investigation in eukaryotes. It may be also relevant for drug testing. We have previously suggested that the archaebacterial plasmid pGRB-1 could be useful in the prescreening of antitumoral drugs active on DNA topoisomerases II, because topological changes induced by VP16 in this plasmid are easy to monitor (Sioud et al., 1987b). Our new finding indicates that this system could be used at the same time to look for new antibiotics active against bacterial DNA gyrase. The main advantage of such in vivo prescreening would be the identification of topoisomerase inhibitors in complex mixtures of natural products containing nuclease activities preventing the testing of topoisomerase activities in vitro.

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Registry No. Ciprofloxacin, 85721-33-1; etoposide, 33419-42-0; DNA topoisomerase, 80449-01-0; quinolone, 13721-01-2; epipodophyllotoxins, 4375-07-9.

REFERENCES

Chen, G. L., Yang, L., Rowe, T. C., Halligan, B. D., Tewey, K. M., & Liu, L. F. (1984) *J. Biol. Chem. 259*, 13560-13566.
Drlica, K., & Franco, R. J. (1988) *Biochemistry 27*, 2253-2259.
Fisher, L. M., Mizuuchi, K., O'Dea, M. H., Ohmori, H., & Gellert, M. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4165-4169.
Fisher, L. M., Barot, H. A., & Cullen, M. E. (1986) *EMBO J. 5*, 1411-1418.
Forterre, P., Nadal, M., Elie, C., Mirambeau, G., Jaxel, C., & Duguet, M. (1986) *Syst. Appl. Microbiol. 9*, 77-82.
Franco, R. J., & Drlica, K. (1988) *J. Mol. Biol. 201*, 229-233.
Gellert, M., O'Dea, M. H., Itoh, T., & Tomizawa, J. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 4474-4478.
Gellert, M., Mizuuchi, K., O'Dea, M. H., Itoh, T., & Tomizawa, j. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4772-4776.
Hsieh, T., & Brutlag, D. (1980) *Cell (Cambridge, Mass.) 21*, 115-125.
Hussy, P., Maass, G., Tummler, B., Grosse, F., & Schomburg, U. (1986) *Antimicrob. Agents Chemother. 29*, 1073-1078.
Marshall, B., Darkin, S. J., & Ralph, R. (1983) *FEBS Lett. 161*, 75-78.
Maxwell, A., & Gellert, M. (1986) *Adv. Protein Chem. 38*, 69-107.
Nelson, E. M., Tewey, K. M., & Liu, L. F. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 1361-1365.
Ross, W., Rowe, T., Glisson, B., Yalowitz, J., & Liu, L. F. (1984) *Cancer Res. 44*, 5857-5860.
Sioud, M., Baldacci, G., Forterre, P., & De Recondo, A. M. (1987a) *Eur. J. Biochem. 169*, 231-236.
Sioud, M., Baldacci, G., Forterre, P., & De Recondo, A. M. (1987b) *Nucleic Acids Res. 15*, 8217-8234.
Sioud, M., Possot, O., Elie, C., Sibold, L., & Forterre, P. (1988a) *J. Bacteriol. 170*, 946-953.
Sioud, M., Baldacci, G., De Recondo, A. M., & Forterre, P. (1988b) *Nucleic Acids Res. 16*, 1379-1391.
Snyder, M., & Drlica, K. (1979) *J. Mol. Biol. 131*, 287-302.
Sugino, A., Peebles, C., Kreuzer, K., & Cozzarelli, N. (1977) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4838-4842.
Tewey, K., Rowe, R., Yand, L., Nelson, E. M., & Liu, L. F. (1984) *Science (Washington, D.C.) 226*, 446-468.
Tindall, B. J., Ross, H. N. M., & Grant, W. D. (1984) *Syst. Appl. Microbiol. 5*, 41-57.
Vosberg, H. P. (1985) *Curr. Top. Microbiol. Immunol. 114*, 19-100.
Wang, J. C. (1985) *Annu. Rev. Biochem. 54*, 665-697.
Wang, J. C. (1987) *Biochim. Biophys. Acta 909*, 1-9.
Woese, C. R. (1987) *Microbiol. Rev. 51*, 221-271.
Yang, L., Rowe, T. C., Nelson, E. M., & Liu, L. F. (1985) *Cancer Res. 45*, 5872-5876.

A Resonance Raman Characterization of the Primary Electron Acceptor in Photosystem II

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ABSTRACT: Low-temperature resonance Raman spectra of D1/D2 particles from spinach excited at 406 and 413 nm contained enhanced contributions from pheophytin *a*. These contributions were partly photobleachable in dithionite-treated particles. Difference resonance Raman spectra calculated on this basis essentially arose from a single environmental population of neutral pheophytin *a*. The 9-keto carbonyls of these bleachable molecules vibrated at 1680 cm^{-1}, a frequency identical, within experimental uncertainty, with that of the 9-keto carbonyl of the acceptor bacteriopheophytin H_L in the reaction centers of *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides*. This constitutes strong evidence that the acceptor pheophytin of the PS II reaction center is H-bonded to the D1-130 glutamic residue, in a relative geometry and environment that must be very close to those of the H_L molecule and the L-104 glutamic residue in the reaction centers of the above two bacterial species.

In photosystem II (PS II) of oxygenic photosynthetic organisms, the sites of the primary electron-transfer steps have been shown to be located in two polypeptides of mass 39 and 39.5 kDa, named D1 and D2, respectively. Indeed, Nanba and Satoh (1986) recently isolated a photosystem II complex from spinach that only contained the D1 and D2 polypeptides

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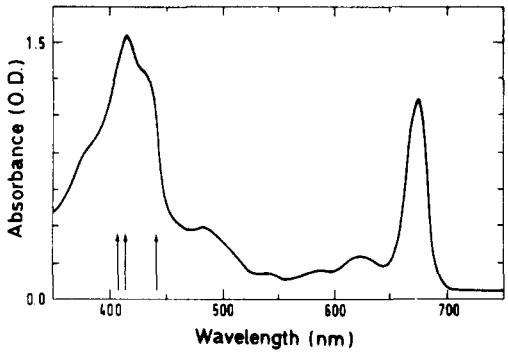

FIGURE 1: Electronic absorption spectrum of D1/D2 particles from spinach used in this study at room temperature. The arrows indicate the excitation wavelengths at which the resonance Raman spectra were obtained.

and cytochrome *b*-559 and that exhibited partial photoactivity. This complex was highly enriched in pheophytin, which is known to act as a primary electron acceptor in PS II (Klimov et al., 1977; Shuvalov et al., 1980), and a reversible reduction of about half of these pheophytins was observed in the presence of dithionite and methylviologen. The preparation contained four to six Chl *a*,[1] about one β-carotene, and one or two cytochrome *b*-559 per two pheophytin *a* molecules. The photochemistry of this preparation has been extensively studied during the last 2 years (Danielius et al., 1987; Takahashi et al., 1987; Van Dorssen et al., 1987; Newell et al., 1988; Wasielewski et al., 1989). Polypeptides D1 and D2 exhibit marked homologies with the L and M subunits of the photochemical reaction center of purple photosynthetic bacteria, respectively (Michel et al., 1986a). Moreover, the stoichiometry of the D1/D2 complexes appears to be similar to that observed in bacterial reaction centers. PS II also shares a number of functional similarities with bacterial reaction centers [for a review, see Robinson et al. (1988)]. These analogies prompted proposals of tentative models of the structure of the D1/D2 subunit, based on sequence homologies and on the crystallographic models obtained for bacterial reaction centers (Trebst, 1986; Michel & Deisenhofer, 1988; Robinson et al., 1988). However, direct structural information on the PS II reaction center is still rather scarce.

Resonance Raman (RR) spectroscopy has proven to be a powerful technique for studying interactions assumed in the ground state by chlorin-type pigments in vivo [for a review, see Lutz and Robert (1988), Robert et al. (1988), and Bocian et al. (1987)]. In this paper, we report the first selective observations of resonance Raman scattering of the pheophytin present in D1/D2 particles. From these results, it unambiguously appears that a single population of pheophytin *a* may be photochemically reduced in the presence of dithionite. Moreover, the molecular interaction assumed by the keto carbonyl group of this population of pheophytin is identical with that assumed by the keto carbonyl group of the acceptor bacteriopheophytins in bacterial reaction centers. Preliminary observations of RR spectra of D1/D2 pheophytin were reported previously (Moënne-Loccoz, 1987).

Experimental Procedures

Materials. The D1/D2–cytochrome *b*-559 complex was isolated from spinach following the procedure described in Nanba and Satoh (1986). Photoactivity in these complexes

[1] Abbreviations: Chl *a*, chlorophyll *a*; H_L, primary electron acceptor in bacterial reaction center; Pheo, pheophytin *a*; PS, photosystem; P_{680}, primary electron donor in PS II; RR, resonance Raman.

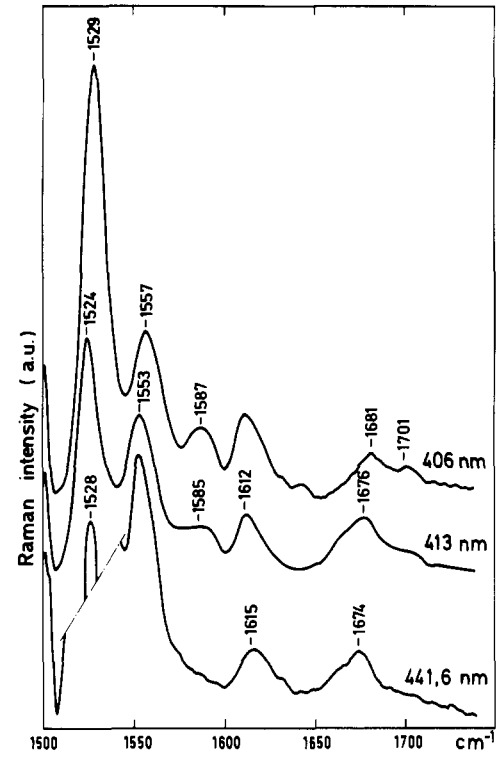

FIGURE 2: Higher wavenumber regions of resonance Raman spectra obtained from D1/D2 particles at 15 K, with excitations at 406, 413, and 441.6 nm [in the last spectrum the strong 1524-cm^{-1} band of the carotenoid has been truncated (peak intensity = $6I_{1553}$)]. Spectral resolution = 7 cm^{-1}.

rapidly disappears if the preparation is stored at temperatures higher than 4 °C or if it is submitted to freezing–thawing cycles. For the Raman experiments, D1/D2 particles were concentrated up to 0.2 mg/mL of Chl with a Centricon (Amincon) system. Room temperature absorption spectra of these particle were almost identical with those reported in Nanba and Satoh (1986) (Figure 1). Relative stoichiometries of the different prosthetic groups present in the preparation were comparable to those previously published (Nanba & Satoh, 1986).

Resonance Raman experiments were conducted on a Jobin Yvon spectrometer (Ramanor HG2S-UV). Excitations at 413 and 406 nm were provided by a krypton laser (Coherent Radiation Inc., Model Innova 90). Excitation at 441.6 nm was provided by a helium cadmium laser (Liconix, Model 4050). During RR experiments, the sample temperature was kept at 15 K by a flow of cold gaseous helium. Detailed description of the Raman apparatus and of the recording procedure of the resonance Raman spectra has been given in Robert and Lutz (1986).

Pheophytin *a* was prepared from chlorophyll *a* and purified and dried following conventional methods (Schwartz & Von Elbe, 1982).

Methods. The possibility of selective observation of pheophytins within D1/D2 particles, via resonance with their specific yellow electronic absorption bands, was prevented by the very strong resonance of β-carotene in these conditions.

The procedure used for accumulating sizable amounts of photoreduced pheophytin in D1/D2 particles was the following: dithionite-treated samples were put into the cryostat at 273 K and were then continuously illuminated during the cooling of the cryostat down to 15 K (ca. 10 min). This illumination was achieved by using the white emission of a 800-W tungsten filament lamp. Actual reduction of the pheophytin was con-

trolled by the weakening of the contributions of neutral pheophytin from RR spectra.

Results and Discussion

Figure 2 displays the higher frequency regions of RR spectra of D1/D2 particles recorded with 406-, 413-, and 441.6-nm excitations. According to Raman excitation profiles of chlorophylls in the Soret region (Lutz, 1974; Lutz & Robert, 1988), excitations at 406 and 413 nm are expected to induce stronger resonance of the pheophytins than those located on the red side of the Soret band of the chlorin pigments, as far as the Soret band of the pheophytin is observed in vitro at 408 nm (Goedheer, 1966). This effect is indeed observed in this set of spectra: A 1585-cm^{-1} band, which is mostly due to pheophytins (Lutz, 1979, 1984), is clearly present in spectra excited at 413 and 406 nm, while it is absent from the spectrum excited at 441.6 nm. Similar wavelength dependence is observed throughout the RR spectra of D1/D2 complexes, for any of the RR bands that are known to be characteristic of pheophytin *a*, e.g., at 350, 676, 988, and 1226 cm^{-1} (data not shown). Thus, it appears that ensuring resonance in the 406–413-nm range leads to RR spectra of D1/D2 particles that involve sizable contributions from the pheophytins contained in the preparation.

The 1640–1710-cm^{-1} region of RR spectra of chlorin pigments contains bands arising from the stretching modes of their conjugated keto carbonyl groups (Lutz, 1979). The wavenumbers of these modes have been shown to be sensitive to the nature and strength of the intermolecular interactions assumed by these groups. They occur at ca. 1700 cm^{-1} when the 9-keto carbonyls are free from intermolecular interactions and are shifted to any lower wavenumber down to 1640 cm^{-1} when the keto groups are interacting with their environment, e.g., through H-bonding. In RR spectra of D1/D2 particles, at any excitation wavelength, the overlapping bands arising from these modes in the various chlorin populations present constitute a complex, broad, unresolved cluster. However, comparison of the three spectra of Figure 2 shows that ensuring resonance on the short-wavelength side of the Soret transition of D1/D2 particles rather than on its long-wavelength side induces relative intensity changes within this cluster and, in particular, a reinforcement of the intensity of its components located around 1680–1700 cm^{-1}. However, because of possible modification of the resonance conditions of the different environmental populations of chlorophylls present in D1/D2 when the excitation wavelength is changed, a simple comparison of these spectra does not permit any firm conclusion to be drawn about the stretching frequency of the keto carbonyl groups of the pheophytins of D1/D2.

In order to unambiguously determine which were the contributions of pheophytin in RR spectra excited at 413 nm, we conducted photoaccumulation experiments on D1/D2 particles, involving stabilization of reduced pheophytin (Nanba & Satoh, 1986). Photoaccumulation of the pheophytin anion is expected to induce a weakening of RR contributions from the neutral species. Indeed, contributions from the anion species are expected to be much weaker than those of the neutral species, because excitation wavelengths chosen for these experiments should favor resonance of the neutral, ground-state pigments (Lutz, 1979). This phenomenon is indeed observed: Figure 3 displays RR spectra (413-nm excitation) of untreated D1/D2 particles (Figure 3, spectrum 1) and of D1/D2 particles in which the pheophytin anion has been preaccumulated by dithionite treatment and white light illumination (Figure 3, spectrum 2). The intensity of the 1585-cm^{-1} band, measured after both of these spectra are normalized with respect to the

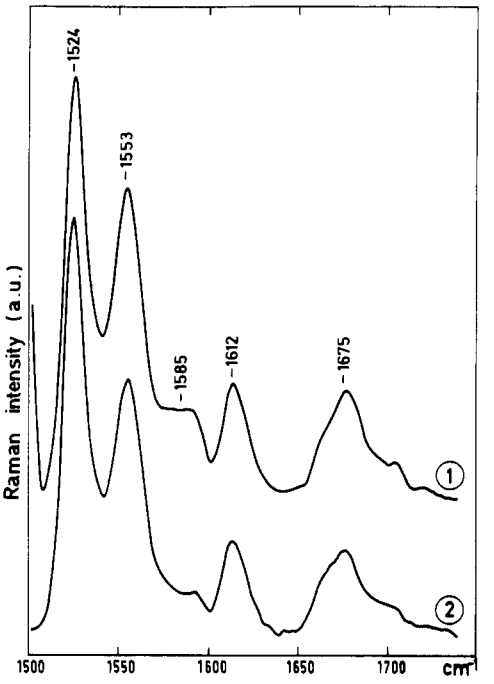

FIGURE 3: Higher wavenumber regions of resonance Raman spectra from D1/D2 particles at 15 K under 413-nm excitation. Spectrum 2 was obtained from dithionite-treated particles illuminated during cooling (see text). Spectrum 1 is from an untreated control.

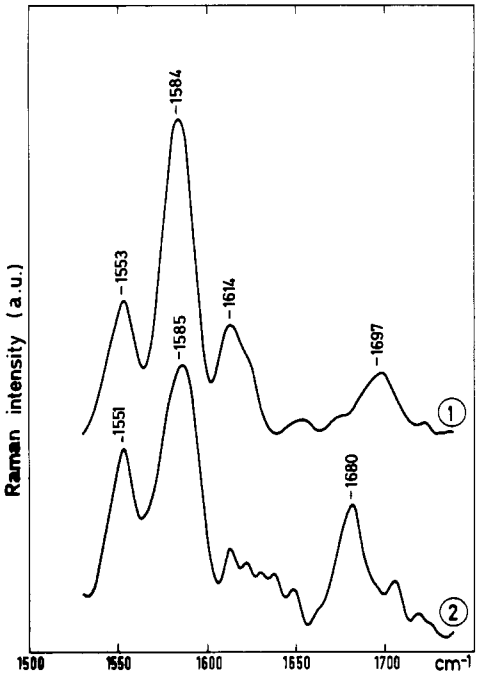

FIGURE 4: Higher wavenumber regions of resonance Raman spectra (413-nm excitation, sample at 15 K). Spectrum 1, isolated pheophytin *a* in CCl_4; spectrum 2, difference between spectrum 1 and spectrum 2 of Figure 3, with normalization on the 1524-cm^{-1} band of the carotenoid, overcompensated by 5% (see text). This difference spectrum has been smoothed by using a Savitsky–Golay algorithm.

intensity of the carotenoid contribution, which is expected to be largely insensitive to formation of the anion, has decreased by an approximate 2 factor in spectrum 2 of Figure 3, as compared to spectrum 1. This effect is observed for all of the RR bands arising from pheophytin. It is possible to compute a normalized difference between spectra 1 and 2 of Figure 3 in order to selectively obtain the contribution of this bleachable pheophytin. Figure 4, spectrum 2, displays such a difference

spectrum obtained with normalization on the 1524-cm^{-1} carotenoid band.[2] For comparison, Figure 4, spectrum 1, displays the 1530–1740-cm^{-1} frequency range of a RR spectrum of isolated pheophytin *a* dissolved in dry CCl_4. These two spectra are very similar, each yielding two major bands at 1553–1551 and 1584–1585 cm^{-1}. This confirms that neutral pheophytin only is sizably contributing in the difference spectrum. No negative contribution, which might have been attributed to the anion, is in particular observed in the 1550–1700-cm^{-1} region. It can be noticed, however, that the difference spectrum of Figure 4, spectrum 2, apparently lacks the medium-intensity component around 1614 cm^{-1} which is expected for pheophytin *a* at this excitation wavelength (Figure 4, spectrum 1). A possible origin for this peculiarity, for which we have no definite explanation, might be found in a specific environmental effect. Only one band is present in the carbonyl stretching region of the difference spectrum of Figure 4, spectrum 2. This band is located at 1680 cm^{-1} and must arise from a keto carbonyl group engaged in intermolecular interactions. Inasmuch as its half-bandwidth is 13 cm^{-1} only, i.e., it is equal to the smallest width observed for a keto stretching mode in the present conditions, it is very likely that it arises from a single environmental population of pheophytin within the D1/D2 complex. This shows, to a first approximation, that a single environmental population of pheophytin is brought to the anion state during preillumination.

It is worth noting that the wavenumber of the stretching mode of the keto carbonyl group of this population is the same, within 1 cm^{-1}, as that observed for the homologous chemical grouping of the acceptor bacteriopheophytin H_L in bacterial reaction centers—1679 cm^{-1} [*Rh. sphaeroides* (Lutz, 1980); *Rps. viridis* (Q. Zhou, B. Robert, and M. Lutz, unpublished results)]. From the present results it is thus possible to conclude that the acceptor pheophytin in PS II has a keto carbonyl group assuming a well-defined intermolecular interaction of exactly the same strength as that of the acceptor bacteriopheophytin *a* or *b* in bacterial RCs (H_L). This conclusion permits the following points to be discussed: (i) the degree of structural homology between PS II and bacterial RCs and (ii) the functional role of the partner amino acid of the keto carbonyl group of the acceptor pheophytin.

Raman spectra of either of the two bacteriopheophytin molecules present in reaction centers of *Rh. sphaeroides* obtained at resonance with their individual Q_X electronic transitions at low temperature have unambiguously shown that the keto carbonyl of the acceptor molecule H_L, vibrating at 1678 cm^{-1}, was engaged in molecular interaction, while that of the second, accessory bacteriopheophytin, vibrating at 1700 cm^{-1}, was free of any interaction (Lutz, 1980, 1984). The same observations were also recently made for the reaction center Bpheo molecules of *Rps. viridis* (Q. Zhou, B. Robert, and M. Lutz, unpublished experiments; Mäntele et al., 1988). X-ray crystallography of reaction centers from *Rps. viridis* confirmed this situation by showing that the keto carbonyl group of H_L was in position to interact with the side chain of the glutamic residue L-104, consistent with the RR observations (Deisenhofer et al., 1984, 1985). No equivalent possibility was observed near the accessory bacteriopheophytin molecule, hence breaking the overall C_2 symmetry shown by X-ray data to relate the pigments and polypeptides L and M (Michel et al., 1986b). It thus appeared likely that the specific interaction involving the (conjugated) keto carbonyl group of the acceptor molecule, H_L, should play a role in the asymmetry of the electron transfer (Michel et al., 1986b; Hanson et al., 1987; Lutz & Robert, 1988).

Sequence alignments between the D1 polypeptide of photosystem II and the L subunit of the bacterial RCs indicated that the L-104 glutamic acid residue might have a homologue in D1, namely, the glutamic D1-130 residue (Michel & Deisenhofer, 1988). The present results constitute the first direct demonstration that this hypothesis is most probably valid and that, in particular, the relative topology observed between bacteriopheophytin H_L and the Glu L-104 residue in bacterial RCs is conserved between the bleachable pheophytin molecule in photosystem II and the Glu D1-130 residue.

Bylina et al. (1988) have recently shown that replacement of the glutamic acid L-104 in *Rhodobacter capsulatus* by a leucine residue has a limited effect on the electron transfer, inducing a slowdown of the first charge transfer between P and the acceptor Bpheo by only a factor of 1.5. This experiment, although directly demonstrating that the H-bonding of the keto carbonyl of H_L indeed has an influence on the electron-transfer kinetics, appears to limit its role to a second-order effect. However, the present results strongly suggest that, inasmuch as the glutamic acid–acceptor pheophytin interaction appears to be strictly conserved in D1/D2, this effect, although small, should actually play a crucial role in electron transfer in both bacterial and photosystem II reaction centers.

Finally, we noted above that resonance Raman spectra of D1/D2 particles excited at 406 and 413 nm presented enhanced components at 1680–1700 cm^{-1}, which were much weaker in the 441.6-nm excited spectra (Figure 2). The excitation profiles of these components hence appeared to be parallel to that of the 1585-cm^{-1} pheophytin band. The above photobleaching experiments showed that the 1680-cm^{-1} component indeed should essentially arise from the acceptor pheophytin. It is thus tempting to propose that the ca. 1701-cm^{-1} component, which is specifically enhanced in spectra excited at 406 and 413 nm, actually arises from the second pheophytin molecule, which is known from stoichiometric measurements to be present in D1/D2 particles (Nanba & Satoh, 1986). The keto carbonyl of this molecule should then be free from any interaction, as is that of the accessory bacteriopheophytin of reaction centers from *Rh. sphaeroides* (Lutz, 1980, 1984) and from *Rps. viridis* (Michel et al., 1986b; Q. Zhou, B. Robert, and M. Lutz, unpublished experiments). If confirmed, this assignment would constitute additional evidence for a close structural homology between PS II and bacterial reaction centers.

[2] Strict normalization on the 1524-cm^{-1} band of the carotenoid resulted in difference spectra exhibiting some variability in the ratio of the intensities of the 1551- and 1585-cm^{-1} bands, depending on the experiment. We ascribe this variability to residual contributions from the 1553-cm^{-1} band, which dominates the RR spectrum of chlorophyll *a* in this region. These residual contributions should be due to slight variations in the absolute intensities of the carotenoid and/or chlorophyll bands upon dithionite treatment and/or preillumination of one of the two samples. This variability, however, never exceeded a few percent and did not result in any qualitative change in the difference spectra here discussed.

References

Bocian, D. F., Boldt, N. J., Chadwick, B. W., & Franck, H. A. (1987) *FEBS Lett. 214*, 92–96.

Bylina, E. J., Kirmaier, C., McDowell, L., Holten, D., & Youvan, D. C. (1988) *Nature 336*, 182–184.

Danielius, R. V., Satoh, K., Van Kan, P. J. M., Plijter, J. J., Nuijs, A. M., & Van Gorkom, H. J. (1987) *FEBS Lett. 213*, 241–244.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., & Michel, H. (1984) *J. Mol. Biol. 180*, 385–398.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., & Michel, H. (1985) *Nature 318*, 618–624.

Goedheer, J. C. (1966) in *The Chlorophylls* (Vernon, L. P., & Seely, J. R., Eds.) pp 147–184, Academic Press, New York.
Hanson, L. K., Fajer, J., Thompson, M. A., & Zerner, R. C. (1987) *J. Am. Chem. Soc. 109*, 4728–4730.
Klimov, V. V., Klevanik, A. V., Shuvalov, V. A., & Krasnovsky, A. A. (1977) *FEBS Lett. 82*, 183–186.
Lutz, M. (1974) *J. Raman Spectrosc. 2*, 497–516.
Lutz, M. (1979) Thèse de Doctorat d'Etat, Université Pierre et Marie Curie, Paris.
Lutz, M. (1980) *C. R.—Conf. Int. Spectrosc. Raman, 7th*, 520–523.
Lutz, M. (1984) *Adv. Infrared Raman Spectrosc. 11*, 211–300.
Lutz, M., & Robert, B. (1988) in *Biological Applications of Raman Spectroscopy* (Spiro, T. G., Ed.) pp 347–411, Wiley, New York.
Mäntele, W., Wollenweber, A., Nabedryk, E., & Breton, J. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 8468–8472.
Michel, H., & Deisenhofer, J. (1988) *Biochemistry 27*, 1–7.
Michel, H., Weyer, K. A., Gruenberg, H., Dunger, I., Oesterhelt, D., & Lottspeich, F. (1986a) *EMBO J. 5*, 1149–1158.
Michel, H., Epp, O., & Deisenhofer, J. (1986b) *EMBO J. 5*, 2445–2451.
Moënne-Loccoz, P. (1987) Diplôme d'études approfondies, Université Pierre et Marie Curie, Paris.
Nanba, O., & Satoh, K. (1986) *Proc. Natl. Acad. Sci. U.S.A. 84*, 109–112.
Newell, W. R., Amerongen, H., Van Grondelle, R., Aalberts, J. W., Drake, A. F., Udvarhelyi, P., & Barber, J. (1988) *FEBS Lett. 228*, 162–166.
Robert, B., & Lutz, M. (1986) *Biochemistry 25*, 2303–2309.
Robert, B., Nabedryk, E., & Lutz, M. (1989) in *Time-Resolved Spectroscopy* (Hester, R. E., Ed.) Wiley, London (in press).
Robinson, H., Yerkes, C., & Crofts, A. (1988) *Photosynth. Res.* (in press).
Schwartz, S. J., & Von Elbe, J. H. (1982) *J. Liq. Chromatogr. 5*, 43–47.
Shuvalov, V. A., Klimov, V. V., Dolan, E., Parson, W. W., & Ke, B. (1980) *FEBS Lett. 118*, 279–282.
Takahashi, Y., Hansson, O., Mathis, P., & Satoh, K. (1987) *Biochim. Biophys. Acta 893*, 49–59.
Trebst, A. (1986) *Z. Naturforsch., C: Biosci. 41C*, 240–245.
Van Dorssen, R. J., Breton, J., Plijter, J. J., Satoh, K., Van Gorkom, H. J., & Amesz, J. (1987) *Biochim. Biophys. Acta 893*, 267–274.
Wasielewski, M. R., Johnson, D. G., Seibert, M., & Govindjee (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 524–528.

Articles

Regulation of $\Delta\tilde{\mu}_H$+-Coupled ATP Synthesis and Hydrolysis: Role of Divalent Cations and of the F_0F_1-β Subunit†

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ABSTRACT: The divalent cation specificity of ATP-linked reactions catalyzed by the H^+-translocating F_0F_1 ATP synthase–ATPase complex has been followed in the *Rhodospirillum rubrum* chromatophore bound complex. In the presence of Mg^{2+} and Mn^{2+} the complex catalyzes ATP synthesis and hydrolysis as well as ATP-driven H^+ translocation, but in the presence of Ca^{2+} it catalyzes only ATP hydrolysis, which is not coupled to H^+ tanslocation. The inability of Ca^{2+} to maintain the coupling process is not due to opening of a proton leak in its presence nor to any release of F_1 from the membrane, because (a) an identical light-induced H^+ translocation is observed in the absence or presence of Ca·ATP and (b) the Ca-ATPase, as well as the Mg- and Mn-ATPase activities, is blocked by specific F_0 inhibitors. These results indicate that the divalent cations play an important role in the regulation of H^+-coupled ATP synthesis and hydrolysis by the F_0F_1 complex. Further tests suggest that their site of action is located on the F_1-β subunit. The isolated β subunit of the *R. rubrum* F_0F_1 has been reported to contain two nucleotide binding sites, a Mg-independent and a Mg-dependent site [Gromet-Elhanan, Z., & Khananshvili, D. (1984) *Biochemistry 23*, 1022–1028]. Addition of Mn^{2+} also enables the binding of 2 mol of ATP/mol of this isolated β subunit. But under identical conditions, Ca^{2+} does not enable ATP binding to this cation-dependent site and inhibits its binding in the presence of Mg^{2+} or Mn^{2+}. In light of these results we propose that the binding of Mg·ATP or Mn·ATP, but not of Ca·ATP, to the *R. rubrum* F_1-β subunit forms the trigger that opens the pathway for H^+ translocation through the F_0F_1 complex during catalysis.

Electron-transport coupled ATP synthesis in respiratory and photosynthetic organisms is catalyzed by a reversible proton-translocating ATP synthase–ATPase complex that is composed of two distinct structures (Senior & Wise, 1983; Merchant & Selman, 1985): a readily solubilized catalytic ATPase sector, F_1, and an intrinsic membrane sector that is involved in proton fluxes, F_0. The F_1-ATPase from many different sources has a $\alpha_3\beta_3\gamma\delta\epsilon$ subunit structure and contains

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