tensity rather than the appearance of new vibrational modes. Apparently the greater planarity of the corrin ring in methylcobinamide or "base-off" methylcobalamin allows more ring vibrations to come into resonance with the electronic transitions. Intensity changes could also arise from changes in the overlap of corrin and cobalt orbitals, accompanying the displacement of benzimidazole, with the possible formation of an essentially five-coordinate square-pyramidal complex.[8,11]

When aquocobalamin is reduced in acid solution to the Co(II) derivative, vitamin B$_{12r}$, the benzimidazole ligand is protonated and dissociates.[12] We find that this product gives the same Raman spectrum as does methylcobinamide. Addition of an electron to the Co(III) center has no discernible influence on the resonance Raman spectrum, nor is there much change in the electronic spectrum (see Figure 2). In neutral solutions of vitamin B$_{12r}$, the benzimidazole is coordinated.[12] Curiously, however, the resonance Raman spectrum is the same as that of the "base-off" derivatives. We note that the electronic spectrum of "base-on" vitamin B$_{12r}$ is also similar to those of the cobinamides.[13] It is conceivable that the corrin ring structure is also closer to that of the "base-off" derivatives, in view of the weakness of the Co(II)–benzimidazole bond.[9]

All of the bands, in both the cobalamin and the cobinamide-type resonance Raman spectra, are polarized, with depolarization ratios, ρ_1 ranging from 0.2 to 0.6. The phenomenon of inverse polarization,[14] exhibited by the resonance Raman spectra of hemoglobin and cytochrome *c*, is not observed here. Inverse polarization ($\rho_1 = \infty$) arises when a vibration whose scattering tensor is antisymmetric ($\alpha_{ij} = -\alpha_{ji}$) is vibronically active.[14] Antisymmetric tensors only arise when the molecular symmetry is axial (threefold or higher rotation axis). Since the highest symmetry available to the corrin ring is C_{2v},[15] it is not surprising that no inverse polarized bands are found in vitamin B$_{12}$ spectra. The A$_2$ vibration in the C_{2v} point group does have an asymmetric tensor[16] and should show anomalous polarization ($\rho_1 > {}^3/_4$).[14] Since all observed bands are polarized, A$_2$ modes are evidently not vibronically active in vitamin B$_{12}$ derivatives.

There is considerable current interest in structural alternations of B$_{12}$ coenzyme which may be involved in enzymatic mechanisms. Both reduction of the cobalt[17] and dissociation of the benzimidazole ligand with attendant changes in corrin conformation[18,19] are possibilities. Inasmuch as the latter alteration has profound effects on the resonance Raman spectrum, it is apparent that Raman spectroscopy may prove useful in probing vitamin B$_{12}$–enzyme reactions.

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Transient Absorption Spectra of the Chlorophyll a Anion and Cation

Sir:

Chlorophyll plays an important role in photosynthesis by virtue of its ability to produce a charge separation and catalyze specific electron transfer reactions in the chloroplast.[1] However, little information has been obtained on the transient ionic intermediates of chlorophyll, though there are a few investigations of the cationic intermediate produced by oxidation with ferric chloride[2] or ferric perchlorate,[3] and by the electrolytic method.[4] We wish to report here the optical absorption spectra of both the anion and cation of chlorophyll a (Chl a) observed by the pulse radiolysis technique at room temperature as well as in the rigid glasses irradiated with γ-rays at 77°K. The decay rates of these ions were also determined by the pulse radiolysis.

Chl a was extracted with methanol from spinach leaves and isolated chromatographically by using a microcrystalline cellulose column and *n*-hexane as a developing solvent. Electron pulses from a Mitsubishi Van de Graaff generator were 2.7 MeV in energy and 0.5–1.0 μsec in duration. Absorption spectra in the γ-irradiated glasses were recorded on a Cary 14R spectrophotometer by using a thin (0.5–1.5 mm thickness) Suprasil cell.[5,6]

The absorption spectrum at 77°K in Figure 1 was obtained with an irradiated *sec*-butyl chloride glass containing Chl a (7 m*M*). This spectrum is regarded as being due to Chl a cation (Chl a$^+$). In the butyl chloride glass, only solute cations are formed, since all the electrons ejected by γ irradiation are stabilized as Cl$^-$ and any excited molecules or neutral radicals cannot migrate to the solutes.[7] The absorption spectrum obtained by the pulse radiolysis is also shown in Figure 1. This spectrum was observed at 20 μsec after pulse for aerated Chl a solutions (0.1–0.2 m*M*) in benzonitrile containing excess biphenyl (0.3 *M*). The aerated benzonitrile solutions have been found to give only solute cations on pulse irradiation, other possible intermediates such as triplet states and anions being completely removed by oxygen.[8] Therefore, the absorp-

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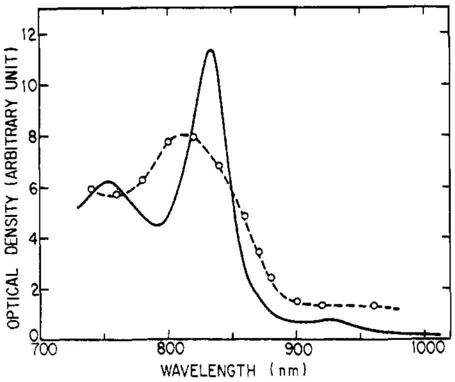

Figure 1. Absorption spectra of Chl a$^+$ formed in the γ-irradiated *sec*-butyl chloride glass at 77°K (—); in the pulse-irradiated benzonitrile solutions at room temperature (- -O).

tion spectrum is concluded to be solely Chl a$^+$ produced by charge transfer from solvent cation *via* biphenyl.[8]

This conclusion is supported by the following experimental results of the charge transfer from Chl a$^+$ to tetramethylbenzidine. When the benzonitrile solution containing Chl a (0.7 mM) and benzidine (0.07 mM) was pulsed, the absorption due to the Chl a$^+$ was replaced rapidly with the absorption of the benzidine cation which has large peaks at wavelengths above 900 nm[6] and little overlapping with that of Chl a$^+$. Kinetically, both rates of the formation of benzidine cation (determined at 980 nm) and the decay of Chl a$^+$ (determined at 750 nm) were found to be approximately the same. It is extremely difficult to determine the spectrum at 300–450 nm and 600–700 nm because of an intense background absorption of Chl a itself.

The absorption spectrum in the γ-irradiated methyltetrahydrofuran (MTHF) glass containing 7 mM Chl a is shown in Figure 2, which is regarded as being due to Chl a anion (Chl a$^-$). In the deaerated MTHF glass, only solute anions are formed since solvent cations are stabilized by rapid ion–molecule reactions with solvent.[6,9] The inset spectrum of Chl a$^-$ was obtained in the irradiated MTHF glass of a much lower concentration of Chl a (0.6 mM) after photobleaching the residual absorption band due to the trapped electrons. Although sharp peaks are seen at about 460 and 650 nm (dashed lines), their optical densities are uncertain because of the background absorption of Chl a.

The transient absorption spectrum observed at 20 μsec after pulse for dearated tetrahydrofuran (THF) solutions (Chl a, 0.1–0.2 mM) is also shown in Figure 2. The spectrum is assignable to that of Chl a$^-$ on the same basis as for the MTHF glass. When p-dinitrobenzene (p-DNB) was added to the solution, this absorption was replaced with the absorption of p-DNB anion which has a sharp peak at 910 nm.[9] In the THF solution containing Chl a (0.7 mM) and p-DNB (0.07 mM), electron transfer from Chl a$^-$ to p-DNB was found to take place. Although the permanent absorption of Chl a in THF solution was reduced after repeated pulse irradiation, it was unchanged in the solution containing p-DNB. Since formation of transient Chl a$^-$ was observed in both solutions, it is apparent that Chl a$^-$ is restored completely to the original Chl a by transferring an electron to p-DNB. For Chl a$^+$, a similar restoration was confirmed in the presence of benzidine.

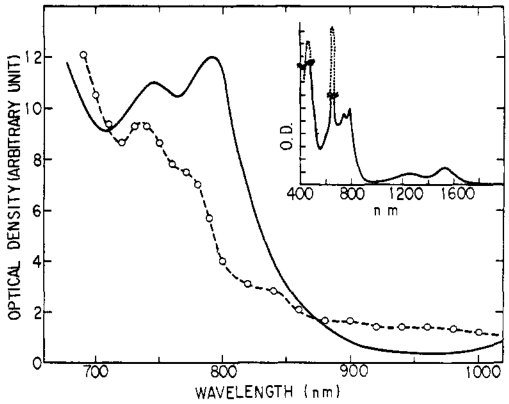

Figure 2. Absorption spectra of Chl a$^-$ formed in the γ-irradiated MTHF glass at 77°K (——) (optical densities of sharp peaks at about 460 nm and 650 nm in the inset (- - - - -) are uncertain); in the pulse-irradiated THF solutions at room temperature (- -O).

Assuming that the radiation chemical yield of the trapped electron in MTHF glass is $G = 2.55$ and all the electrons are scavenged with solutes in the glass,[9] one can calculate the molar extinction coefficient (ϵ) of Chl a$^-$ at 790 nm to be $1.1 \times 10^4\ M^{-1}\ \mathrm{cm}^{-1}$ at 77°K. The $\epsilon_{835\ \mathrm{nm}}$ of Chl a$^+$ is roughly estimated to be $7 \times 10^3\ M^{-1}\ \mathrm{cm}^{-1}$ at 77°K. It is noted that the half-lives of these transient ions (about 500 μsec, at room temperature) are much longer than those of radical ions arising from a majority of aromatic hydrocarbons ($\lesssim$20 μsec[10]). Rapid electron transfer from Chl a$^-$ to vitamin K_3, phenazine methosulfate, or flavine mononucleotide was observed by pulse radiolysis for methanol solution. The rate constant is approximately $1 \times 10^9\ M^{-1}\ \mathrm{sec}^{-1}$ for vitamin K_3 at room temperature.

The absorption spectra of Chl a$^+$ obtained here are very similar to those prepared by other methods[3,4] at wavelengths above 700 nm. It should be also noted that P-700[11] gives a weak absorption around 800 nm by light irradiation,[12,13] which might be ascribed to Chl a$^+$ or Chl a$^-$. Both Chl a$^+$ and Chl a$^-$ formed in the irradiated glasses show singlet esr spectra with line widths of about 10 G.

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Effects of Base Strength and Size upon Orientation in Base-Promoted Elimination Reactions

Sir:

The relative importance of base strength and size in determining orientation for olefin-forming elimination reactions has been a subject of controversy for nearly 2