll *a* · dipyridinate diminishes the absorption at 619 nm and increases the absorption at 633 nm (Fig. 5). The absorption maximum at 633 nm appears to be the best indicator of 6-coordination in chlorophyll *a*. The peak at 633 nm has been often seen in spectra recorded on chlorophyll *a* solutions, and has generally been interpreted in terms of changes in the population of thermally excited vibrational levels, or even on occasion as a result of exciton splitting. Neither of these explanations for this absorption maximum can be valid in light of the experimental evidence presented here. The changes in the chlorophyll *a* spectrum in this region are complex, and the problem of an accurate determination of the relative amounts of 5- and 6-coordinated Mg when both are present in a chlorophyll *a* solution is still a severe one, but in the limiting cases the criterion advanced here would appear to have considerable utility.

For chlorophyll *b* the situation is likewise complex. Freed and Sancier [16] deduced a value of 10 lmol^{-1} for the formation of chlorophyll *b* · dipyridinate. This equilibrium constant was calculated from absorbance changes at 480 and 467 nm. We observe that self-aggregated chlorophyll *b* or chlorophyll *b* · monopyridinate absorb at 457 nm, whereas chlorophyll *b* · dipyridinate absorbs at 460 nm. The red shift on forming 6-coordinated Mg in chlorophyll *b* systems is much smaller and more difficult to use than that observed for bacteriochlorophyll, but changes in the Soret region

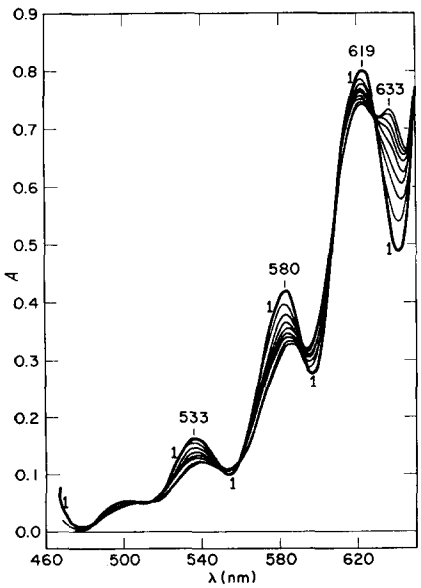

Fig. 5. Spectral changes in chlorophyll *a* as a function of chlorophyll/pyridine ratio. The initial curve indicated by 1 is that of chlorophyll · monopyridinate, [chlorophyll *a*], 62 μM, [pyridine], 1 mM. The final [pyridine] is 0.44 M. The spectral changes at 619 and 633 nm appear to show the largest coordination dependence.

appear to offer possibilities for establishing the coordination status of the Mg in chlorophyll *b* species.

Applications of the coordination criterion

We have shown that the yellow region of the visible absorption spectrum can be used to identify the coordination state of Mg in bacteriochlorophyll, and that 5-coordinated Mg species absorb near 580 nm, whereas 6-coordinated species are red shifted to 610 nm. Computer deconvolution techniques assist in the interpretation in this region of the spectrum, but often qualitative conclusions can be arrived at by simple inspection of the position of a peak and its symmetry. The utility of the coordination criterion can be illustrated from some recent results of Goedheer [24], who has examined bacteriochlorophyll spectra both in vivo and in vitro.

Goedheer [24] found that bacteriochlorophyll *a* dissolved in propan-1-ol absorbs at 605 nm with a blue shoulder, whereas bacteriochlorophyll *a* in propan-2-ol absorbs at 585 nm with a red shoulder. Secondary and tertiary alcohols are known to be weaker coordinating agents than primary alcohols for Mg in chlorophyll [8]. In Fig. 5C of ref. 24 it is evident from the coordination criterion we have proposed that bacteriochlorophyll in propan-1-ol is largely 6-coordinated, whereas bacteriochlorophyll in the sterically less accessible propan-2-ol is principally 5-coordinated.

Chromatophores of a number of different species of photosynthetic bacteria absorb in the yellow. *Rhodopseudomonas spheroides* is unusual in that it is the only photosynthetic bacterium examined by Goedheer that does not have significant absorption to the red of the $\approx$ 590 nm peak (Fig. 1 of ref. 24). Thus, it appears that in *R. spheroides* bacteriochlorophyll is largely 5-coordinated, whereas the other photosynthetic bacteria contain mixtures of 5- and 6-coordinated Mg. This may be indicative of a significant structural difference in the bacteriochlorophyll aggregates between *R. spheroides* and the other purple photosynthetic bacteria. It is also evident that the spectral changes produced by cooling to 77 °K do not appear to involve major changes in the coordination behavior of the Mg in the bacteriochlorophyll species present in these organisms.

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