An Electrochemical Investigation of the Redox Properties of Bacteriochlorophyll and Bacteriopheophytin in Aprotic Solvents

Therese M. Cotton[1a,b] and Richard P. Van Duyne*[1a]

Contribution from the Department of Chemistry, Northwestern University, Evanston, Illinois 60201. *Received May 21, 1979*

Abstract: Knowledge of solvent effects on the redox properties of bacteriochlorophyll (BChl) and bacteriopheophytin (BPheo) is important for understanding their possible role(s) as intermediate electron acceptors in the primary photochemistry of photosynthetic bacteria. In the present study, an investigation of the electrochemical behavior of these compounds by cyclic voltammetry (CV) and cyclic differential pulse voltammetry (CDPV) in several aprotic solvents has shown that BChl aggregation and ligation interactions have a significant effect on its redox potentials. In methylene chloride, the one-electron reduction potential of BChl was found to shift positively by 200 mV to a value nearly identical with that of BPheo in the same solvent. The shift is most readily explained by the presence of BChl aggregates in this solvent. The one-electron oxidation potential is relatively unaffected by aggregation. In contrast, the formation of six-coordinate BChl in tetrahydrofuran (two molecules of solvent coordinated to the Mg atom of BChl) affects both the one-electron reduction and one-electron oxidation potential, with the greatest effect on the latter. Solvent effects on the redox properties of BPheo were found to be much smaller, a finding consistent with its inability to undergo aggregation and coordination interactions similar to those of BChl.

During the past decade, considerable progress has been made toward understanding the primary photochemical events in photosynthetic bacteria.[2] The reaction center (RC) complex has been purified[3] and found to contain four bacteriochlorophyll (BChl) and two bacteriopheophytin (BPheo) molecules in addition to one quinone and three polypeptides. (See recent reviews in ref 4-8.) Two of the BChl molecules, the special pair, which absorb at 870 nm (P870) are now generally accepted as the primary electron donor in photoinduced charge separation.[9-12] The quinone molecule has been shown to act as the primary electron acceptor[13,14] as defined on a millisecond time scale.[15] However, with the advent of picosecond (ps) absorption spectroscopy techniques, an intermediate electron acceptor with a lifetime of 250 ps has been detected.[16,17] The intermediate was identified by Fajer and co-workers[18] as one of the BPheo molecules present in the RC by a comparison of the optical properties of the intermediate with those of the BPheo anion radical prepared electrochemically in methylene chloride. Subsequently, BPheo has been identified as the intermediate electron acceptor in a variety of photosynthetic bacterial species.[19-23] The picosecond data have recently been reviewed,[24,25] and possible artifacts of the method have been discussed.[26,27] Although the role of the remaining two BChl molecules and the BPheo molecule in the charge separation process has not been unambiguously demonstrated, there are some experimental observations that suggest BChl (P800) may be involved in some manner in the transfer of an electron from P870 to PBheo. These observations include the picosecond absorption study of Shuvalov et al.,[28] which has been interpreted by these authors to indicate that electron transfer proceeds from P870 to P800 and then to BPheo within 35 ps. Steady-state optical spectra of the reduced intermediate have indicated the presence of a BChl anion radical as well as that of BPheo.[29]

Because BChl and BPheo participate directly in the initial photosynthetic charge separation, it is important that their redox properties be known. This information is necessary for a detailed understanding of the mechanism of charge separation. For example, an estimate of the efficiency of the primary step in photochemical energy conversion has been made by comparing the electrochemical potential difference between the oxidized donor and reduced acceptor with the energy of a single photon absorbed by P870.[18] In this calculation, the oxidation potential of the special pair BChl, as determined by potentiometric titrations on in vivo preparations, was used as the donor potential. Since the reduction potential of BChl or BPheo has not been determined directly in the RC,[30] values obtained electrochemically for these compounds in organic solvents were used for the acceptor potential. The results indicated an efficiency of 70% for electron transfer from P870 to BPheo and an efficiency of 90% for electron transfer from P870 to BChl. The former value is in good agreement with previous estimates of photosynthetic efficiency and constitutes additional support for the much stronger optical evidence of BPheo as the intermediate electron acceptor. If, however, P800 BChl functions as an extremely short-lived electron acceptor between P870 and BPheo as proposed by Shuvalov et al.,[28] it is necessary to consider the potential difference created by the transfer of an electron from P870 to BChl. An accurate value for the reduction potential of BChl is needed for this purpose. There have been several literature reports for the one-electron reduction potential of BChl in organic solvents,[31-33] but the effect of BChl interactions has not been considered. In the case of P870 BChl, the oxidation potential in vivo is lowered by approximately 200 mV relative to monomeric BChl in solution, no doubt as a result of the interaction between the two special pair BChl molecules. Therefore, it seems reasonable that the reduction potential of BChl or BPheo may be affected by their interactions in the RC, since there is strong coupling of the RC pigments as evidenced by CD studies.[34] Consequently, we have endeavored to determine the effect of BChl aggregation and ligation interactions on its redox properties in organic solvents. The effect of solvent on BPheo redox properties was also examined. The results, which are presented here, indicate both aggregation and ligation interactions affect the redox properties of BChl, although in a significantly different manner.

Experimental Section

Materials. BChl was isolated from *Rhodospirillum rubrum* according to the procedure of Strain and Svec.[35] BPheo was prepared by standard methods. The isolation and chromatography of both BChl and BPheo were performed under subdued light and the final product was stored under vacuum at 0 °C.

The solvents used in this study were the purest available grade (Burdick and Jackson, distilled in glass). Both ACN and CH_2Cl_2 were dried by repeated vacuum distillation onto activated 3- or 4-Å molecular sieves. According to a recent study, this treatment results in water contents below 10^{-4} M in many organic solvents.[36] In the case

0002-7863/79/1501-7605$01.00/0

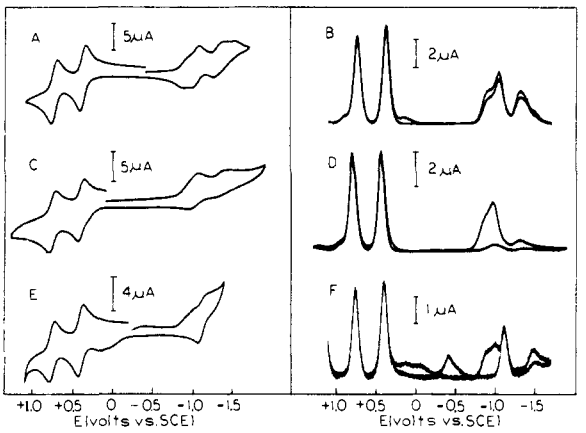

Figure 1. Cyclic and cyclic differential pulse voltammograms of BChl (5×10^{-4} M) in acetonitrile with 0.1 M TBAP as supporting electrolyte: (A, B) very dry acetonitrile; (C, D) acetonitrile plus less than 1% water; (E, F) very dry acetonitrile plus 2×10^{-3} M hydroquinone. Working electrode was a platinum bead with surface area equal to 0.105 cm^2. Cyclic voltammetry parameters were as follows: scan rate = 0.1 V s^{-1}, V_{step} = 1.0 mV, $t_{step} = t_{samp}$ = 1.00 ms. Cyclic differential pulse voltammetry parameters were as follows: scan rate = 0.02 V s^{-1}, V_{step} = 1.0 mV, t_{step} = 50 ms, V_{pulse} = 10 mV, t_{pulse} = 20 ms. The initial scan direction was cathodic, and data shown are of the first scan.

of THF, the initial drying over molecular sieves was followed by refluxing the THF over metallic Na and distilling into a round-bottomed flask. The THF was then subjected to three vacuum distillations onto activated sieves as described for ACN and CH_2Cl_2.

The electrolytes tetrabutylammonium tetrafluoroborate (TBAF) and tetrabutylammonium perchlorate (TBAP) (Southwestern Analytical Chemicals, Austin, Tex.) were twice recrystallized from ethyl acetate and dried under vacuum at 100 °C for 48 h.

Apparatus. Electrochemical Cell. The cell used in these experiments was designed for high vacuum operations. In CV and CDPV the working electrode consisted of a Pt or Au bead sealed in glass. A Pt mesh working electrode was used for controlled potential bulk electrolysis. Other aspects of the cell design were identical with those described previously.[37]

Electronics and Data Collection. The potentiostat, computer hardware, and programming used in performing CV and CDPV have been described.[38] The latter technique is comparatively new, and certain aspects of the data collection will be reviewed here to facilitate interpretation of the voltammograms. Both the CV and CDPV data are collected on a Raytheon 500 minicomputer. The data collection and analysis are performed in real time by two separate programs. The CV program utilizes a staircase potential ramp with a variable step height and width. The current is sampled near the end of each step. Data are displayed in conventional plots of potential vs. current. The CDPV program also utilizes a staircase potential ramp. However, a single potential pulse of short duration compared to the step width is superimposed on each step. Current is sampled immediately before (i_1) and at the end (i_2) of each pulse. Data are displayed in plots of potential vs. the difference in current ($i_2 - i_1$) (hence, differential pulse voltammetry).

Methods. Solutions of the electrolyte and BChl were prepared by two procedures. In one procedure the electrolyte solution was prepared in a nitrogen-purged glovebox and pretreated with activated alumina (Woelm W200, Activity Super 1) for 24 h to remove electrophilic impurities.[39,40] This pretreatment has been shown to enhance greatly the stability of Chl *a* anion radicals in DMF.[41,42] The BChl was dried as described by Ballschmiter et al.[43] in the side arm of the electrolysis cell. The electrolyte solution was decanted from the alumina into the cell in a N_2-purged glovebox, and the cell was returned to the vacuum line for final degassing by repeated freeze-pump-thaw cycles. In the second procedure, the electrolyte was added to the side arm of the electrochemical cell, and the BChl was cast as a film from CH_2Cl_2 onto the walls of the optical cell portion of the electrochemical cell. The cell was evacuated, and the electrolyte was heated to 100 °C overnight. The following day, dry solvent was vacuum distilled into the side arm of the cell and the contents mixed by tilting the cell. Both procedures resulted in comparable electrochemical behavior of the BChl on the CV and CDPV time scale.

The electrolysis cell used in these studies employs a platinum quasireference electrode rather than the commonly used SCE reference electrode. This choice of reference is necessary to permit high-vacuum degassing of the cell and its contents and to avoid contamination of the electrolysis solution with water. The anions of BChl and BPheo are extremely sensitive to oxygen and water. In order to relate the potentials obtained with the quasireference electrode to a SCE reference electrode, the pilot ion method of calibration was used.[44] Ferrocene was added to the auxiliary side arm of the cell, which was also evacuated and isolated from the electrolysis solution. Following the electrochemical studies on the compound of interest, ferrocene was added to the electrolysis solution while the cell was kept under vacuum. The potential for the one-electron oxidation of ferrocene to the ferricinium ion was then determined by CV and CDPV against the Pt reference. Finally, the cell was filled with nitrogen and the quasireference was replaced with a SCE electrode. The potential for the ferrocene oxidation was again determined. The difference determined between the oxidation potential of ferrocene vs. Pt and ferrocene vs. SCE was used to correct the BChl and BPheo redox potentials. An attempt was also made to compensate for differences in the junction potentials of ferrocene vs. SCE for the various solvents by relating all of the reported potentials to ferrocene oxidation in tetrahydrofuran (THF).

Results

Comparison of Cyclic and Cyclic Differential Pulse Voltammetry. In the data to be presented, both CV and CDPV were used on the same solutions of BChl or BPheo. A direct comparison of the results, as shown in the figures which follow, illustrates the advantages of CDPV over CV. However, the theory for cyclic differential pulse voltammetry is still in the state of development. For this reason, conventional cyclic voltammograms, the theory of which is well developed, were also recorded for comparison purposes. The charging current is not observed in the cyclic differential pulse voltammogram, since it is effectively subtracted from the total current in the differential response. The oxidation and reduction processes of the electroactive species, or faradaic response, are manifest as peaks on a flat base line in a manner identical with that in the related technique differential pulse voltammetry.[45] The determination of base-line current, a problem in CV, is eliminated. Chemical reversibility can be readily assessed by comparing the peak heights in the forward and reverse scans. Quasireversible charge transfer causes a separation in the peak potentials with scan direction.[46] A disadvantage in the differential pulse procedure is the lower signal-to-noise ratio, especially in solvents of low dielectric constant. In order to offset this problem, it was necessary to scan at a slower rate (0.02 V/s) than in cyclic voltammetry (0.1 V/s).

Electrochemistry of BChl in Acetonitrile. Nitrile-containing solvents are often used in electrochemical measurements because they have a broad potential range in both the anodic and cathodic scan directions. Butyronitrile (BuCN) was used as a solvent for the coulometric generation of the anion radical of BChl.[31,47] Acetonitrile (ACN) was used as a solvent in a CV study of the oxidation behavior of BChl.[32] In the present study, ACN was chosen over BuCN as a solvent for evaluating the electrochemical and chemical reversibility of the BChl anion radical due to its greater volatility, which permits relatively rapid vacuum distillation of this solvent. Anhydrous BChl dissolved slowly in dry ACN, often taking as long as 1 h to reach a concentration of 5×10^{-4} M. The solutions were stable, however, once the BChl had dissolved, and no precipitation was observed.

Figures 1A and 1B are typical first scan cyclic and cyclic differential pulse voltammograms for a 5×10^{-4} M solution of BChl in dry ACN. The initial potential was −0.3 V, and the scan direction was cathodic. On examining CV results, it may be seen that the first reduction peak at −0.99 V has a pronounced anodic shoulder, which appears reversible. The $E_{1/2}$ values are listed in Table 1. The second reduction peak also

Table I. Oxidation and Reduction Potentials of Bacteriochlorophyll in ACN[a,b]

sample	$E_{1/2}^{2+}$	$E_{1/2}^{+\cdot}$	$E^{-\cdot}$ peak 1		$E_{1/2}^{2-}$ peak 3	$E_{1/2}^{2-}$ peak 2	$E_{1/2}^{2-}$ peak 4
ACN, dry							
CV	0.78	0.42	−0.88 (sh)	−0.99		−1.28	
CDPV	0.78	0.42	−0.88 (sh)	−1.00		−1.26	
ACN, wet							
CV	0.78	0.42	0.90 (sh) (I)	−0.98 (I)		−1.32 (I)	
CDPV	0.78	0.42	0.90 (sh) (I)	−0.98 (I)		−1.32 (I)	
ACN/HQ, dry							
CV	0.78	0.42	(I)	(I)			
CDPV	0.78	0.42	0.90 (sh) (I)	−0.99 (I)	−1.10		1.46
ACN/THF, dry							
CV	0.84	0.42		−1.01		−1.35	
CDPV		0.42		−1.00			

[a] Potentials are in volts vs. SCE. [b] sh = shoulder, I = irreversible.

contains a shoulder, but it is cathodic to the main peak. The one- and two-electron oxidation of BChl appears electrochemically and chemically reversible in this solvent on the basis of peak separation and relative heights in the forward and reverse scans. The same conclusions are reached on examining the reduction and oxidation processes by the CDPV procedure (Figure 1B), but the reduction peaks are better resolved and the reversibility is easier to assess than in CV. Both cathodic peaks and their respective shoulders show reverse scan peaks approximately 90% of the current value observed in the forward scan. The peak current for the one-electron reduction of BChl is only 50% that of the one-electron oxidation.

The origin of the shoulders on the reduction peaks of BChl in ACN was initially puzzling. Several explanations were considered, including adsorption of the BChl on the Pt electrode surface. Adsorption of the product of an electron transfer is known to cause prewaves, as observed in the one-electron reduction of BChl on Hg in Me_2SO.[33] The separation between the peaks due to adsorption in the forward and reverse scans is <59 mV for a reversible one-electron transfer process if the electroactive compound is strongly adsorbed. The relative intensity of an adsorption peak to those associated with diffusion-controlled electron transfer is scan rate and concentration dependent. An increase in scan rate, or a decrease in the bulk concentration of the electroactive compound, should result in an increase in peak current for the adsorbed species relative to that of the solution species.[48] None of these diagnostic criteria applied to the shoulder on the reduction peak of BChl in ACN, however.

Another possible explanation considered was that the shoulders are due to the interaction of BChl with an impurity in the solvent or electrolyte. Therefore, the effect of two common impurities, water and proton donors, known to interact with porphyrin anions,[49] was examined. The CV and CDPV response of BChl in ACN in the presence of low concentrations of water (less than 1%) is shown in Figures 1C and 1D. From both figures it is immediately obvious that water has no effect on the one- and two-electron oxidation but decreases the chemical reversibility of the one- and two-electron reduction of BChl in ACN. The anodic shoulder on the first reduction peak is observed to shift negatively in Figure 1D and overlap more with the main reduction peak (see Table I). The effect of an added proton source, hydroquinone (HQ), is shown in Figures 1E and 1F. The concentration of HQ is 4 times that of BChl. The electrochemistry of BChl in this solution is quite different from that in either dry or wet ACN solutions with respect to the reduction processes. The initial potential was −0.25 V, and, in the cathodic scan, the shoulder and first reduction peak are identical with that observed in dry ACN (see Table I). Immediately following reduction peak 1 is another peak (peak 3) that may reflect a protonation reaction or adsorption of BChl on the electrode surface. The second major reduction peak present in dry ACN (peak 2) is no longer observed when HQ is present, and a new peak is seen at a lower potential, or −1.6 V (peak 4). On the reverse scan in the anodic direction, only peak 3 appears reversible and a broad peak is present near −0.4 V (peak 5). The behavior of this peak is similar to that observed near −0.5 V in the irreversible reoxidation of the protonated products of ZnTPP[50] and Chl *a*[41] reduction. Hence, it is assigned to the oxidation of protonated BChl dianion.

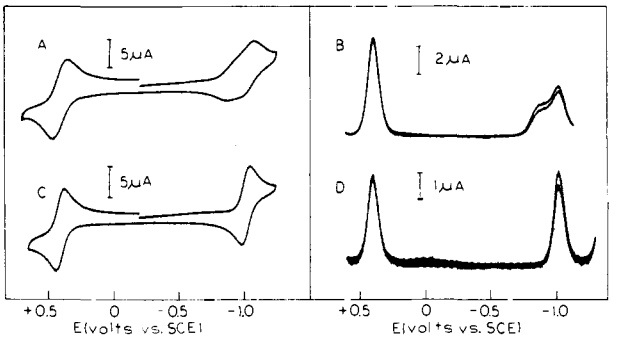

Figure 2. Cyclic and cyclic differential pulse voltammograms of BChl (5×10^{-4} M) in acetonitrile with 0.1 M TBAP as supporting electrolyte: (A, B) very dry acetonitrile; (C, D) very dry acetonitrile plus less than 1% tetrahydrofuran. Working electrode was a platinum bead electrode with surface area equal to 0.105 cm^2. Cyclic voltammetry and cyclic differential pulse voltammetry parameters were identical with those in Figure 1. The initial scan direction was cathodic, and data shown are of the first scan.

From the above experimental results, it may be concluded that the electrochemical behavior of BChl in ACN in the presence of water or the proton donor, HQ, is significantly different than in dry ACN. Residual amounts of water or proton impurities in the ACN do not appear to be the cause of the shoulders on the BChl reduction peaks. A third possibility considered was that the shoulders result from BChl self-aggregation in ACN. (The nature of BChl aggregates has been reviewed recently.[51]) To explore this hypothesis a small amount of tetrahydrofuran (THF) (less than 1% v/v) was distilled into the electrochemical cell containing BChl in ACN. The CV and CDPV measurements were repeated, and the one-electron reduction and one-electron oxidation processes are shown in Figures 2C and 2D. For purposes of comparison, the cyclic and cyclic differential pulse voltammograms of BChl in dry ACN are also shown in Figures 2A and 2B. The effect of THF on the one-electron reduction of BChl in ACN is significant. The anodic shoulder is observed to disappear completely on the addition of THF, and the current magnitude of the main peak is increased to equal that of the first oxidation peak. Though not shown, the second electron reduction peak also shows a loss

Table II. Oxidation and Reduction Potentials of Bacteriochlorophyll and Bacteriopheophytin in CH_2Cl_2[a,b]

sample	$E_{1/2}^{2+}$	$E_{1/2}^{+\cdot}$	$E_{1/2}^{-\cdot}$		$E_{1/2}^{2-}$	
BChl, CH_2Cl_2						
CV	0.82	0.42	−0.83	−1.08	−1.39	−1.57
CDPV	0.79	0.42	−0.83	−1.08	−1.34	−1.56
BChl, CH_2Cl, THF						
CV	0.79	0.42		−1.03	−1.43	
CDPV	0.79	0.42		−1.05	−1.41	
BPheo, CH_2Cl_2						
CV		0.82	−0.85		−1.10 (I)	
CDPV		0.82	−0.83		−1.11 (I)	
BPheo + BChl, CH_2Cl_2						
CV		0.85,[c] 0.42[d]	−0.82[e]		−1.10	
CDPV		0.85,[c] 0.42[d]	−0.81[e]		−1.09	
ferrocene						
CV		0.52				
CDPV		0.51				

[a] Potentials are in volts vs. SCE. [b] I = irreversible. [c] BPheo $E_{1/2}^{+\cdot}$. [d] BChl $E_{1/2}^{+\cdot}$. [e] BPheo $E_{1/2}^{-\cdot}$.

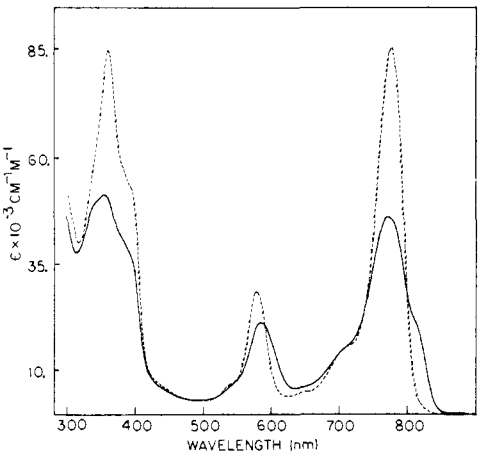

Figure 3. Electronic absorption spectrum of a 1.3×10^{-4} M solution of BChl in dry methylene chloride (solid line) and in the presence of less than 1% tetrahydrofuran (dashed line).

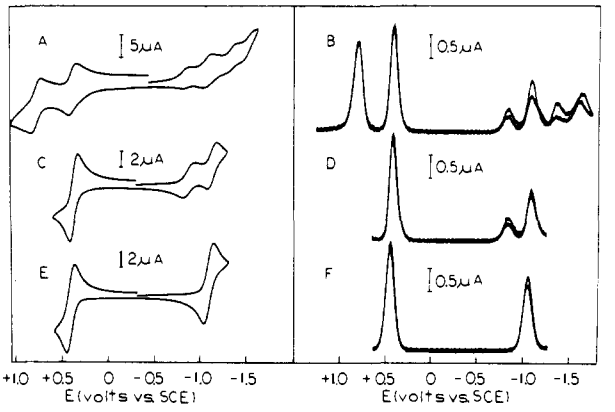

Figure 4. Cyclic and cyclic differential pulse voltammograms of BChl (4×10^{-4} M) in dry methylene chloride with 0.1 M TBAP as supporting electrolyte: (A, B) dry methylene chloride with scan range including all four reduction peaks and both oxidation peaks; (C, D) dry methylene chloride with scan range including only the first two reduction peaks and the first oxidation peak; (E, F) dry methylene chloride plus less than 1% tetrahydrofuran distilled into the solution. Working electrode was a platinum bead with surface area equal to 0.105 cm^2. Cyclic voltammetry and cyclic differential pulse voltammetry parameters were identical with those in Figure 1. The initial scan direction was cathodic, and data shown are of the first scan.

of its shoulder and an increase in peak current. These results are consistent with BChl self-aggregation as the cause of the shoulder on the reduction peaks, since THF is an excellent nucleophile and readily dissociates BChl aggregates as discussed in more detail below.

Electrochemistry of BChl in CH_2Cl_2. The electrochemical behavior of BChl in CH_2Cl_2 was examined, since this solvent is similar in properties to CCl_4, a solvent known to promote aggregation of the chlorophylls. (It is not possible to perform electrochemical measurements in CCl_4, due to the high solution resistance.) In order to interpret the data to be presented, a brief review will be given concerning the aggregation behavior of BChl in CCl_4.[51] From IR and NMR spectroscopy, it has been deduced that aggregation is the result of coordination of the carbonyl functional groups of one molecule of BChl with the central Mg atom of a second molecule in solvents lacking nucleophilic properties, such as CCl_4 and benzene. It is a consequence of the requirement for a coordination number of five for the central Mg atom of BChl. This interaction can be perpetuated, and BChl in CCl_4 has an average aggregate size of three over the concentration range from 10^{-2} to 10^{-1} M as determined by vapor phase osmometry measurements.[52] In BChl there are two carbonyl functional groups on the periphery of the porphyrin macrocycle that are involved in self-aggregation: there are the C-9 keto and the C-2 carbonyl groups. Evidence for their interaction can be had from shifts of their infrared vibrational frequencies to lower values on aggregation. Self-aggregation of BChl also affects its electronic absorption spectrum. Figure 3 (solid line) illustrates the spectrum of BChl in CH_2Cl_2; it is identical with that in CCl_4 at the same concentration. The $Q_y(0,0)$ transition near 780 nm is most affected by aggregation. A decrease in intensity is noted together with the formation of a shoulder near 810 nm. The addition of sufficient quantities of a nucleophile such as THF causes disruption of the BChl aggregates and coordination of the nucleophile to the central Mg of BChl as shown by IR and NMR spectroscopy. The disaggregation is manifest in the electronic absorption spectrum by a loss of the 810-nm shoulder together with an increase in the 780-nm peak intensity (dashed line in Figure 3). Equilibrium constants have been obtained for the interaction of nucleophiles with BChl or Chl *a* following the changes in the IR or visible absorption spectrum with incremental additions of the nucleophile.[53,54] Values obtained by the two techniques agree well, indicating that the effects of aggregation on the IR and visible absorption spectra are of the same origin.

Examples of typical first-scan CV and CDPV results for BChl solutions in dry CH_2Cl_2 are shown in Figures 4A and 4B. The midpoint potentials for the reduction and oxidation peaks are listed in Table II. In both techniques four distinct peaks

are evident in the cathodic scan. The fourth peak is near the solvent limit and is not as discernible in the CV results (Figure 4A). Both the second and third peaks show irreversibility—the current values are much lower in the reverse scan relative to the forward scan. The reversibility of the second peak is substantially improved if the cathodic scan limit includes only the first two peaks (Figures 4C and 4D), which suggests a chemical reaction follows the third peak.

The current magnitude for the reduction peaks is considerably less than that of the one- and two-electron oxidation peaks of BChl in CH_2Cl_2 (Figures 4A and 4B). Since only the first two reduction peaks are chemically reversible, the effect of BChl concentration and scan rate on the relative heights of these two peaks was investigated. The effect of concentration was examined to determine whether these peaks are related by a chemical equilibrium, i.e., the formation of BChl dimers and trimers from monomers. Extrapolation of data obtained by vapor phase osmometry to infinite dilution has indicated that the trimer is the limiting size of the BChl aggregate.[52] However, small amounts of water or other nucleophilic impurities in the electrolyte solution could serve to disaggregate the BChl.

Peak currents for a series of solutions containing BChl in the concentration range between 10^{-4} and 10^{-3} M were normalized by ratioing the values to the current magnitude of the first oxidation peak in the same solution. The ratios were found to be independent of BChl concentration with the value of the first peak equal to 0.25 ± 0.05 and that of the second peak equal to 0.50 ± 0.05. Variation of the scan rate between 0.02 and 10 V/s had no effect on the relative heights of the two reduction peaks, although the first peak showed a greater increase in ΔE, the difference between the forward and reverse scan peak maxima, with increasing scan rate.

Distillation of the THF into the CH_2Cl_2 solution in sufficient quantities to disaggregate the BChl produces significant changes in the electrochemical reduction behavior. The four reduction peaks collapse into two reversible peaks. Figures 4E and 4F show the cyclic and cyclic pulse voltammograms of the first oxidation and first reduction peaks in the presence of THF. A comparison with the above figure for BChl in CH_2Cl_2 in the absence of THF shows that the two reduction peaks are replaced by one peak of enhanced current magnitude. A comparison of the reduction potentials for aggregated and disaggregated BChl (Table II and Figures 4D and 4F) indicates that the first electron reduction peak in the disaggregated BChl has a potential slightly more positive than the second peak in the aggregated BChl.

To characterize further the nature of the BChl species giving rise to the first reduction peak in dry CH_2Cl_2, a constant potential bulk electrolysis experiment was performed, and absorption changes were monitored by difference absorption spectroscopy. Before electrolysis a zero base line was established between 300 and 1100 nm by placing an aliquot of the neutral BChl solution in the reference compartment of the Cary 17D spectrophotometer. The cell pathlength was chosen to match that of the BChl solution in the electrochemical cell, which was placed in the sample compartment of the spectrophotometer. Electrolysis at −0.92 V, or 90 mV past the first reduction peak, resulted in the difference spectrum shown in Figure 5A. A negative absorbance is seen at 780 and 812 nm, indicating that the species reduced at the first peak in CV or CDPV has an absorption spectrum corresponding to aggregated BChl. The absorbance decrease at 812 nm corresponds to a complete loss of the 812-nm shoulder in the normal absorption spectrum (see Figure 3). A positive absorbance is seen at 1050 nm, which is red-shifted by 50 nm relative to the previously reported near-infrared transition of monomeric $BChl^{-\cdot}$ in DMF.[31] Other bands that appear red-shifted in the anion radical include the 650 (from 620 nm) and the 940 (from 920

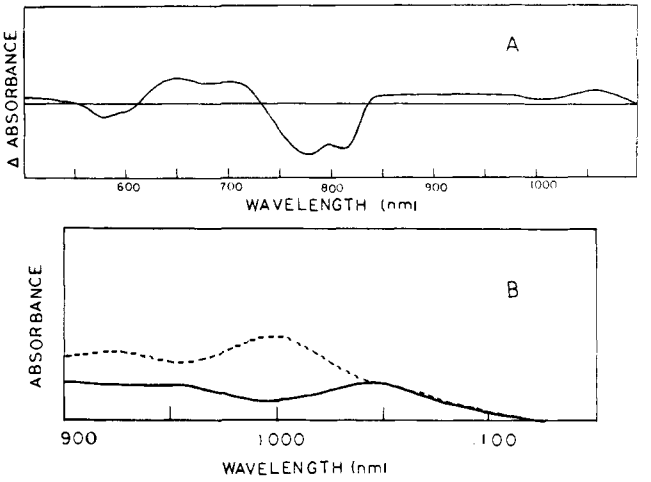

Figure 5. Electronic absorption spectra of bulk electrolyzed BChl (4×10^{-4} M) in dry methylene chloride with 0.1 M TBAP as the supporting electrolyte: (A) difference absorption spectrum (BChl reduced at −0.92 V in sample compartment minus neutral BChl in reference compartment of Cary 17D spectrophotometer); (B) absorption spectrum of BChl reduced at −0.92 V (solid line) and −1.16 V (dashed line).

nm). The solution was then electrolyzed at −1.16 V or 80 mV past the $E_{1/2}$ value of the second reduction peak. At this potential the difference absorption spectrum showed a decrease in BChl absorbing at 780 nm, as would be expected, since this is the only spectral form of BChl remaining. An increase in absorbance occurred at 1000 nm (Figure 5B).

An examination of the electrochemical behavior of BPheo in CH_2Cl_2 provides further evidence that BChl self-aggregation is responsible for the multiple reduction peaks observed in CV and CDPV. BPheo lacks Mg and, therefore, cannot undergo the C=O···Mg interactions possible in BChl. Rather, BPheo self-associates by much weaker π-π interactions, which apparently do not affect its redox behavior. The electrochemical reduction of BPheo in CH_2Cl_2 showed only two peaks (Table II). The one-electron reduction potential was found to be very near that of the first of the four reduction peaks observed for BChl in CH_2Cl_2. In order to determine very accurately the relative values for the reduction potential of BChl and BPheo in CH_2Cl_2, the CV and CDPV studies were repeated with BChl added to the solution of BPheo in CH_2Cl_2. It is not possible to resolve all of the observed electron-transfer steps, since the reduction peaks overlap considerably. However, the oxidation peaks are clearly resolved and can be used to calibrate the reduction potentials obtained for the isolated compounds in CH_2Cl_2. These values are listed in Table II under the heading BChl plus BPheo. It may be noted that the reduction potential of BPheo is only 20 mV less negative than that of the first reduction peak of isolated BChl in CH_2Cl_2.

Electrochemistry of BChl in THF. In the previously discussed electrochemical studies of BChl in ACN and CH_2Cl_2, THF was added in sufficient quantities to disaggregate the BChl. Care was taken to avoid adding an excess of THF, as the objective was to form monomeric, five-coordinate BChl, i.e., one molecule of THF coordinated to the central Mg atom of BChl, i.e., BChl·THF. If additional THF is titrated into a solution of BChl in CH_2Cl_2, it is possible to follow the addition of a second molecule of THF to the Mg atom resulting in the formation of six-coordinate BChl, i.e., $BChl\cdot(THF)_2$. This interpretation is based on changes in the visible absorption spectrum similar to those observed on titration of pyridine into a BChl solution in CCl_4.[55] The $Q_x(0,0)$ band of BChl gradually shifts from 580 to 593 nm with the incremental addition of a concentrated THF solution in CH_2Cl_2. In neat THF the $Q_x(0,0)$ band is also at 593 nm, and the BChl is predominantly

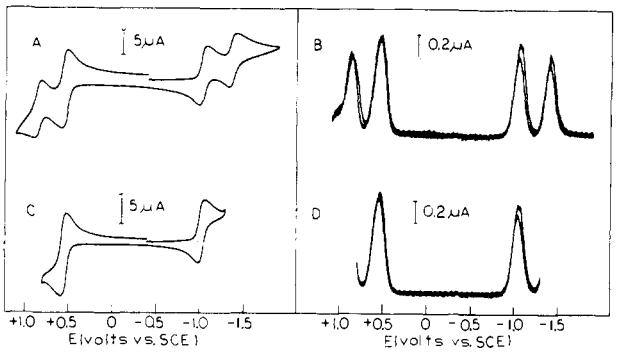

Figure 6. Cyclic and cyclic differential pulse voltammograms of BChl (2×10^{-4} M) in dry tetrahydrofuran with 0.1 M TBAP as the supporting electrolyte: (A, B) scan range includes both reduction peaks and both oxidation peaks, (C, D) scan range includes only the first reduction and first oxidation peak. Working electrode was a platinum bead with surface area equal to 0.105 cm^2. Cyclic voltammetry and cyclic differential pulse voltammetry parameters were identical with those in Figure 1, except IR compensation was included in the potentiostat circuit. The initial scan direction was cathodic, and the data shown are of the first scan.

six coordinate. The electrochemical response of BChl in THF was examined to determine the effect of ligand coordination on its redox properties.

Both CV and CDPV studies of BChl in THF show two reversible one-electron reduction peaks and two reversible one-electron oxidation peaks (Figure 6 and Table III). All four peaks are of approximately the same current magnitude, and, in each case, ΔE, the difference in potential between the current maximum in the forward and reverse scans in CV, is equal to 59 mV. Variation of the CV sweep rate gives cathodic/anodic current ratios near 1.0 for each electron transfer. The CDPV verified reversible behavior for all of the electron-transfer steps as shown by nearly identical peak heights in the forward and reverse scans.

As discussed in the Experimental Section, ferrocene was used as a standard to correct for junction potential differences between the various solvents and the SCE reference. Ideally, this permits a meaningful comparison of the BChl redox potentials in the different solvents. From the values listed in Tables I and II for BChl·THF in ACN and in CH_2Cl_2, it may be seen that an increase in ligand coordination to BChl·$(THF)_2$ results in cathodic shifts of 60 and 80 mV, respectively, for the one- and the two-electron reduction of BChl. The one-electron oxidation potential is even more affected and shifts positively by 100 mV on formation of six-coordinate BChl. Recently, the one-electron oxidation potential of Chl *a* in THF was also found to shift anodically in THF.[56] However, it may be that the shift in BChl redox potentials in THF is not entirely due to an increase in ligand coordination. The $E_{1/2}$ for the one-electron oxidation potential of BPheo in THF also shifts positively by 50 mV relative to that in CH_2Cl_2 (see Tables II and III), and it obviously does not undergo metal coordination interactions.

Discussion

The experimental results presented here emphasize the considerable effects BChl interactions can have on its redox properties. The significant findings, together with their interpretation, are summarized as follows:

(1) BChl self-aggregation affects its reduction processes. Shoulders on the major one- and two-electron reduction peaks (in ACN solutions) or four distinct reduction peaks (in CH_2Cl_2) are observed in CV and CDPV experiments. Both phenomena are typical of uncomplicated multistep charge processes.[57] The CH_2Cl_2 solutions were investigated in detail because the reduction peaks are well resolved and, also, because BChl aggregates in similar solvents have been well characterized. Scan rate and concentration studies show that the first peak is not due to absorption of BChl on the electrode surface, nor are the first two peaks related by a chemical equilibrium (at least on the time scale of the electrochemical experiment). An equilibrium process that might be considered is the formation of dimers and trimers from monomeric BChl. If this were present an increase in BChl concentration should shift the equilibrium toward the aggregated state and result in an increase in current magnitude of the first peak. This is not observed. Therefore, a tentative explanation is that the first peak represents the addition of one electron to the BChl aggregate and the second is due to the addition of one (or two) additional electrons to the aggregate.

Table III. Oxidation and Reduction Potentials of BChl and BPheo in THF[a]

sample	$E_{1/2}^{2+}$	$E_{1/2}^{+\cdot}$	$E_{1/2}^{-\cdot}$	$E_{1/2}^{2-}$
BChl				
CV	0.83	0.52	−1.07	−1.41
CDPV	0.85	0.52	−1.08	−1.43
BPheo				
CV		0.88	−0.84	−1.17
CDPV		0.87	−0.85	−1.20
ferrocene				
CV		0.56		
CDPV		0.57		

[a] Potentials are in volts vs. SCE.

(2) The first electron-transfer step in the reduction of the BChl aggregate has a midpoint potential that is 200 mV more positive than monomeric five-coordinate BChl. A plausible explanation for this effect is that one of the BChl molecules in the aggregate, the keto C=O donor, is easier to reduce as a result of its interaction with a second molecule, the acceptor. In practical terms, the shift equalizes the one-electron reduction potential of aggregated BChl with that of BPheo in the same solvent. The effect of aggregation on the reduction potential is important because, in the RC, P800 is known to involve at least two interacting BChl molecules. A similar shift in the $E_{1/2}$ value for P800 would decrease the potential difference created by electron transfer from P870 to P800. This decrease in potential would, in turn, reconcile the thermodynamic considerations[18] with the picosecond observations of Shuvalov et al.,[28] indicating P800 as a transient electron acceptor prior to BPheo. In the RC, however, it is not necessary, or desirable, that the P800 reduction potential be shifted so far as to equal that of BPheo. A positive shift in the one-electron reduction potential of Chl *a* has been noted previously and attributed to the presence of aggregates, but this point was not verified experimentally.[58]

(3) The absorption spectrum of the anion radical of aggregated BChl is red-shifted relative to that of the monomeric anion radical, as shown by bulk electrolysis of the CH_2Cl_2 solution. The shifts are comparable energetically to that observed for the Q_y transition in the neutral BChl aggregate. Apropos of the RC, it has been noted that all of the picosecond and steady-state absorption spectra of I^-, the intermediate electron acceptor, have absorption bands indicative of $BChl^{-\cdot}$ as well as $BPheo^{-\cdot}$.[59] However, the bands in the 600-700 and 800-950 nm region are red-shifted by approximately the same amount as the aggregated anion radical described here. Also, the absorption spectrum of the intermediate electron acceptor of PSI of green plants was observed recently to correspond to a red-shifted anion radical of Chl *a*. A Chl dimer was proposed as the intermediate to account for the red shift.[60]

(4) BChl self-aggregation has no significant effect on the oxidation processes. This finding is surprising in view of the results of Wasielewski et al.[61] in which the midpoint potential

for the one-electron oxidation and one-electron reduction of the covalently linked bis(chlorophyll) cyclophane was observed to shift positively by 70 mV and negatively by 150 mV, respectively. It should be emphasized, however, that the BChl aggregates in the present study are structurally distinct from the covalent dimer of Wasielewski. The BChl molecules interact by coordination interactions of the donor C=O of one molecule with the acceptor Mg atom of a second molecule. Consequently, *these noncovalent aggregates are asymmetrical.* Moreover, the aggregates discussed here have been prepared under anhydrous conditions, unlike the dimeric Chl *a*,[62,63] pyrochlorophyll *a*,[64] and BChl *a*[65] reaction center models, which contain two molecules of coordinated ethanol or water per dimer. These latter models are highly symmetrical and on one-electron oxidation exhibit ESR spectra that indicate equal sharing of the remaining unpaired electron over both molecules of the dimer. As of the present time, we do not have ESR measurements to indicate whether spin delocalization occurs in the BChl aggregates studied here. Since these are structurally different and asymmetrical, equal sharing of the unpaired spin in the cation or anion radical cannot be assumed a priori. Indeed, if these aggregates are representative of the interactions present in P800, delocalization should not occur, as the ESR measurements on trapped I^- have clearly shown that the spin is localized on one molecule.[29,47,66]

(5) The redox properties of BPheo are relatively unaffected by solvent. BPheo, which lacks Mg, and is incapable of aggregating in the same manner as BChl, does not display unusual reduction behavior in CH_2Cl_2. The much weaker π-π interactions that are possible in BPheo are not observed to an appreciable extent in the concentration range used in the CV and CDPV experiments. It might be noted, however, that BPheo is capable of functioning as a C=O donor to BChl. In the RC this type of interaction between P800 and BPheo could conceivably affect the redox behavior of BPheo.

(6) Coordination of a sixth axial ligand to the Mg of BChl affects both its oxidation and reduction potentials. The addition of a sixth ligand was shown to shift the one-electron reduction cathodically by approximately 60 mV. A much larger effect was observed for the one-electron oxidation potential which shifted anodically by 100 mV relative to five-coordinate BChl. The exact magnitude of these shifts is dependent upon the junction potential correction and, hence, there is some uncertainty associated with these values. A more meaningful comparison of the effects of solvent on the redox properties might be obtained by calculating the δ value, or the difference between the one-electron oxidation and one-electron-reduction potentials within a given solvent, since it is independent of junction potential variations. In CH_2Cl_2 this value is smallest and equal to 1.25 V, if the first reduction peak is used in the calculation. Five-coordinate BChl in CH_2Cl_2 has a value equal to 1.45 V and six-coordinate BChl has the largest value, 1.60 V. Solvent effects on the δ value of BPheo are much smaller with a difference of only 70 mV between the value in CH_2Cl_2 and THF.

In conclusion, although the data we have obtained are not based upon measurements of BChl and BPheo redox potentials in vivo, the need for an understanding of the redox properties of these molecules in solution has been recognized.[59] Such data are relevant to considerations of the energetics of primary events, and BChl and BPheo reduction potentials in solution have been used extensively in calculations involving the kinetics and proposed mechanisms of photoinduced charge separation in the RC (see, for example, ref 66). The data presented describe the influence of BChl interactions on its midpoint potentials and, thus, predict the effect of similar interactions on these values in the RC. Those molecules capable of interacting with BChl in the RC include other BChl's, BPheo, or protein side chains that can function as axial ligands.

Acknowledgments. The authors wish to thank Professor Paul Loach for helpful discussions and Professor Donald E. Smith for use of the computer facilities. The support of this research by the National Science Foundation under Grant CHE 78-00877 is gratefully acknowledged. In addition, T.M.C. acknowledges support from IBM and National Institutes of Health Postdoctoral Fellowships.

References and Notes

(1) (a) Northwestern University. (b) IBM Fellow, 1978. NIH Fellow, 1979.
(2) Clayton, R. K. "The Photosynthetic Bacteria"; Clayton, R. K., Sistrom, W. R., Eds.; Plenum Press: New York, 1978; pp 387–396.
(3) Reed, D. W.; Clayton, R. K. *Biochem. Biophys. Res. Commun.* **1968,** *30,* 471–475.
(4) Loach, P. A. *Prog. Bioorg. Chem.* **1976,** *4,* 91–192.
(5) Parson, W. W.; Cogdell, R. J. *Biochim. Biophys. Acta* **1975,** *416,* 105–149.
(6) Parson, W. W. *Annu. Rev. Microbiol.* **1974,** *28,* 41–59.
(7) Blankenship, R. E.; Parson, W. W. *Annu. Rev. Biochem.* **1978,** *47,* 635–653.
(8) Clayton, R. K. *Annu. Rev. Biophys. Bioeng.* **1973,** *2,* 131–156.
(9) Norris, J. R.; Uphaus, R. A.; Crespi, H. L.; Katz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1971,** *68,* 625–628.
(10) Norris, J. R.; Druyan, M. E.; Katz, J. J. *J. Am. Chem. Soc.* **1973,** *95,* 1680–1682.
(11) Norris, J. R.; Scheer, H.; Druyan, M. E.; Katz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1974,** *71,* 4897–4900.
(12) Feher, G.; Hoff, A. J.; Isaacson, R. A.; Ackerson, L. C. *Annu. Rev. N.Y. Acad. Sci.* **1975,** *244,* 239–259.
(13) Loach, P. A.; Hall, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1972,** *69,* 786–790.
(14) Feher, G.; Okamura, M.; McElroy, J. D. *Biochim. Biophys. Acta* **1972,** *267,* 222–226.
(15) Feher, G.; Okamura, M. Y. *Brookhaven Symp. Biol.* **1977,** *28,* 183–194.
(16) Rockley, M. G.; Windsor, M. W.; Cogdell, R. J.; Parson, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1975,** *72,* 2251–2255.
(17) Kaufmann, K. J.; Dutton, P. L.; Netzel, T. L.; Leigh, J. S.; Rentzepis, P. M. *Science* **1975,** *188,* 1301–1304.
(18) Fajer, J.; Brune, D. C.; Davis, M. S.; Forman, A.; Spaulding, L. D. *Proc. Natl. Acad. Sci. U.S.A.* **1975,** *72,* 4956–4960.
(19) Kaufmann, K. J.; Petty, K. M.; Dutton, P. L.; Rentzepis, P. M. *Biochem. Biophys. Res. Commun.* **1976,** *70,* 839–845.
(20) Netzel, T. L.; Dutton, P. L.; Petty, K. M.; Degenkolb, E. O.; Rentzepis, P. M. *Adv. Mol. Relaxation Int. Processes* **1977,** *11,* 217–220.
(21) Peters, K.; Avouris, Ph.; Rentzepis, P. M. *Biophys. J.* **1978,** *23,* 207–217.
(22) Shuvalov, V. A.; Klimov, V. V.; Krakhmaleva, I. N.; Moskalenko, A. A.; Krasnovskii, A. A. *Dokl. Chem. (Engl. Transl.)* **1976,** *227,* 984–987.
(23) Van Grondelle, R.; Romijn, J. C.; Holmes, N. G. *FEBS Lett.* **1976,** *72,* 187–192.
(24) Holten, D.; Windsor, M. W. *Annu. Rev. Biophys. Bioeng.* **1978,** *7,* 189–227.
(25) Campillo, A. J.; Shapiro, S. L. "Ultrashort Light Pulses"; Shapiro, S. L., Ed.; Springer-Verlag: New York, 1977; pp 317–376.
(26) Mauzerall, D. *Photochem. Photobiol.* **1979,** *29,* 169–170.
(27) Loach, P. A. *Photochem. Photobiol.* **1977,** *26,* 87–94.
(28) Shuvalov, V. A.; Klevanik, A. V.; Sharkov, A. V.; Matveetz, Ju. A.; Krukov, P. G. *FEBS Lett.* **1978,** *91,* 135–139.
(29) Tiede, D. M.; Prince, R. C.; Dutton, P. L. *Biochim. Biophys. Acta* **1976,** *449* 447–467.
(30) An estimate of the reduction of BPheo in vivo was made by measuring the BChl special pair triplet yield as a function of potential in: Prince, R. C.; Leigh, J. S.; Dutton, P. L. *Biochim. Biophys. Acta* **1976,** *440,* 622–636.
(31) Fajer, J.; Borg, D. C.; Forman, A.; Dolphin, D.; Felton, R. H. *J. Am. Chem. Soc.* **1973,** *95,* 2739–2741.
(32) Drozdova, N. N.; Kuznetsov, B. A.; Mestechkina, N. M.; Shumakovich, G. P.; Pushkina, E. M.; Krasnovskii, A. A. *Dokl. Chem. (Engl. Transl.)* **1977,** *235,* 1437–1440.
(33) Berg, H.; Kramarczyk, K. *Biochim. Biophys. Acta* **1967,** *131,* 141–146.
(34) Philipson, K. D.; Sauer, K. *Biochemistry* **1973,** *12,* 535–539.
(35) Strain, H. H.; Svec, W. A. "The Chlorophylls"; Vernon, L. P., Seely, G. R., Eds.; Academic Press: New York, 1966; pp 21–66.
(36) Barbetta, A.; Edgell, W. *Appl. Spectrosc.* **1978,** *32,* 93–98.
(37) Van Duyne, R. P.; Suchanski, M. R.; Lakovits, J. M.; Siedle, A. R.; Parks, K. D.; Cotton, T. M. *J. Am. Chem. Soc.* **1979,** *101,* 2832–2837.
(38) Drake, K. F.; Van Duyne, R. P.; Bond, A. M. *J. Electroanal. Chem.* **1978,** *89,* 231–246.
(39) Hammerich, O.; Parker, V. D. *Electrochim. Acta* **1973,** *18,* 537–541.
(40) Jensen, B. S.; Parker, V. D. *J. Chem. Soc., Chem. Commun.* **1974,** 367–368.
(41) Saji, T.; Bard, A. J. *J. Am. Chem. Soc.* **1977,** *99,* 2235–2240.
(42) Fujita, I.; Davis, M. S.; Fajer, J. *J. Am. Chem. Soc.* **1978,** *100,* 6280–6282.
(43) Ballschmiter, K.; Cotton, T. M.; Strain, H. H.; Katz, J. J. *Biochim. Biophys. Acta* **1969,** *180,* 347–359.
(44) Headridge, J. B. "Electrochemical Techniques for Inorganic Chemists"; Academic Press: New York, 1969; pp 71–73, and references therein.
(45) Flato, J. B. *Anal. Chem.* **1972,** *44*(11), 75A–87A.
(46) Rifkin, S. C.; Evans, D. H. *Anal. Chem.* **1976,** *48,* 1616–1618, 2174–2180.
(47) Fajer, J.; Forman, A.; Davis, M. S.; Spaulding, L. D.; Brune, D. C.; Felton, R. H. *J. Am. Chem. Soc.* **1977,** *99,* 4134–4140.

(48) Brown, E. R.; Large, R. F. "Physical Methods of Chemistry"; Weissberger, A., Rossiter, B. W., Eds.; Wiley-Interscience: New York, 1971; Vol. I, Part IIA, pp 502–508.
(49) Dryhurst, G. "Electrochemistry of Biological Molecules"; Academic Press: New York, 1977; pp 392–472, and references therein.
(50) Lanese, J. G.; Wilson, G. S. *J. Electrochem. Soc.* **1972**, *119*, 1039–1043.
(51) Katz, J. J.; Shipman, L. L.; Cotton, T. M.; Janson, T. R. "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. 5, Part C, pp 401–458 and references therein.
(52) Ballschmiter, K.; Truesdell, K.; Katz, J. J. *Biochim. Biophys. Acta* **1969**, *184*, 604–613.
(53) Ballschmiter, K.; Katz, J. J. *J. Am. Chem. Soc.* **1969**, *91*, 2661–2677.
(54) Cotton, T. M. Ph.D. Dissertation, Northwestern University, 1976.
(55) Evans, T. A.; Katz, J. J. *Biochim. Biophys. Acta* **1975**, *396*, 414–426.
(56) Fajer, J., personal communication.
(57) Polcyn, D. S.; Shain, I. *Anal. Chem.* **1966**, *38*, 370–375.
(58) Kiselev, B. A.; Kozlov, Yu. N.; Yevstigneyev, V. B. *Biophysics* **1970**, *15*, 620–628.
(59) Dutton, P. L.; Prince, R. C.; Tiede, D. M.; Petty, K. M.; Kaufmann, K. J.; Netzel, T. L.; Rentzepis, P. M. *Brookhaven Symp. Biol.* **1977**, *No. 28*, 213–237.
(60) Shuvalov, V. A.; Ke, B.; Dolan, E. *FEBS Lett.* **1979**, *100*, 5–8.
(61) Wasielewski, M. R.; Svec, W. A.; Cope, B. T. *J. Am. Chem. Soc.* **1978**, *100*, 1961–1962.
(62) Shipman, L. L.; Cotton, T. M.; Norris, J. M.; Katz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1791–1794.
(63) Wasielewski, M. R.; Studier, M. H.; Katz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4282–4286.
(64) Boxer, S. C.; Closs, G. L. *J. Am. Chem. Soc.* **1976**, *98*, 5406–5408.
(65) Wasielewski, M. R.; Smith, U. H.; Cope, B. T.; Katz, J. J. *J. Am. Chem. Soc.* **1977**, *99*, 4172–4173.
(66) Okamura, M. Y.; Isaacson, R. A.; Feher, G. *Biochim. Biophys. Acta* **1979**, *546*, 394–417.

Communications to the Editor

Reversed Phase Chromatographic Resolution of Amino Acid Enantiomers with Metal–Aspartame Eluants

Sir:

The resolution of amino acid enantiomers is of importance in peptide synthesis and structure determinations. While such methods as selective crystallization and enzyme degradation have been used, they are of limited use, frequently owing to their specificity. Chromatographic methods of analysis offer the possible advantage of resolving not only a pair of enantiomers but also a mixture of several amino acid enantiomers. Gas chromatographic separation of D- and L-amino acids has been described by, among others, Gil-Av,[1] Feibush,[2] and Bayer,[3] who have used chiral stationary phases. The method, however, requires the derivatization of the amino acids to more volatile compounds suitable for GC separations. For this reason, modern high performance liquid chromatography (HPLC) can be a more attractive tool for enantiomeric resolutions. Several approaches have been advanced. Davankov and his co-workers[4] and Lefebvre et al.,[5] to name a few, have used ligand exchange chromatography. There the separation of the enantiomers was achieved with an α-amino acid-copper(II) complex grafted onto a resin. Frequently the grafted amino acid of choice is proline, although, as Angelici[6] has shown, other amino acids can form stereoselective complexes. Cram and his group[7] have used chiral crown ethers bonded to chromatographic support for the separation of the optical isomers. A similar approach was reported by Blasius.[8] More recently, Hara[9] has reported the separation of the enantiomers of N-protected amino acid esters on L-valyl derivatives bonded to silica gel. Pirkle[10] has demonstrated the resolution of 3,5-dinitrobenzoyl derivatives of amino acids on chiral fluoro alcohols. Gaal and Inczedy[11] as well as Yoneda and Yoshizawa[12] have utilized optically active Co(III) compounds to achieve optical resolution. In an entirely different approach, Karger and his group[13] have used the zinc(II) complex of L-2-alkyl-4-octyldiethylenetriamine in an aqueous mobile phase to obtain the resolution of the densyl derivatives of amino acids. Hare and Gil-Av[14] have reported very recently the use of a proline-copper(II) complex in an aqueous mobile phase as the resolving reagent in the ion-exchange separation of D- and L-amino acids.

The work described above, while augmenting greatly the arsenal of the chemist, suffers from such disadvantages as (a) applicability to only one or two pairs of amino acids, (b) the need for derivatized amino acids, and (c) harsh conditions such as high temperature. We report here preliminary results of a chromatographic separation which has the potential of eliminating most of these restrictions.

To resolve optical isomers directly by chromatography, diastereomers must be formed in situ while the resolution takes place. To form the diastereomers, a chiral reagent can be introduced either to the mobile or the stationary phase. Since the mobile phase is inherently easier to manipulate, we prefer to add the chiral reagent to it, in accordance with the approach of Karger[13] and of Hare and Gil-Av.[14] Like other workers we have utilized the fact that amino acids form complexes with metal cations and that the stability of the isomers can be stereodependent (viz., ref 6 and 15 and references therein). We have chosen the metal complex of the dipeptide L-aspartyl-L-phenylalanine methyl ester as the resolving agent. The rationale for choosing this particular dipeptide is as follows. (a) This dipeptide is available commercially under the tradename

Table I. The Capacity Ratios k' and Selectivity Factors α[a] of Some Amino Acid Enantiomers as a Function of the Acetonitrile (ACN) in the Mobile Phase. The Mobile Phase Contains 10^{-3} M Copper–Aspartame Complex

solute	0% ACN		7% ACN		8% ACN		10% ACN	
	k'	α	k'	α	k'	α	k'	α
L-Dopa	3.2	1.5						
D-Dopa	4.8							
L-tyrosine	5.4	1.6	1.2	1.5	1.0	1.7		
D-tyrosine	8.9		1.8		1.7			
L-phenylalanine	*b*		4.8	1.6	4.3	1.3		
D-phenylalanine	*b*		7.8		5.4			
L-tryptophan	*b*		*b*		13.2	1.3	5.6	1.2
D-tryptophan	*b*		*b*		16.7		6.8	

[a] See note 18. [b] Retention times too long for accurate measurements.

Table II. The Capacity Ratios k' and Selectivity Factors α[a] of Some Amino Acid Enantiomers as a Function of the Concentration of the Zinc–Aspartame Complex in the Mobile Phase

solute	10^{-3} M		5×10^{-4} M		2.5×10^{-4} M	
	k'	α	k'	α	k'	α
L-tyrosine	1.9	1.3	2.9	1.4	5.3	1.5
D-tyrosine	2.5		4.2		7.9	
L-phenylalanine	5.5	1.6	9.3	1.7	16	1.9
D-phenylalanine	9.0		16.0		30	
L-tryptophan	19.4	1.2	34	1.2	*b*	
D-tryptophan	23.0		42		*b*	

[a] See note 18. [b] Retention times too long for accurate measurements.

0002-7863/79/1501-7612$01.00/0