Structure of the Primary Electron Donor in Photosystem I: A Resonance Raman Study

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ABSTRACT: Low-temperature resonance Raman (RR) spectra have been obtained at resonance with the Soret transition of chlorophyll *a* in photosystem I particles containing large amounts either of the triplet state of P_{700} or of its radical cation state. Subtracting these spectra from those of resting reaction centers yielded RR spectra of P_{700} in its neutral, ground state. These spectra arise from two distinct chlorophyll *a* molecules differing by the strengths of the bonding interactions assumed by their keto carbonyl groups, the stretching frequencies of which are found at 1655 and 1675 cm^{-1}. The present results rule out previous hypotheses that P_{700} might have consisted of a single, chemically modified chlorophyll *a* molecule. Neither of the bonding interactions assumed by the keto carbonyls of the P_{700} chlorophylls most probably involves chlorophyll–chlorophyll bridging through water molecules, as surmised in the so-called special pair models, but likely consists of H bonds with distinct protein sites. The magnesium atoms of the two P_{700} chlorophylls are 5-coordinated. Hence, the structural model of P_{700} provided by the present data is qualitatively the same, in terms of bonding interactions, as that currently accepted for the bacterial primary donor.

Because of their obvious relevance to the understanding of the mechanisms of the primary, photoinduced charge separation, the structures of the primary electron donors in photosynthetic systems have been the subject of much interest in recent years.

In photosystem I of oxygenic photosynthetic organisms, the primary step of the photoinduced electron transfer consists of a charge separation between a primary electron donor, P_{700}, and an electron acceptor, A_0, and both are probably chlorophyllic in nature (Nuijs et al., 1986). From A_0, the electron is rapidly transferred to a series of electron carriers with increasing redox potentials [for a recent review, see, e.g., Lagoutte and Mathis (1989)]. P_{700} has long been thought to consist of a pair of chlorophyll *a* (Chl[1] *a*) molecules in close mutual excitonic interaction (Norris et al., 1971; Philipson et al., 1972). More recently, the dimeric nature of P_{700} has been questioned on the basis of EPR (Wasielewski et al., 1981a) and ENDOR (O'Malley & Babcock, 1984) experiments. However, absorption experiments still support the dimer hypothesis (Den Blanken & Hoff, 1983; Ikegami & Itoh, 1988). The chemical nature of the P_{700} constituent molecule(s) also has been discussed by different groups (Wasielewski et al., 1981b; Maggiora & Maggiora, 1984; Watanabe et al., 1985; Senger et al., 1987).

Resonance Raman (RR) spectroscopy allows selective observations of the chlorin pigments present in photosynthetic structures and yields direct information about their chemical nature, their conformations, and their environmental interactions in the ground state [for recent reviews, see Lutz (1984), Lutz and Robert (1988), and Robert et al. (1989)]. Earlier RR experiments on particles enriched in photosystem I, particularly CP1 preparations, have permitted detailed studies of their constituent Chl *a* populations (Lutz et al., 1979). However, in these studies it was not possible to identify individual contributions from the P_{700} molecules due, in part, to their low concentration relative to that of bulk Chl *a* in CP1 preparations (typically 1:45). A similar, yet less difficult, situation was recently overcome in resonance Raman studies of the bacterial reaction center. Pump–probe difference methods were devised, which allowed selective observations of the Raman scattering of the primary donor molecules in these proteins and permitted precise descriptions of their conformations and interaction states (Robert et al., 1985; Robert & Lutz, 1986, 1988; Robert, 1987; Zhou et al., 1987).

To obtain specific information on the nature and structure of P_{700}, we performed the same type of experiments on CP1, as well as on diethyl ether treated PS I particles (Ikegami & Katoh, 1975). These particles were preferred to more native preparations because of their relatively high P_{700}/Chl *a* ratios. From electronic and paramagnetic spectroscopic studies (Sétif et al., 1981a, 1982; Den Blanken & Hoff, 1983; Breton & Ikegami, 1989), it is clear that, in these particles, the structure of P_{700} is not significantly altered. In this paper we report the first selective resonance Raman observations of P_{700}, in a native, functional state. From these spectra, it unambiguously appears that two inequivalent Chl *a* molecules constitute the P_{700} structure. A preliminary account of this work has been given in Moënne-Loccoz et al. (1989a).

Experimental Procedures

Materials. CP1 particles were prepared from fresh spinach leaves according to the procedure described in Sétif et al. (1980). According to this method, thylakoid membrane fragments are solubilized with sodium dodecyl sulfate (SDS) by using a SDS:Chl ratio (w/w) of 10:1; CP1 complexes were then purified by using preparative polyacrylamide gel electrophoresis [0.1% SDS, 15% acrylamide, acrylamide:bis(acrylamide) ratio = 120] and dialyzed for 48 h against 50 mM Tris-HCl buffer at pH 8.0. For RR experiments, CP1 particles were concentrated to ca. 0.2 mg of chlorophyll/mL by using a Centricon (Amincon) system. The carotenoid:P_{700}

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[1] Abbreviations: Chl, chlorophyll; PS I, photosystem I; P_{700}, primary donor of photosystem 1 reaction center; RC, reaction center; RR, resonance Raman; Tris, tris(hydroxymethyl)aminomethane.

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and Chl:P_{700} ratios in these CP1 particles were ca. 6 and 45, respectively.

Diethyl ether treated PS I particles were prepared as described previously (Ikegami & Katoh, 1975; Ikegami, 1976): PS I particles, prepared by digitonin treatment of spinach chloroplasts, were lyophilized and then extracted twice with diethyl ether containing water in 80% saturation. The so-enriched PS I particles used during this work had a typical Chl *a*:P_{700} ratio of 11.

Spectroscopy. Resonance Raman experiments were conducted on a Jobin Yvon spectrometer (Ramanor HG2S-UV) using 441.6-nm excitation from a CW helium–cadmium laser (Liconix Model 4050). This excitation wavelength has proven effective for selectively promoting resonance of the RR scattering of Chl *a* in chloroplasts and chlorophyll–protein complexes (Lutz, 1977; Lutz et al., 1979). This wavelength thus also appeared likely to promote resonant scattering of P_{700} through coupling with its major 431-nm transition (Ke, 1973). During RR experiments, the sample temperature was kept at ca. 15 K by a flow of cold gaseous helium. Grazing incidence of the excitation laser beam was used to prevent reabsorption of the Raman photons by the sample. In these conditions, and with laser radiant power weaker than 20 mW, ca. 1 mW penetrated the sample. The spot of the laser beam on the frozen sample was carefully unfocused until its size reached ca. 10 mm^2. Spectral resolution at 1000 cm^{-1} was 8 cm^{-1}. Each RR spectrum presented here resulted from the summation of 10–100 individual spectra added in a multichannel analyzer (Tracor Northern 1710).

Methods. Raman scattering of chlorophylls obtained at resonance with the Soret transitions exhibits high cross sections, ensuring selective observations of these molecules in complex media (Lutz, 1974, 1977, 1984). In such resonance conditions these spectra readily reveal the intermolecular binding states of both the conjugated carbonyl(s) and the central Mg atom. A problem in studying chlorophyll–protein complexes in these conditions is that limited selectivity is obtained on the contributions of the various chlorin pigments which can be present in the sample. We recently developed difference methods permitting selective RR observations of bleachable chlorins in their ground, neutral states (Lutz & Robert, 1985; Robert & Lutz, 1986). These methods involve control of the dynamic equilibria built up during continuous illumination of photoactive complexes, and they have been successfully applied to the study of the primary donors in reaction centers of various strains of Rhodospirillales (Robert & Lutz, 1986; Zhou et al., 1987, 1989). In CP1 particles, as well as in diethyl ether treated PS I in the presence of ascorbate, the primary steps of electron transfer result in the formation of a triplet state by charge recombination involving Chl *a* molecule(s), named ^3P. At 10 K, the lifetimes of ^3P are 800 μs and 1 ms in CP1 particles and in diethyl ether treated PS I, respectively (Sētif et al., 1981b; Ikegami et al., 1987). These lifetimes allow easy control of the amount of the ^3P species in the probed volume by varying the laser irradiance at the sample from about 1 photon to 100 photons per second per P_{700} (Robert & Lutz, 1986). Our previous experiments on bacterial reaction centers indicated that at resonance with the Soret bands of the bacteriochlorins the RR scattering of the triplet-state P^R was undetectably small (Robert & Lutz, 1986; Zhou et al., 1989). This apparently low RR scattering cross section of the triplet state might partly originate from a lower extinction coefficient at the probe wavelength. For this reason, and since the extinction coefficient of state $^3P_{700}$ at 441.6 nm is smaller than that of the ground state (Den Blanken & Hoff, 1983), its contribution in the RR spectra excited at this wavelength may be expected to be small. Hence, differences between RR spectra obtained at low and high irradiance from CP1 and/or diethyl ether treated PS I particles should primarily arise from the neutral, ground states of the molecules that can reach the triplet state.

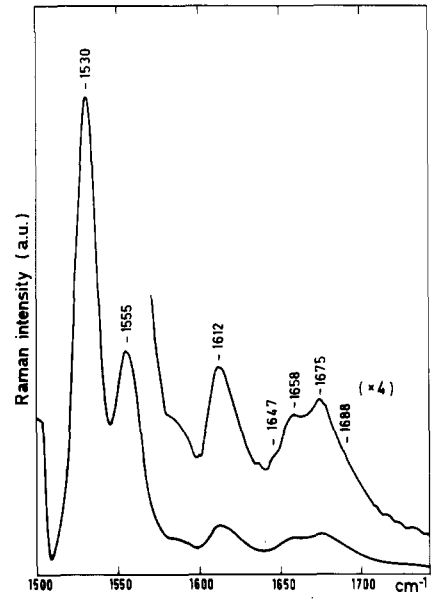

FIGURE 1: Resonance Raman spectra (1500–1740-cm^{-1} region) of untreated CP1 particles from spinach at 15K. Excitation wavelength: 441.6 nm. Spectral resolution: 5 cm^{-1} (sum of 35 scans).

We also conducted experiments on CP1 particles (200 μg of Chl mL^{-1}) that had been treated with 200 μM ferricyanide at room temperature to ensure chemical oxidation of P_{700}. The P_{700}^+ radical which is formed in these conditions also has a lower extinction coefficient at 441.6 nm than the neutral species (Ke, 1973). For the same reasons as above (Zhou et al., 1989) it is also expected to be a poorer scatterer than the latter species under these conditions.

Results

A wealth of experimental and theoretical data [reviews: Katz et al. (1978) and Lutz and Robert (1988)] has shown that the key information permitting a diagnosis of chlorophyll states in its native environment consists of the ligation of its magnesium atom and of the conformations and interaction states of the conjugated carbonyls.

Resonance Raman spectra of CP1 particles excited at 441.6 nm contain two strong bands in the 1500–1750-cm^{-1} range (Figure 1). A 1530-cm^{-1} band arises from the stretching modes of C=C bonds of β-carotene (Rimai et al., 1973; Saito & Tasumi, 1983). A 1555-cm^{-1} band, arising from chlorophyll *a*, has been assigned to a mode primarily involving C=C stretching (Lutz, 1977; Fujiwara & Tasumi, 1986). A weaker band at 1615 cm^{-1} essentially arises from the stretching modes of the methine bridges of Chl *a* (Lutz, 1977). Both of the latter bands have been shown to be sensitive to the number of external ligands on the central Mg of the Chl molecules, being observed at ca. 1545 and 1600 cm^{-1} when this atom binds two external ligands and at 1555 and 1615 cm^{-1} when it binds a single axial ligand, respectively (Fujiwara & Tasumi, 1986). The frequencies of the 1555- and 1615-cm^{-1} bands in CP1 spectra indicate that most if not all of the Chl molecules present in CP1 particles have a singly ligated central Mg. The same conclusion had been obtained by Lutz and co-workers (1979) from the observation of the lower frequency range of RR spectra of spinach CP1 particles in the same conditions

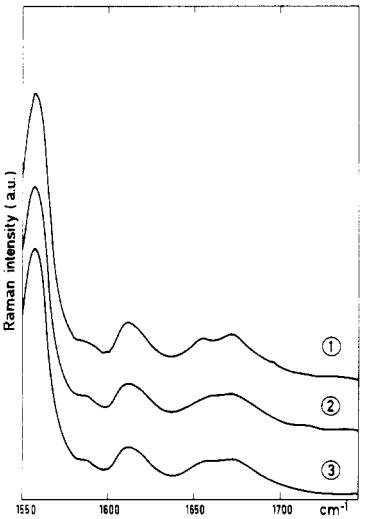

FIGURE 2: Resonance Raman spectra (1540–1740-cm^{-1} region) of spinach CP1 particles. (1) Untreated, low irradiance (see text); (2) untreated, high irradiance (see text); (3) ferricyanide treated, low irradiance. For other experimental conditions, see Figure 1.

of excitation. The 1640–1710-cm^{-1} region contains bands arising from the stretching modes of the 9-keto carbonyls (Lutz, 1977, 1984; Lutz & Robert, 1988) of Chl *a*. The frequency of this mode is sensitive to the nature and strength of intermolecular interactions assumed by the C=O group. As observed previously (Lutz et al., 1979), RR spectra of CP1 particles contain a broad cluster of bands in this spectral region (Figure 1).

Spectrum 1 of Figure 2 displays a RR spectrum of CP1 particles (1545–1740-cm^{-1} region) recorded at a low irradiance level (ca. 100 photons per second per reaction center). In these conditions of irradiation, no significant variations in RR spectra can be observed when the radiant power of the laser beam reaching the sample is decreased or increased by a factor of 2. Nevertheless, a slow evolution of the RR spectra could be observed, resulting in a sizable modification of the signal after more than 30 min of continuous irradiation. This evolution was most likely due to irreversible charge separation occurring with a low probability in CP1 particles, which has already been described by other groups (Hoarau et al., 1977; Sétif, 1984). To avoid any contribution from this phenomenon in the present RR experiments, the laser spot was moved to another sample site every 15 min of irradiation, well before the modifications are observable in the spectra. Spectrum 2 displays the same 1545–1740-cm^{-1} spectral range, recorded at a ca. 100 times higher irradiance: under these conditions, a partial, reversible bleaching of the Chl contributions relative to the carotenoid contributions is observed. This bleaching represents ca. 15% of the intensity of the total Chl contribution as measured, e.g., with the 1555-cm^{-1} band. Returning to low irradiance conditions on the same sample area restored the initially observed spectrum, thus demonstrating the complete reversibility of this bleaching in these conditions (cf. Figure 3, spectrum 3).

Spectrum 3 of Figure 2 displays a RR spectrum (1545–1740 cm^{-1}) of ferricyanide-treated CP1 particles using the low-irradiance conditions. When CP1 particles are treated with potassium ferricyanide, their RR spectra are very similar to spectrum 2 of Figure 2. No further modification of these RR spectra can be observed when the irradiance at the sample is varied in the range used for these experiments (data not shown).

Spectrum 2 of Figure 3 displays a computed difference between RR spectra 1 and 2 of Figure 2, i.e., between RR spectra of untreated CP1 particles obtained at low and high

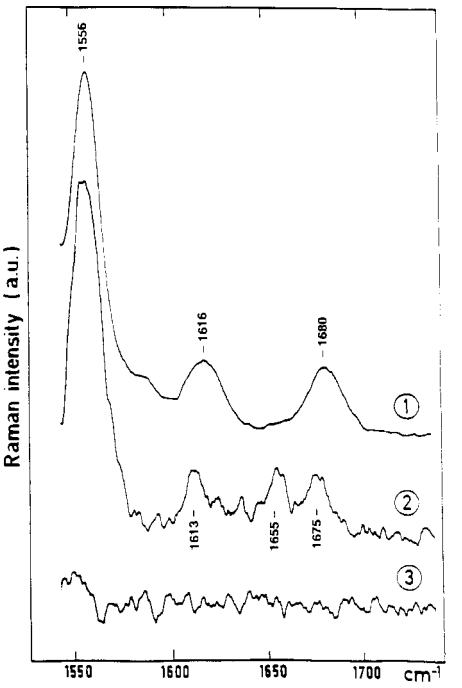

FIGURE 3: High-frequency region (1540–1740 cm^{-1}) of (1) RR spectrum of monomeric chlorophyll *a* dissolved in acetone; (2) difference RR spectrum of CP1 particles, low-irradiance minus high-irradiance conditions; and (3) difference of two low-irradiance RR spectra of CP1 particles recorded before and after the recording of a high-irradiance spectrum (see text for details). For other experimental conditions, see Figure 1.

irradiance, respectively. In this computation, the spectra have been normalized by using the strong 1530-cm^{-1} carotenoid Raman band as a standard. The use of this band as a reference is justified, because the yield of formation of carotenoid triplet in CP1 particles at 15 K is very low as compared to that of formation of ^{3}P (90%) (Sétif et al., 1981b). The difference spectrum 2 of Figure 3 consists of positive contributions only, located at 1555, 1613, 1655, and 1675 cm^{-1}. This spectrum arises from Chl *a* molecules in their neutral, ground state, as shown by its close similarity with RR spectra of Chl *a* (Figure 3, spectrum 1; Lutz, 1977).

The complete reversibility of this light-induced bleaching in the RR spectra of CP1 is demonstrated by spectrum 3 of Figure 3. This difference spectrum was calculated from RR spectra recorded under low-irradiance conditions prior to and immediately after the recording of a high-irradiance spectrum at the same sample site and in the same conditions as those used in recording spectrum 2 of Figure 2. The laser spot was moved to another site every 15 min, the time required to record the above sequence of spectra. The trace of spectrum 3 of Figure 3 is flat, thus demonstrating the reversibility of the features displayed in spectrum 2 of Figure 3.

Spectrum 2 of Figure 4 displays a difference RR spectrum between untreated and potassium ferricyanide treated CP1 particles under the same low-irradiance conditions and using the same 1530-cm^{-1} band for normalizing the spectra. This difference spectrum is very similar to spectrum 1 of Figure 4, exhibiting no negative band, and the same number of positive bands, at the same frequencies.

Spectrum 3 of Figure 4 displays a computed difference between RR spectra of ascorbate (final concentration 10 mM) and diethyl ether treated PS I particles obtained at low and high irradiance, respectively. Although of lower signal to noise ratio than the preceding ones, this difference spectrum is again very similar to spectrum 1 of Figure 4, exhibiting no negative Raman band, and the same number of positive bands, at the

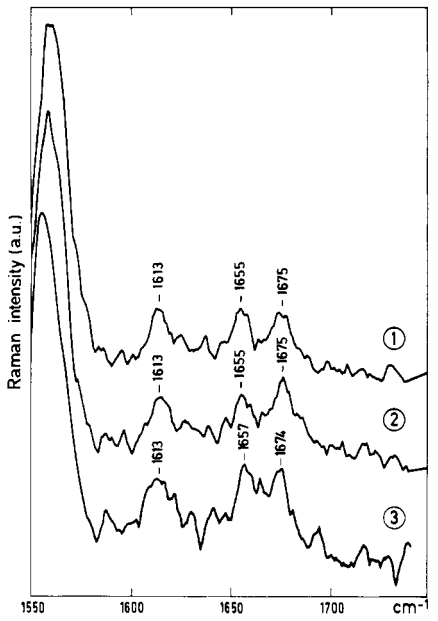

FIGURE 4: Difference resonance Raman spectra (1540–1740 cm^{-1}): (1) CP1 low-irradiance minus high-irradiance spectra (same as spectrum 2 of Figure 3); (2) CP1 low-irradiance minus ferricyanide-treated spectra; (3) diethyl ether treated PS I particles (10 mM ascorbate added), low irradiance minus high irradiance. For other experimental conditions, see Figure 1.

same frequencies within experimental uncertainty.

Changing the excitation wavelength from 441.6 to 413 nm tunes resonance with the B_Y, rather than the B_X, transition of the Soret band of chlorophyll *a*. This did not result in any qualitative change in the difference RR spectra (data not shown).

Discussion

Difference resonance Raman spectra obtained in the 1545–1740-cm^{-1} region from spinach CP1 and PS I particles do not contain any negative bands (Figure 4). Hence, they arise from molecules in their neutral, ground states only and do not involve any contributions from the excited and/or radical states created by illumination or ferricyanide treatment. These molecules in their neutral, ground states can be safely identified as Chl *a*, on the basis of the number of bands observed and of their frequencies and relative intensities (Figure 3, spectrum 1; Lutz, 1974, 1977, 1984; Lutz & Robert, 1988). The presence of two distinct bands at 1655 and 1675 cm^{-1}, which can only arise from the stretching modes of the 9-keto carbonyls, unambiguously indicates that two, and only two, distinct populations of Chl *a* contribute to these difference spectra. Indeed, the width of both bands (13 cm^{-1}) is exactly that expected for the carbonyl stretching bands of chlorophylls in uniquely defined environments such as those provided by protein binding sites (Robert & Lutz, 1986; Moënne-Loccoz et al., 1989b). This width is actually smaller than that observed for monomeric Chl *a* in organic solution at low temperature (Figure 3, spectrum 1; Lutz, 1977).

In these difference experiments, neutral P_{700} is the only molecular species whose concentration is expected to decrease through the formation of P_{700}^+ in the presence of ferricyanide and to reversibly decrease through the photoaccumulation of $^3P_{700}$ in high-irradiance experiments. Possible artifacts of such experiments on the bacterial reaction center have been discussed by Robert and Lutz (1986), who concluded that the primary donor alone was contributing in the difference spectra. Their conclusions about its structure agreed extremely well with the refined models drawn from X-ray crystallographic data (Zhou et al., 1989). The same discussion as that presented in Robert and Lutz (1986) applies to the present difference spectra and permits us to conclude that they most probably arise from P_{700} alone. In particular, it appears unlikely that one of the two molecules observed to bleach in these difference Raman spectra might do so because of it being electronically coupled with P_{700} or because of sizable perturbation of its Soret levels upon formation of both the cation and triplet states of P_{700} (this molecule, for instance might be the primary acceptor A_0). Indeed, in the bacterial RC, none of the accessory bacteriochlorophylls, although most likely electronically coupled with the primary donor (Parson et al., 1985), appeared to undergo any detectable change in its RR cross section when the P^+ or ^3P states were formed (Robert & Lutz, 1986). Hence, strong electronic coupling with the bleached species is probably necessary to induce any detectable changes in the RR spectrum of an adjacent molecule under our experimental conditions. No such couplings are expected between P_{700} and any chlorophyllic species of the reaction center, and hence the spectra of Figure 4 should arise from P_{700} alone.

It also might be argued that difference RR spectra 1 and 2 of Figure 4 could, at least partly, arise from selective effects of illumination and of ferricyanide treatment on certain of the ca. 45 antenna chlorophylls present in CP1 preparations, respectively. However, the close similarity of these two difference spectra, which were obtained under markedly different conditions, as well as their very close similarity with those obtained with ether-treated PS I particles, in which the antenna Chl:P_{700} ratio is only 11, indicates that such an origin is very unprobable. We thus identify the two Chl *a* molecules present in the difference RR spectra of Figure 4 with those constituting the primary electron donor (P_{700}) of photosystem I and conclude that neutral P_{700} consists of a pair of Chl *a* molecules.

Recently, Tavitian et al. (1986) conducted $P_{700}^+ - P_{700}$ FTIR difference spectroscopy on PS I particles. These authors observed a large bleaching at 1700 cm^{-1} upon P_{700}^+ cation formation and tentatively assigned this bleached band to the stretching mode of the 9-keto carbonyl of Chl *a* molecule(s) constituting P_{700}. However, no sizable contribution is observed at this frequency in the difference RR spectra reported here. Because of the high selectivity afforded by the resonance Raman technique, the latter spectra involve only two populations of molecules belonging to a single chemical species, chlorophyll *a*. Because no such selectivity is involved in difference IR spectra, we suggest that the 1700-cm^{-1} infrared signal should actually originate, at least in part, from contributions other than those of chlorophylls. For instance, it might be due to slight perturbations of the protein structure surrounding the first electron carriers, which might be induced by the oxidation of P_{700}, similarly to recent RR observations on bacterial reaction centers (Robert & Lutz, 1988).

Several authors have proposed that in the triplet and/or radical cation state of P_{700} the unpaired electron and/or hole should be more or less localized on one of the chlorophyll molecules (Rutherford & Mullet, 1981; Den Blanken & Hoff, 1983; Wasielewski et al., 1981a; O'Malley & Babcock, 1984). The present results indicate that in both these states the asymmetry of the charge repartition must be limited, since two molecules are "Raman bleached" in these experiments. It cannot be totally excluded that one of these molecules remains in the ground or neutral state but is Raman-bleached because of perturbations of its Soret levels by the charge or unpaired electron located on the other molecule. However, such perturbations would manifest themselves in the Raman spectrum

by more complex changes rather than by a mere weakening of all the bands. The involvement of two molecules in the present difference RR spectra leads, in any case, to the conclusion that P_{700} consists of two closely spaced Chl *a* molecules.

A number of chemical variants of chlorophyll *a* have been proposed to occur in P_{700}. If the existence of 10-hydroxy-δ-chlorochlorophyll *a* in PS I preparations has been proven to be artifactual (Senge et al., 1988), other proposals still have not received unambiguous experimental testing. The present RR spectra of P_{700} permit this testing for some of them.

The fact that P_{700} is actually observed to involve two chlorophyll *a* molecules rather than a single one weakens, if not rules out, alternative hypotheses according to which its electronic and/or magnetic properties should result from chemical particularities of a monomer (Maggiora & Maggiora, 1984; Wasielewski et al., 1981b; Senger et al., 1987).

Wasielewski and co-workers (Wasielewski et al., 1981b) have proposed that P_{700} should consist of a C9-enol form of Chl *a*, which might occur either in an excited, transient state preceding the oxidation of P_{700} or in the ground state as well. The enolization of a C9=O group of a monomeric Chl *a* in the neutral, ground state should result in the vanishing of the C=O stretching RR band from the 1640–1700-cm^{-1} region of its replacement by modes of the C6C10C9–OH group, none of which is expected to fall in this range (Colthup et al., 1975). On the other hand, as discussed by Heald et al. (1988), the increased π character of the C9–C10 bond involved in the enol form should result in partial conjugation of the C10=O ester carbonyl, which in turn might result in RR activity of its stretching mode, conceivably in the 1640–1700-cm^{-1} range. Hence, a monomer of a stable, C9-enolic form of Chl *a* should yield, at best, a single band in the 1640–1700-cm^{-1} region of RR spectra. P_{700} displays two RR-active modes in this range; thus it is not a monomer of a C9-enolic Chl *a*.

The present RR data (Figure 4, spectrum 2) also indicate that the P_{700}^+ state involves two Chl *a* molecules. This, together with recent interpretations of $P_{700}^+ - P_{700}$ difference FTIR spectra (Nabedryk et al., personal communication), makes improbable the fact that P_{700}^+ may involve an enolic Chl *a*, which hence might only occur as a transitory form preceding the ionization of P_{700} with a lifetime of less than 14 ps (Wasielewski et al., 1987).

From chromatographic analyses of the pigments present in various subchloroplast preparations, Kobayashi et al. (1988) proposed that a C10 epimer of Chl *a* is present in the PS I reaction center. This isomerization of Chl *a*, which involves a largely nonconjugated part of the molecule, is expected to have only minor effects on its RR spectra. To check whether this molecule is involved in P_{700} structure, comparisons of RR spectra of Chl *a* and Chl *a'*, as well as the careful recording of specific spectral regions of P_{700}, are required.

Noting that chlorophyll complexes with bifunctional ligands such as water may result in large red shifts of the Q_Y transition, and may present some photoactivity, several authors, and in particular the Argonne group [review: Katz et al. (1978)], have proposed that P_{700} might consist of a Chl *a* dimer bridged by one or two water molecules or other small bifunctional molecules. Vibrational spectroscopy (Ballschmiter & Katz, 1968, 1972; Lutz, 1974, 1977, 1984) as well as X-ray crystallography (Chow et al., 1975) has shown that hydrated oligomers of Chl *a* primarily involve the magnesium atom and the 9-keto carbonyl through C=O··H(H)O··Mg bonds. On this basis, unsymmetrical as well as symmetrical (C2) models have been proposed for P_{700}, involving one or two such bonds, respectively [low-symmetry type, see Ballschmiter and Katz (1968), Katz and Norris (1973), and Katz et al. (1978); C2 type, see Boxer and Closs (1976) and Shipman et al. (1976)]. Our RR data, which demonstrate inequivalent interactions on the 9-keto groups of the P_{700} pair, readily exclude the C2-type models.

The unsymmetrical models of P_{700} involving water can be rejected as well, considering the stretching frequencies of the C9=O groups observed for P_{700}. All of the hydrated oligomers of Chl *a* studied by IR or RR spectroscopy presented an unusually low keto carbonyl stretching frequency at 1640 cm^{-1} (Ballschmiter & Katz, 1968, 1969, 1972; Boxer & Closs, 1976; Lutz, 1974, 1977; Koyama et al., 1986). Ballschmiter and Katz (1969) attributed this large downshift occurring upon H-bond formation to a polarization of the water OH bond by the Mg atom of the partner Chl *a* binding the water oxygen atom. This 1640-cm^{-1} wavenumber value is thus characteristic of the C=O··H(H)O··Mg bonding, independent of the actual structure of the dimer or oligomer. The lowest of the two carbonyl stretching frequencies of P_{700}, 1655 cm^{-1}, is well over this value, hence excluding bridging of the two chlorophylls by water, at the level of any of their keto carbonyls.

In addition, the formation of the water aggregates resulted in very large intensity changes and wavenumber shifts in RR spectra of Chl *a* (Lutz, 1974, 1977, 1984; Koyama et al., 1986). In particular, the relative intensity of the 1555-cm^{-1} band was 0.12 that of the monomer, the relative intensity of the 1615-cm^{-1} band was increased, and an upshifted, enhanced band arose at 1596 cm^{-1}. None of these features is observed in RR spectra of P_{700}, in which the relative intensities of the 1555- and 1615-cm^{-1} carbonyl bands are comparable to those of monomeric Chl *a*.

This discussion leaves open the possibility that bridging between the two P_{700} chlorophylls involving their keto carbonyls and Mg atoms might occur via molecule(s) other than water, e.g., amino acid side chains, as suggested by Shipman et al. (1976). However, it may be expected that if the inductive effects that we have mentioned above occur for the C=O··H(H)O··Mg system, they should also be transmitted through any other bifunctional protein group (Sagarik & Rode, 1981) and should also result in unusually low keto C=O stretching frequencies, which is not observed.

RR spectra of P_{700} contain two skeletal bands at 1555 and 1613 cm^{-1}. As mentioned above (cf. Results), these two wavenumber values are typical of Chl *a* molecules with 5-coordinated magnesium atoms. The half-bandwidths of these 1555- and 1613-cm^{-1} bands are 20 and 10 cm^{-1}, respectively. Both these values are not higher, and are actually slightly smaller, than those observed under similar conditions for monomeric Chl *a* in solution (Lutz, 1977). This demonstrates that both of the P_{700} chlorophylls have a single axial ligand on their respective magnesium atoms. Hence, if any molecule is bridging them via their Mg and keto groups, they cannot form any additional bond with the protein via their Mg atoms.

Because the stretching modes of the keto carbonyls of both P_{700} chlorophylls fall in the range generally observed for monomeric, H-bonded Chl *a* (Lutz, 1984), we actually favor the hypothesis that P_{700}, as the bacterial primary electron donor (Robert & Lutz, 1986; Michel et al., 1986), consists of two closely spaced but not interbonded Chl *a* molecules. Moreover, we recently proposed that a significant homology might exist between α helices VI (Fish et al., 1985) of the psa A and psa B subunits of PS I on one hand and α helices B of the L and M subunits of bacterial RCs and helices IV of the D1 and D2 subunits of PS II on the other (Robert & Moënne-Loccoz, 1989). This homology actually may extend further and in-

dicates that histidines psa A 535 and psa B 521 of PS I are likely homologous to histidines L 173 and M 200 of the bacterial RC (*Rps. viridis* numbering). We thus propose that histidines psa A 535 and psa B 521 each bind the magnesium atom of one of the two P_{700} chlorophylls.

In this picture, the keto carbonyls of the P_{700} chlorophylls should each bind another protein site, likely through H bonds. Badger-type calculations (Zadorozhnyi & Ishchenko, 1965) indicate that the carbonyl vibrating at 1655 cm^{-1} should be engaged in an approximately 7 kcal/mol bond, while that vibrating at 1675 cm^{-1} should be engaged in a weaker, 4 kcal/mol, bond. We also note that these two frequencies are close to, although not identical with, the 1660–1665- and 1680–1685-cm^{-1} values observed for the bonded keto carbonyls of the two BChl molecules constituting the bacterial primary electron donor (Robert & Lutz, 1986; Zhou et al., 1987, 1989). As mentioned above, it appears extremely likely that all the features of P_{700} which we describe here from our observations on CP1 and ether-treated particles are genuine to the native structure, inasmuch as previous spectroscopic studies have shown that the preparation of these particles does not significantly alter the structure of P_{700} (Sêtif et al., 1981a, 1982; Den Blanken & Hoff, 1983; Breton & Ikegami, 1989).

In summary, RR spectra of P_{700} demonstrate that it is constituted of a pair of chlorophyll *a* molecules which are involved in inequivalent bonding interactions through their keto carbonyls. These interactions most probably do not involve Chl–Chl bridging through water molecule(s) or other small bifunctional groups, as surmised in the so-called special pair models, but most likely consist of H bonds with the protein. The magnesium atoms of these two molecules each bind a single axial ligand, possibly the histidines psa A 535 and psa B 521.

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CORRECTIONS

Exchange and Flip-Flop of Dimyristoylphosphatidylcholine in Liquid-Crystalline, Gel, and Two-Component, Two-Phase Large Unilamellar Vesicles, by William C. Wimley and T. E. Thompson*, Volume 29, Number 5, February 6, 1990, pages 1296–1303.

Page 1298. Equation 2 should read

$$^3\bar{H}_d(t) = \left[1 - \frac{k_2k_4D}{k_1k_3A + k_2k_4D}\right] \times \exp\left[-\frac{f(k_1k_3A + k_2k_4D)t}{k_2D + k_3A}\right] + \frac{k_2k_4D}{k_1k_3A + k_2k_4D} \quad (2)$$

Mechanism of Spontaneous, Concentration-Dependent Phospholipid Transfer between Bilayers, by Jeffrey D. Jones and T. E. Thompson*, Volume 29, Number 6, February 13, 1990, pages 1593–1600.

Page 1596. Equations 4 and 5 should read

$$k_1 = D_m/l_b^2 \exp(-\Delta G_d^{\ddagger\circ}/RT) \quad (4)$$

$$\ln k_1 = \frac{-\Delta H_d^{\ddagger\circ}}{RT} + \frac{\Delta S_d^{\ddagger\circ}}{R} + \ln [D_m/l_b^2] \quad (5)$$