Resonance Raman Studies of Genetically Modified Reaction Centers from *Rhodobacter capsulatus*†

Jeffrey M. Peloquin,‡ Edward J. Bylina,§,‖ Douglas C. Youvan,§ and David F. Bocian*,‡

Department of Chemistry, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received January 29, 1990; Revised Manuscript Received June 11, 1990

ABSTRACT: Resonance Raman (RR) spectra are reported for the photosynthetic reaction center (RC) proteins from *Rhodobacter capsulatus* wild type and the genetically modified systems $Glu^{L104} \rightarrow Leu$ and $His^{M200} \rightarrow Leu$. The spectra were obtained with a variety of excitation wavelengths, spanning the UV, violet, and yellow–green regions of the absorption spectrum, and at temperatures of 30 and 200 K. The RR data indicate that the structures of the bacteriochlorin pigments in RCs from *Rb. capsulatus* wild type are similar to those in RCs from *Rhodobacter sphaeroides* wild type. The data also show that the amino acid modifications near the primary electron acceptor ($Glu^{L104} \rightarrow Leu$) and special pair ($His^{M200} \rightarrow Leu$) perturb only those bacteriochlorin pigments near the site of the mutation and do not influence the structures of the other pigments in the RC. In the case of the $Glu^{L104} \rightarrow Leu$ mutant, elimination of the hydrogen bond to the C_9 keto group of BPh_L results in frequency shifts of RR bands of certain skeletal modes of the macrocycle. This allows the assignment of bands to the individual BPh_L and BPh_M pigments. In the case of the $His^{M200} \rightarrow Leu$ mutant, in which the special pair is comprised of a bacteriochlorophyll (BChl)–bacteriopheophytin (BPh) heterodimer rather than the $BChl_2$ unit bound in the wild type, certain skeletal vibrations due to the additional BPh unit are identified. The frequencies of these modes are similar to those of the analogous vibrations of BPh_L and BPh_M, which indicates that the structure of the BPh in the heterodimer is not unusual in any discernible way. For *Rb. capsulatus* wild type and the $His^{M200} \rightarrow Leu$ mutant, the frequencies of the structure-sensitive skeletal modes of one of the two BPhs shift with temperature as has been previously observed for the analogous RR bands of RCs from *Rb. sphaeroides* [Peloquin, J. M., Violette, C. A., Frank, H. A., & Bocian, D. F. (1990) *Biochemistry 29*, 4892–4898]. The RR data obtained for the $Glu^{L104} \rightarrow Leu$ mutant confirm that this pigment is BPh_L. In addition, no temperature dependence is observed for the RR bands of the skeletal modes of the BPh_L in RCs from the $Glu^{L104} \rightarrow Leu$ mutant. This indicates that the global nature of the interactions between BPh_L and the protein matrix is altered when the L104 glutamic acid is replaced by leucine.

The X-ray crystal structures reported for the reaction center (RC)[1] proteins from *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* provide detailed information regarding the distances between and arrangement of the photophysically active pigments in the protein (Deisenhofer et al., 1984, 1985; Michel et al., 1986b; Chang et al., 1986; Allen et al., 1987, 1988). These data also provide a bench mark for spectroscopic and theoretical studies of the electronic properties of the pigments and the mechanism of electron transfer in RCs [for recent reviews and monographs, see Kirmaier and Holten (1987), Breton and Vermeglio (1988), Hanson (1988), Norris (1988), and Friesner and Won (1989)]. The X-ray studies and other studies indicate that many of the protein residues are conserved between *Rb. sphaeroides* and *Rps. viridis* (Michel et al. 1986a; Williams et al., 1983, 1984, 1986) and between these species and *Rhodobacter capsulatus* (Youvan et al., 1984). The crystal structure of the RC from this latter species has not been determined; however, it has a well-characterized genetic system (Scolnick & Marrs, 1987) which has allowed the preparation of a number of site specifically modified mutants (Bylina & Youvan, 1988; Bylina et al., 1988). Two of these mutants include $Glu^{L104} \rightarrow Leu$ and $His^{M200} \rightarrow Leu$. In the former system, the glutamic acid residue which hydrogen bonds to the C_9 keto group of the primary electron acceptor, BPh_L, is replaced by a non-hydrogen-bonding residue (Bylina et al., 1988b). In the latter, the histidine which ligates to one of the bacteriochlorophyll (BChl) molecules which comprises the special pair, $BChl_2$, is replaced by a nonligating residue. This converts $BChl_2$ into a heterodimer wherein a bacteriopheophytin (BPh) replaces one of the two BChls (Bylina & Youvan, 1988).

The perturbations on the primary electron donor and acceptor which occur in the $His^{M200} \rightarrow Leu$ and $Glu^{L104} \rightarrow Leu$ mutants make these systems ideal for examining how changes in pigment structural and electronic properties and changes in pigment–protein interactions influence the electron-transfer process. In this regard, recent linear dichroism and absorption studies indicate that the pigment orientation in these genetically modified RCs is identical with that of the wild type (Breton et al., 1989). Optical studies on the $Glu^{L104} \rightarrow Leu$ mutant indicate that much of the red shift of the Q_x absorption band of BPh_L with respect to that of BPh_M is due to the

† This work was supported by Grants GM-39781 (D.F.B.) and GM-42645 (D.C.Y.) from the National Institute of General Medical Sciences.

‡ Carnegie Mellon University.

§ Massachusetts Institute of Technology.

‖ Present address: Biotechnology Program, Pacific Biomedical Research Center, University of Hawaii at Manoa, Honolulu, HI 96822.

[1] Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; C_aC_m, stretching modes of the carbon–carbon bonds in the methine bridges of the bacteriochlorin macrocycle; LDAO, lauryldimethylamine *N*-oxide; L and M, light and medium polypeptides of the reaction center; Q_A, quinone; RR, resonance Raman; RC, reaction center.

0006-2960/90/0429-8417$02.50/0

presence of the glutamic acid residue (Bylina et al., 1988b). However, time-resolved optical measurements on this mutant have shown that elimination of this asymmetry between the L and M subunits does not change the path of electron transfer (Bylina et al., 1988b) [through the L branch (Kirmaier et al., 1985b)]. The path specificity is also retained in the $His^{M200} \rightarrow$ Leu mutant (Kirmaier et al., 1988) despite the fact that the electronic structure of the heterodimer is different from $BChl_2$ (Bylina et al., 1990; DiMagno et al., 1990; Kirmaier et al., 1989). Regardless, the rates of electron transfer in RCs from both the $Glu^{L104} \rightarrow$ Leu and $His^{M200} \rightarrow$ Leu mutants are different from those observed in RCs from the wild type (Kirmaier & Holten, 1988a).

Resonance Raman (RR) spectroscopy affords another means for probing the structural features of photosynthetic RCs (Lutz, 1984; Lutz & Robert, 1985, 1988; Robert & Lutz, 1986, 1988; Zhou et al., 1985; Bocian et al., 1987; Schick & Bocian, 1987; Peloquin et al., 1989, 1990). Lutz and coworkers have used the RR technique to examine the extent of hydrogen bonding between the C_2 acetyl and C_9 keto groups of the bacteriochlorin pigments and the amino acid residues of the protein (Lutz & Robert, 1985, 1988; Robert & Lutz, 1986, 1988). These workers have also assigned the coordination numbers of the Mg(II) ions in the various BChls in the RC on the basis of the frequencies of the core-size sensitive vibrations of ca. 1610 cm^{-1} (Lutz & Robert, 1985; Robert & Lutz, 1986; Zhou et al., 1987). Our group has also been using RR spectroscopy to probe the structure of bacteriochlorin macrocycles (Boldt et al., 1987; Schick & Bocian, 1987; Donohoe et al., 1988; Peloquin et al., 1989, 1990). In a recent study of RCs from *Rb. sphaeroides*, we found that the frequencies of certain skeletal modes are different for BPh_L and BPh_M and that the frequencies of these modes for one of the two BPhs are sensitive to changes in temperature (Peloquin et al., 1990). However, specific assignments of the RR bands to either BPh_L or BPh_M were not possible on the basis of our data. The temperature-dependent RR data were interpreted to indicate that the out-of-plane distortions of the macrocycle are less severe at low than at high temperatures. The temperature sensitivity of the structure was attributed to unique interactions between the BPh and the protein matrix.

In this paper, we report RR spectra of RCs from *Rb. capsulatus* wild type and the genetically modified systems $Glu^{L104} \rightarrow$ Leu and $His^{M200} \rightarrow$ Leu. The spectra were acquired with a variety of excitation wavelengths, spanning the UV, violet, and yellow–green regions of the absorption spectrum, and at both high (200 K) and low (30 K) temperatures. Collectively, the RR data allow an assessment of structural changes that occur in the bacteriochlorin pigments as a consequence of altering amino acid residues near the primary electron donor and acceptor. These data also provide insight into the nature of the pigment–protein interactions and the factors that might influence the electron-transfer process in RCs.

Materials and Methods

The RCs from *Rb. capsulatus* were purified as previously described (Prince & Youvan, 1987; Bylina & Youvan, 1988a) and solubilized in 0.01 M phosphate (pH 7.4)/0.05% lauryldimethylamine *N*-oxide (LDAO). Wild-type RCs were isolated from a pU2922-containing U43 derivative which is a plasmid-complemented deletion background (Bylina & Youvan, 1987; Bylina et al., 1988c). RCs from several independent preparations were examined during the course of the studies. Chemically oxidized and reduced RCs were prepared by the addition of potassium ferricyanide or sodium ascorbate, respectively.

The RR spectra were acquired in a 90° scattering configuration on a computer-controlled Spex Industries 1403 double monochromator equipped with a photon counting detection system and a Hamamatsu R928P photomultiplier tube. The excitation wavelengths (λ_{ex} = 363.8, 406.7, 530.9, and 548.5 nm) were provided by an argon ion (Coherent Radiation INNOVA 15UV), krypton ion (Coherent Radiation INNOVA 200-K3), or continuous-wave dye (Coherent Radiation CR590-03) laser. The incident laser power was typically 15 mW for all excitation wavelengths; however, the power at the sample was substantially less because the beam was loosely focused to an apparent area of 2–3 mm^2. The corresponding beam diameter is 1.5–2 times larger than the diameter of capillary tubes in which the samples were contained. As a consequence, the total flux through the illuminated volume corresponded to approximately 500–750 photons s^{-1} $molecule^{-1}$. Spectra were acquired at 0.5 cm^{-1} intervals with a count time of 1 s/point. The spectral slit width was ~4 cm^{-1} for all excitation wavelengths. The scan to scan precision is better than the maximum theoretical resolving power of the monochromator (~0.15 cm^{-1}).

The relatively low light levels and small slit widths used in the RR experiments necessitated spectral averaging. The spectra reported are typically the average of 20 scans. During the course of data acquisition, on the order of 10 independent data sets were acquired for each sample. In order to prevent photodegradation during data collection for any given scan, the sample was frequently repositioned in the laser beam. Even minor amounts of photodamage (undetectable by absorption spectroscopy) are readily discernible in the RR experiments because loss of sample integrity invariably leads to the production of highly fluorescent impurities. In addition, all spectra were acquired at reduced temperatures (200 K and below). Temperature control was achieved by mounting the samples, contained in capillary tubes, via a home-built holder onto the cold tip of an ADP cryogenics DE-202 Displex closed-cycle refrigeration system. The samples were diluted 1:3 in ethylene glycol in order to obtain high-quality glasses at the reduced temperatures. The laser line and/or the Raman bands of the glycol were used for frequency calibration.

Results

The RR spectra observed for RCs from *Rb. capsulatus* wild type, the $Glu^{L104} \rightarrow$ Leu mutant, and the $His^{M200} \rightarrow$ Leu mutant upon UV (λ_{ex} = 363.8 nm) excitation are shown in Figures 1, 2, and 3, respectively. The RR spectra observed for these three types of RC upon green (λ_{ex} = 530.9 nm) excitation are shown in Figures 4–6, respectively. Spectra observed at both 200 and 30 K are shown in the figures. RR spectra were also obtained for the three types of RC at the two different temperatures with violet excitation (λ_{ex} = 406.7 nm) (not shown). Selected spectra were also obtained with yellow–green excitation (λ_{ex} = 548.5 nm) (not shown). Inspection of Figures 1–6 reveals that many of the general features of the RR spectra of the RCs from *Rb. capsulatus* wild type and the two genetically modified systems are similar to one another. Many of these features are also similar to those observed in the RR spectra of RCs from *Rb. sphaeroides* wild type (Lutz et al., 1976; Lutz, 1984; Lutz & Robert, 1988; Peloquin et al., 1990). We first summarize the features which are common to the RR spectra of the various RCs. We then describe specific differences between the spectra of the systems.

General Features of the RR Spectra. UV excitation (near the Soret band maximum) results in scattering from all of the bacteriochlorin pigments in the RCs (cf. Figures 1–3). RR bands ascribable to the ν(C=O) vibrations of the C_2 and C_9

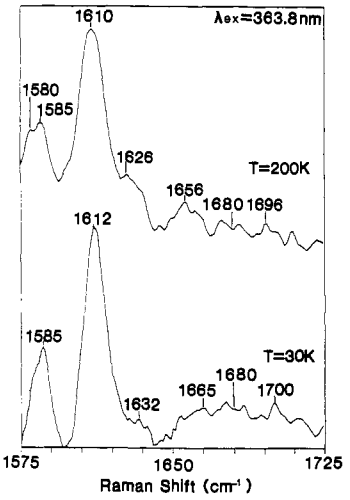

FIGURE 1: RR spectra of RCs from *Rb. capsulatus* wild type in ethylene glycol glasses at 200 and 30 K with λ_{ex} = 363.8 nm.

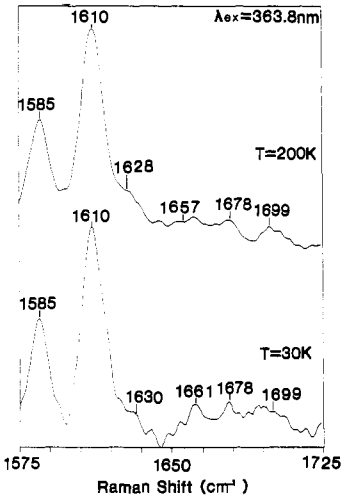

FIGURE 2: RR spectra of RCs from the Glu^{L104} → Leu mutant of *Rb. capsulatus* in ethylene glycol glasses at 200 and 30 K with λ_{ex} = 363.8 nm.

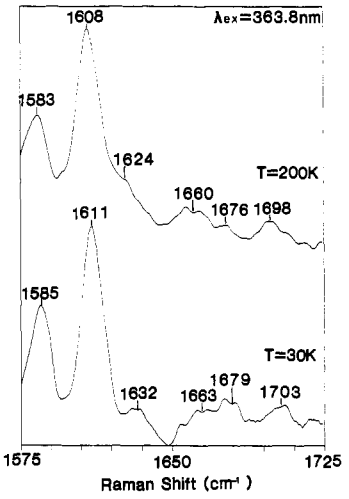

FIGURE 3: RR spectra of RCs from the His^{M200} → Leu mutant of *Rb. capsulatus* in ethylene glycol glasses at 200 and 30 K with λ_{ex} = 363.8 nm.

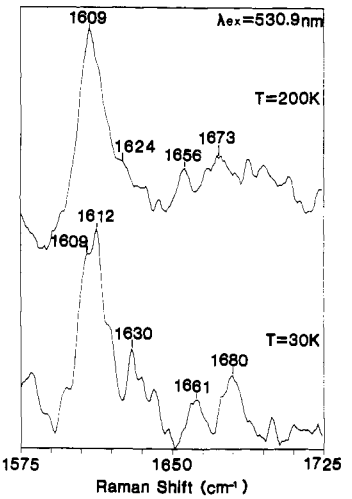

FIGURE 4: RR spectra of RCs from *Rb. capsulatus* wild type in ethylene glycol glasses at 200 and 30 K with λ_{ex} = 530.9 nm.

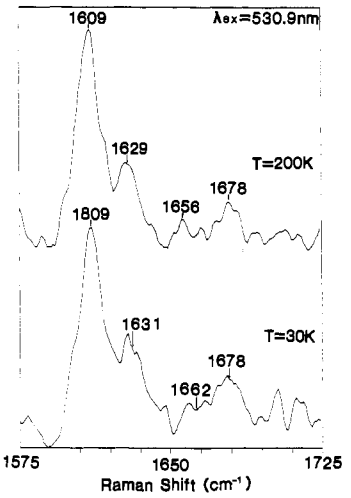

FIGURE 5: RR spectra of RCs from the Glu^{L104} → Leu mutant of *Rb. capsulatus* in ethylene glycol glasses at 200 and 30 K with λ_{ex} = 530.9 nm.

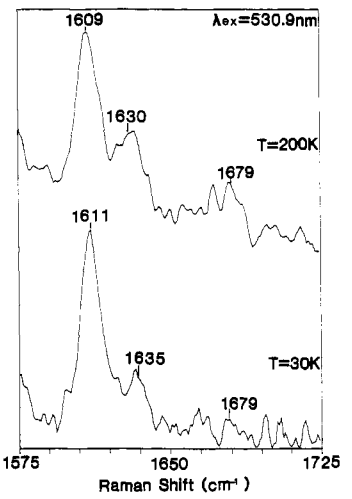

FIGURE 6: RR spectra of RCs from the His^{M200} → Leu mutant of *Rb. capsulatus* in ethylene glycol glasses at 200 and 30 K with λ_{ex} = 530.9 nm.

carbonyl groups of the six bacteriochlorin pigments are observed in the 1630–1710 cm^{-1} region (Lutz & Robert, 1985; Robert & Lutz, 1986, 1988). This spectral region is very congested, and many of the individual ν(C=O) bands are overlapped, particularly in the 1650–1665 and 1675–1690 cm^{-1} regions (Robert & Lutz, 1988). Broad features due to the C_2 acetyl groups are observed near 1630 and 1660 cm^{-1} while features due to the C_9 keto groups are seen near 1680 and 1700 cm^{-1}. The peaks labeled in the figures are those previously assigned by Lutz and co-workers.

The UV excitation RR spectra also exhibit bands due to the ring skeletal vibrations of the various bacteriochlorin pigments. For example, the strong band observed near 1610 cm^{-1} is a characteristic C_aC_m vibration of both BChl and BPh (Lutz, 1984; Donohoe et al., 1988). This mode is the analogue of the ν_{10} band of porphyrins and is extremely sensitive to the core size and degree of ruffling of the macrocycle (Callahan & Cotton, 1987). Shoulders are observed on the high- and low-energy sides of this band which are due to other C_aC_m vibrations of the ring (Donohoe et al., 1988). The relatively strong band in the 1580–1585 cm^{-1} region is due to scattering from another C_aC_m mode of the ring skeleton (Donohoe et al., 1988). This band is characteristically strong in BPh and weak in BChl.

Excitation into the green region of the absorption spectrum allows selective resonance enhancement of the Raman bands of the BPhs (Lutz et al., 1976; Lutz, 1984). The bands observed near 1610 cm^{-1} in the green excitation spectra of all three RCs from *Rb. capsulatus* are due to scattering from the ν_{10}-like skeletal modes of the BPh pigments. Shoulders on this band due to other C_aC_m vibrations are also observed with this excitation wavelength. The 1580–1585 cm^{-1} RR band of the BPhs is absent with green excitation because this mode is not resonance-enhanced with Q_x excitation (Lutz et al., 1976). For all three RCs, green excitation also results in resonance enhancement of modes in the 1630–1710 cm^{-1} region (cf. Figures 4–6). Certain of these bands have frequencies similar to those observed with UV excitation (for example, those near 1630 and 1680 cm^{-1}) and are undoubtedly due to ν(C=O) vibrations of the C_2 and C_9 carbonyl groups of the BPhs in the RCs. However, there appear to be more features in the 1630–1710 cm^{-1} region that can be accounted for in terms of the C_2 and C_9 keto groups of BPh_L and BPh_M (and also the BPh in the heterodimer of the $His^{M200} \rightarrow$ Leu mutant).

The RR spectra obtained with violet (λ_{ex} = 406.7 nm) excitation, on the low-energy side of the composite Soret band, exhibit bands due primarily to the BChls (Peloquin et al., 1990). The only prominent feature observed in these spectra is the RR band due to the ν_{10}-like skeletal mode. This band occurs at approximately 1610 cm^{-1} for all three types of RC from *Rb. capsulatus*.

Comparison of the RR spectra of the RCs from *Rb. capsulatus* obtained at 200 K versus 30 K with either UV or green excitation reveals that the frequencies and line shapes of the bands due to a number of the carbonyl and skeletal modes are sensitive to temperature. In contrast, no temperature-dependent spectral changes are observed with violet excitation. Similar effects are observed in the UV, green, and violet excitation RR spectra of RCs from *Rb. sphaeroides* (Peloquin et al., 1990). For these latter RCs, the temperature-dependent spectral changes observed for the ν(C=O) modes appear to involve several or all of the bacteriochlorin pigments in the RCs. The bands due to the C_2 acetyl groups (1630–1660 cm^{-1} region) appear to be the most sensitive to changes in temperature. This also appears to be the case for the RCs from *Rb. capsulatus*. Regardless, it is difficult to identify any general trends in the behavior of the ν(C=O) RR bands as a function of temperature due to the complexity of this spectral region. In the case of the skeletal modes (1580 and 1610 cm^{-1} regions), selective excitation (UV vs violet vs green) RR studies on RCs from *Rb. sphaeroides* revealed that the temperature-dependent spectral shifts involve only the BPh pigments (Peloquin et al., 1990). Analogous experiments on the RCs from *Rb. capsulatus* indicate that this is also the case for these systems. In general, the frequencies of certain skeletal modes increase as the temperature is lowered from 200 to 30 K. However, the exact changes are dependent on the specific RC and will be described in more detail below.

As was the case for our RR studies on *Rb. sphaeroides* (Peloquin et al., 1990), a number of additional studies were performed on the RCs from *Rb. capsulatus* in order to investigate the factors which might influence the frequencies of the skeletal modes and their temperature dependence. As previously noted, a number of data sets were obtained for each RC. These typically included samples from different preparations. In addition, RR spectra were acquired after each of several cycles of the temperature between 200 and 30 K. In all cases, it was found that the skeletal mode frequencies for a given RC at a given temperature were identical with those indicated in the figures to within ±1 cm^{-1}.

Because the light levels required to obtain RR spectra (500–750 photons s^{-1} $molecule^{-1}$) are sufficient to induce complete photooxidation of the special pair, spectra were also obtained for wild-type and mutant RCs in which the special pair was chemically oxidized or the electron-transfer chain was blocked via chemical reduction of Q_A. [The heterodimer of RCs from the $His^{M200} \rightarrow$ Leu mutant cannot be oxidized with ferricyanide (D. Holten, private communication).] In chemically reduced samples, substantial amounts of $^3BChl_2$ are expected to be formed (Parson & Monger, 1976). RR spectra were also compared at 100 and 30 K where differing amounts of $^3BChl_2$ and $BChl_2$ are observed to be present in chemically reduced RCs from *Rb. sphaeroides* (Robert & Lutz, 1986). Despite the different populations of $BChl_2$, $BChl_2^+$, $^3BChl_2$ (and the heterodimer counterparts), Q_A and Q_A^- which should be present under these various conditions, the skeletal mode frequencies observed for each type of RC from *Rb. capsulatus* were found to be identical with those shown in the figures to within ±1 cm^{-1}. (It should be noted that the exact populations of the various photoinduced intermediates cannot be readily ascertained for the RCs from *Rb. capsulatus* because detailed kinetic measurements at different temperatures have not been performed on these systems. Provided that the lifetimes of the intermediates are of the same order of magnitude in RCs from *Rb. capsulatus* and *Rb. sphaeroides*, chemical reduction should provide a reasonable means for altering the populations of $BChl_2$, $BChl_2^+$, and $^3BChl_2$ present under the illumination conditions of the RR experiment.) Collectively, these observations indicate that the frequencies of the skeletal modes of the BPhs and the accessory BChls are insensitive to the electronic and/or redox states of the special pair and the redox state of Q_A. These results also suggest that the skeletal mode frequencies of the "neutral" BChl molecule in $BChl_2^+$ and the "neutral" BPh molecule in the oxidized heterodimer (presuming these are adequate descriptors of the electronic system on the time scale of the RR experiment) are rather insensitive to the electronic and/or redox state of the neighboring species in the dimer.

Specific Features of the RR Spectra. Both the ν(C=O) and the skeletal modes of the bacteriochlorin macrocycles should be sensitive indicators of any specific structural differences which might exist in analogous bacteriochlorin pigments of RCs from *Rb. sphaeroides* wild type vs *Rb. capsulatus* wild type of between the pigments of this latter RC and those of the genetically modified systems. The frequencies of the ν(C=O) modes should reflect the extent of hydrogen bonding between the carbonyl groups of the bacteriochlorins and the amino acid residues of the protein (Lutz & Robert, 1985, 1988; Robert & Lutz, 1986, 1988). On the other hand, the frequencies of the skeletal modes of the bacteriochlorin

pigments should be indicative of the structure of the macrocycles (Callahan & Cotton, 1987; Peloquin et al., 1990).

(1) *Carbonyl Stretching Modes.* Comparison of the carbonyl regions of the RR spectra of RCs from the *Rb. capsulatus* wild type with those of *Rb. sphaeroides* wild type suggests that the frequencies of analogous ν(C=O) vibrations are very similar. However, as noted above, the complexity of the spectra in the carbonyl region makes it difficult to ascertain whether there are subtle differences in the frequencies of analogous ν(C=O) vibrators. This complexity also makes it difficult to determine whether the genetic modifications significantly affect the frequencies of any of the ν(C=O) modes. In this regard, one ν(C=O) mode which would be expected to exhibit a frequency difference in the wild-type versus the genetically modified RCs is the C_9 keto group stretch of BPh_L in the $Glu^{L104} \rightarrow$ Leu mutant. In RCs from *Rb. sphaeroides*, the presence of a hydrogen bond between the glutamic acid residue and the keto oxygen reportedly downshifts this ν(C=O) mode from ~1710 to ~1680 cm^{-1} (Lutz & Robert, 1985; Robert & Lutz, 1986, 1988). This hydrogen bond is also present in RCs from *Rb. capsulatus* wild type; thus, its absence in RCs from the $Glu^{L104} \rightarrow$ Leu mutant should result in a significant upshift in the frequency of the ν(C=O) mode of the C_9 keto group of BPh_L. Comparison of the UV excitation RR spectra of RCs from the wild type and this mutant (cf. Figures 1 and 2) reveals that there appears to be somewhat more RR intensity in the 1700–1710 cm^{-1} region of the spectrum of the latter RCs. However, there is no well-characterizable diminution in intensity in the 1680 cm^{-1} region of the spectrum of the mutant compared with that of the wild type (although there are clearly differences between the spectra in this region). Interestingly, the green excitation RR spectra of RCs from both the mutant and the wild type reveal a band near 1680 cm^{-1} (cf. Figures 4 and 5). Collectively, these observations suggest that the interpretation of the RR spectra in the 1630–1710 cm^{-1} region may be more complicated than previously assumed. Consequently, we will not attempt to draw any conclusions regarding structure based on the RR bands observed in this region.

(2) *Bacteriochlorin Skeletal Modes.* The frequencies of the bacteriochlorin skeletal modes observed in the RR spectra of RCs from *Rb. capsulatus* wild type (Figures 1 and 2) are similar to those observed for the analogous modes of the pigments in the RCs from *Rb. sphaeroides* wild type (Peloquin et al., 1990). The temperature-dependent frequency shifts observed for the skeletal modes of the BPhs in the former RCs also parallel those observed for the latter. In particular, with UV excitation at 200 K, the BPh bands in the 1580–1585 cm^{-1} region appear as a doublet with maxima near 1580 and 1585 cm^{-1} (Figure 1, top). The composite band due to the ν_{10}-like modes of the various pigments is observed as a single band near 1610 cm^{-1}. With green excitation at 200 K, the composite band due to the ν_{10}-like modes of the BPhs appears as a single band at 1609 cm^{-1} (Figure 4, top). At 30 K, the 1580 cm^{-1} BPh band is upshifted under its 1585 cm^{-1} partner (Figure 1, bottom). Concomitantly, the composite band due to the ν_{10}-like modes of the various pigments upshifts by several wavenumbers (Figure 1, bottom). With green excitation at 30 K, the ν_{10}-like band of the BPhs is observed as a clear doublet with maxima at 1609 and 1612 cm^{-1} (Figure 4, bottom). [Note that the shoulder observed on the high-frequency side of the band is not due to the ν_{10}-like mode (vide supra).] We have previously ascribed similar temperature-dependent shifts observed in the RR spectra of RCs from *Rb. sphaeroides* wild type as arising from a conformational change in one of the two BPhs in the RC (Peloquin et al., 1990). The RR spectra obtained for RCs from *Rb. capsulatus* wild type are also consistent with this interpretation and suggest that the skeletal mode frequencies of one BPh are at ~1585 and 1609 cm^{-1} at all temperatures whereas those of the other are at ~1580 and 1609 cm^{-1} at 200 K and at ~1585 and 1612 cm^{-1} at 30 K.

Comparison of the skeletal mode regions of the RR spectra of RCs from the $Glu^{L104} \rightarrow$ Leu mutant (Figures 2 and 5) with those from the wild type (Figures 1 and 4) reveals several striking differences. In particular, the 1580–1585 cm^{-1} band of the BPhs of the mutant appears as a single peak at 1585 cm^{-1} at 200 K (Figure 2, top). Upon lowering the temperature to 30 K, this band does not shift, nor does the composite ν_{10}-like band which is observed near 1610 cm^{-1} at both temperatures. With green excitation, the ν_{10}-like mode of the BPhs in the $Glu^{L104} \rightarrow$ Leu mutant is observed as a single band centered near 1609 cm^{-1} both at 200 K and at 30 K (Figure 5). Collectively, these observations suggest that the skeletal mode frequencies for the two BPhs in the RCs from the $Glu^{L104} \rightarrow$ Leu mutant are essentially identical and that BPh_L is the pigment whose skeletal mode frequencies shift with temperature. The existence of a single versus double set of RR bands in the spectra of the mutant versus wild-type RCs with both UV and green excitation cannot be attributed to selective excitation of one versus both of the BPhs in the two different proteins. This is so because the elimination of the hydrogen bond to the C_9 keto group of BPh_L does not appreciably shift the Soret absorption maximum (E. J. Bylina and D. C. Youvan, unpublished experiments). The Q_x absorption maximum is shifted in the $Glu^{L104} \rightarrow$ Leu mutant versus the wild type; however, the Q_x maxima of the two BPhs are much closer to one another in the mutant (Bylina et al., 1988b). This makes selective excitation less likely in the mutant than in the wild type.

Comparison of the skeletal mode regions of the RR spectra of RCs from the $His^{M200} \rightarrow$ Leu mutant (Figures 3 and 6) with those of the wild type (Figures 1 and 4) indicates that there are certain differences; however, these differences are much less pronounced than those observed for the $Glu^{L104} \rightarrow$ Leu mutant versus the wild type. For the $His^{M200} \rightarrow$ Leu mutant at 200 K, the 1580–1585 cm^{-1} skeletal mode is observed as a single band at 1583 cm^{-1} (Figure 3, top). This band is considerably broader than the single band observed for this mode in the RR spectra of the $Glu^{L104} \rightarrow$ Leu mutant (cf. Figures 2 and 3, top). Upon lowering the temperature to 30 K, the band narrows and the maximum upshifts slightly to ~1585 cm^{-1}. The composite ν_{10}-like RR band observed for the $His^{M200} \rightarrow$ Leu mutant also upshifts upon lowering the temperature; however, the position of the maximum at both temperatures is slightly lower than that observed for the wild type. With green excitation, the ν_{10}-like mode of the BPhs in this mutant is observed at 1609 cm^{-1} at 200 K and at 1611 cm^{-1} at 30 K (Figure 6). Unlike the wild type, however, the band observed for the mutant does not appear to be doubled at the lower temperature.

The RR spectra obtained for the RCs from the $His^{M200} \rightarrow$ Leu mutant with violet and yellow-green excitation do not reveal any additional features which are not present in the UV and green excitation spectra. As was previously noted, selective excitation of the BChls (with violet excitation) shows that the composite RR band due to the ν_{10}-like modes of these pigments occurs at 1610 cm^{-1} in the $His^{M200} \rightarrow$ Leu mutant and does not shift with temperature. These features are identical with those observed for the wild type and the $Glu^{L104} \rightarrow$ Leu mu-

tant. Likewise, excitation in the yellow–green, closer to the Q_x absorption maximum of the BPh in the heterodimer (~550 nm; Breton et al., 1989), does not reveal a discernible shift in the frequency of the composite ν_{10}-like band of the BPhs. As we have previously discussed, however, selective excitation of the different BPhs (which might reveal slight differences in their skeletal mode frequencies) is not possible due to the close proximity of their Q_x absorption maxima (Peloquin et al., 1990).

Discussion

Rb. capsulatus Wild Type. The studies reported here indicate that both the general and many of the specific features of the RR spectra of RCs from *Rb. capsulatus* wild type are similar to those of the RR spectra of RCs from *Rb. sphaeroides*. This observation suggests that the structures of the bacteriochlorin macrocycles are similar in the two species. Likewise, any pigment–protein interactions which are capable of significantly influencing the structure of the ring skeleton must be similar in the two species. These conclusions are consistent with those drawn from linear and circular dichroism studies (Breton et al., 1989), time-resolved optical studies (Kirmaier & Holten, 1988a), and the observed amino acid sequence homologies between the L and M polypeptides of the two species (Youvan et al., 1984; Williams et al., 1986). The similarity of the pigment–protein interactions in the RCs from the two organisms in the region of the BPhs is further supported by the observation of essentially identical temperature-dependent changes in the structure of one of the two BPhs in the RCs. The RR studies on the RCs from the $Glu^{L104} \rightarrow$ Leu mutant identify this pigment as BPh_L (vide infra).

$Glu^{L104} \rightarrow$ Leu Mutation. The general features observed in the RR spectra of the RCs from the $Glu^{L104} \rightarrow$ Leu mutant indicate that changes in the nature of the interaction between residue L104 and BPh_L influence the structure of BPh_L but do not perturb the structures of any of the BChls or the BPh_M in the RC. This conclusion is justified by the fact that skeletal mode frequencies of the BChls in the mutant are identical with those observed for the wild type. Furthermore, the skeletal mode frequencies for the two BPhs in the mutant are identical with one another and also identical with those observed for one of the two BPhs in the wild type. The appearance of a single set of BPh bands at 1585 and 1609 cm^{-1} in the RR spectra of the RCs from the $Glu^{L104} \rightarrow$ Leu mutant indicates that the bands observed at these same frequencies in the RR spectra of the RCs from the wild type are assignable to BPh_M while the temperature-sensitive set of bands (1580 and 1609 cm^{-1} at 200 K; 1585 and 1612 cm^{-1} at 30 K) are due to BPh_L. The fact that the genetic modification affects only BPh_L and not the other pigments idicates that the effects of the modification are quite site-specific.

The fact that the skeletal mode frequencies of the two BPhs in RCs from the $Glu^{L104} \rightarrow$ Leu mutant are identical indicates that the structures of these pigments are more similar to one another than are those of the two BPhs in RCs from the wild type. The question remains as to the nature of the interactions between the glutamic acid residue and BPh_L and how these interactions alter the structure of BPh_L. It is unlikely that the hydrogen bond between the glutamic acid residue and the C_9 keto group of BPh_L is directly responsible, because RR studies of bacteriochlorin pigments in solution indicate that the formation of hydrogen bonds to the C_9 and/or the other carbonyl groups on the macrocycle does not significantly affect the structure of the ring skeleton (Callahan & Cotton, 1987). Given this, the effect of the glutamic acid must be either to force BPh_L into a unique position in the protein pocket (via steric interactions) or to produce a structure for the pocket in which the conformation of BPh_L is uniquely affected. Regardless of the detailed nature of these interactions, a more interesting question is whether the exact conformation of BPh_L has any influence on the electron-transfer rates in RCs (Woodbury et al., 1985; Breton et al., 1986; Martin et al., 1986; Wasielewski & Tiede, 1986; Kirmaier & Holten, 1988a) or their temperature dependence (Martin et al., 1988; Fleming et al., 1988; Kirmaier et al., 1985a; Kirmaier & Holten, 1988b). Such questions can only be answered by additional time-resolved optical studies on genetically modified RCs. Such experiments are currently in progress (D. Holten, L. McDowell, and C. Kirmaier, private communication).

$His^{M200} \rightarrow$ Leu Mutation. The observation that the RR spectra are less perturbed by the $His^{M200} \rightarrow$ Leu than the $Glu^{L104} \rightarrow$ Leu mutation makes a unique description of the effects of the mutation more difficult for the former RCs. The fact that the two accessory BChls also contribute to the RR spectra further complicates the interpretation. Nevertheless, a self-consistent interpretation is possible for the RR spectral differences observed for the $His^{M200} \rightarrow$ Leu mutant versus the wild type. The most straightforward interpretation of the RR data is that the $His^{M200} \rightarrow$ Leu mutation affects only the structure of the special pair dimer and not that of the accessory BChls or the BPhs. The conclusion that the structure of the BChls is not affected is well justified by the observation that the frequencies of the ν_{10}-like skeletal modes of these pigments are identical with those of the wild type. [It should be noted that stuble structural changes in the other pigments, such as loss of a hydrogen bond, would not be manifested in shifts in the skeletal mode frequencies. For example, the crystallographic data for *Rsp. viridis* suggest that a water molecule forms a bridge between His^{M200} and $BChl_L$ (Diesenhofer & Michel, 1989). This interaction, presuming it exists in *Rb. capsulatus* wild type, would necessarily be absent in the $His^{M200} \rightarrow$ Leu mutant.] The conclusion that the structures of BPh_L and BPh_M are not affected by the mutation in the vicinity of the special pair is somewhat more tenuous; however, it is supported by the observation that the absorption maxima for these pigments are not shifted in the mutant relative to the wild type (Kirmaier et al., 1988; Breton et al., 1989).

Presuming that the structures of BPh_L and BPh_M are not altered by the $His^{M200} \rightarrow$ Leu mutation, the RR spectral features can be interpreted as follows: The single broad BPh mode observed at 1583 cm^{-1} at 200 K arises because a new band, due to the BPh in the heterodimer, lies approximately in between the analogous modes of BPh_L and BPh_M (Figure 3). The presence of this new band could also account for the observed attenuation in the magnitude of the temperature shift (2 cm^{-1} in the mutant vs 5 cm^{-1} in the wild type) if the frequency of the new mode is not temperature-sensitive. The appearance of a new, relatively strong RR band due to the ν_{10}-like band of the BPh in the special pair could also account for the slight downshift (relative to the wild type) of the composite band observed with UV excitation at both 200 and 30 K (cf. Figures 1 and 3). The contribution of the ν_{10}-like mode of the BPh in the heterodimer to the composite band observed with green excitation could also be responsible for the absence of doublet structure at 30 K (cf. Figures 4 and 6). The presence of this band could also account for the observed attenuation in the temperature shift of this band envelope.

The question remains as to the structure of the BChl molecule in the heterodimer in the RCs from the $His^{M200} \rightarrow$ Leu mutant. It is possible that RR bands are not observed

from this pigment with either UV or violet excitation although it is not obvious why this should be the case. Alternatively, RR bands from the BChl in the heterodimer may contribute to the spectra but at frequencies essentially identical with those exhibited by the accessory BChls. This latter possibility seems more reasonable given that dimerization does not result in significantly different frequencies for analogous skeletal modes of the $BChl_2$ unit and the accessory BChls in RCs from *Rb. sphaeroides* (and presumably *Rb. capsulatus*) wild type (Lutz & Robert, 1985; Robert & Lutz, 1986, 1988). In addition, the skeletal mode frequencies of the BPh pigment in the heterodimer occur at frequencies essentially identical with those of the analogous modes of BPh_L and BPh_M.

The observation that the skeletal mode frequencies of the BPh and presumably the BChl molecules of the heterodimer are apparently similar to those of the other BPhs and BChls in the RC suggests that the conformations of the pigments in the heterodimer are not unusual in any discernible way. Accordingly, the RR data also suggest that the coordination number of the BChl in the heterodimer is the same as that of the $BChl_2$ unit of the wild type. Perhaps these observations are not surprising inasmuch as the dimer is noncovalently linked and held together only through constraints imposed by the protein matrix.

Acknowledgments

We thank Professors D. Holten and H. Frank for stimulating discussions.

References

Allen, J. P., Feher, G., Yeates, T. O., Komiya, H., & Rees, D. C. (1987a) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5730–5734.
Allen, J. P., Feher, G., Yeates, T. O., Komiya, H., & Rees, D. C. (1987b) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6162–6166.
Allen, J. P., Feher, G., Yeates, T. O., Komiya, H., & Rees, D. C. (1987c) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6438–6442.
Allen, J. P., Feher, G., Yeates, T. O., Komiya, H., & Rees, D. C. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 8487–8491.
Bocian, D. F., Boldt, N. J., Chadwick, B. W., & Frank, H. A. (1987) *FEBS Lett. 214*, 92–96.
Boldt, N. J., Donohoe, R. J., Birge, R. R., & Bocian, D. F. (1987) *J. Am. Chem. Soc. 109*, 2284–2298.
Breton, J., & Vermeglio, A., Eds. (1988) *NATO ASI Ser., Ser. A 149*, 1–443.
Breton, J., Martin, J. L., Mingus, A., Antonetti, A., & Orszag, A. (1986a) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5121–5125.
Breton, J., Martin, J. L., Petrich, J., Mingus, A., & Antonetti, A. (1986b) *FEBS Lett. 209*, 37–43.
Breton, J., Bylina, E. J., & Youvan, D. C. (1989) *Biochemistry 28*, 6423–6430.
Bylina, E. J., & Youvan, D. C. (1987) *Z. Naturforsch. 42C*, 769–774.
Bylina, E. J., & Youvan, D. C. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7226–7230.
Bylina, E. J., Jovine, R., & Youvan, D. C. (1988a) *NATO ASI Ser., Ser. A 149*, 113–118.
Bylina, E. J., Kirmaier, C., McDowell, L., Holten, D., & Youvan, D. C. (1988b) *Nature 336*, 182–184.
Bylina, E. J., Robles, S., & Youvan, D. C. (1988c) *Isr. J. Chem. 28*, 73–78.
Bylina, E. J., Kowlaczkowski, S. V., Norris, J. R., & Youvan, D. C. (1990) *Biochemistry 29*, 6203–6210.
Callahan, P. M., & Cotton, T. M. (1987) *J. Am. Chem. Soc. 109*, 7001–7007.
Chang, C. H., Tiede, D. M., Tang, J., Smith, U., Norris, J. R., & Schiffer, M. (1986) *FEBS Lett. 205*, 82–86.
Deisenhofer, J., & Michel, H. (1989) *EMBO J. 8*, 2149–2170.
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.
DiMagno, T. J., Bylina, E. J., Angerhofer, A., Youvan, D. C., & Norris, J. R. (1990) *Biochemistry 29*, 899–907.
Donohoe, R. J., Frank, H. A., & Bocian, D. F. (1988) *Photochem. Photobiol. 48*, 531–537.
Fleming, G. R., Martin, J. L., & Breton, J. (1988) *Nature 333*, 190–192.
Friesner, R. A., & Won, Y. (1989) *Biochim. Biophys. Acta 977*, 99–122.
Hanson, L. K. (1988) *Photochem. Photobiol. 47*, 903–921.
Kirmaier, C., & Holten, D. (1987) *Photosynth. Res. 13*, 225–260.
Kirmaier, C., & Holten, D. (1988a) *FEBS Lett. 239*, 211–218.
Kirmaier, C., & Holten, D. (1988b) *NATO ASI Ser., Ser. A 149*, 219–228.
Kirmaier, C., Holten, D., & Parson, W. W. (1985a) *Biochim. Biophys. Acta 810*, 33–49.
Kirmaier, C., Holten, D., & Parson, W. W. (1985b) *Biochim. Biophys. Acta 810*, 49–61.
Kirmaier, C., Holten, D., Bylina, E. J., & Youvan, D. C. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7562–7566.
Kirmaier, C., Bylina, E. J., Youvan, D. C., & Holten, D. (1989) *Chem. Phys. Lett. 159*, 251–257.
Lutz, M. (1984) *Adv. Infrared Raman Spectrosc. 11*, 211–300.
Lutz, M., & Robert, B. (1985) in *Antennas and Reaction Centers of Photosynthetic Bacteria* (Michel-Beyerle, M. E., Ed.) pp 138–146, Springer-Verlag, Berlin.
Lutz, M., & Robert, B. (1988) in *Biological Applications of Raman Spectroscopy* (Spiro, T. G., Ed.) Vol. III, pp 347–411, Wiley, New York.
Lutz, M., Kleo, J., & Reiss-Husson, F. (1976) *Biochem. Biophys. Res. Commun. 69*, 711–717.
Martin, J. L., Breton, J., Hoff, A. J., Mingus, A., & Antonetti, A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 957–961.
Martin, J. L., Breton, J., Lambry, J. C., & Fleming, G. (1988) *NATO ASI Ser., Ser. A 149*, 195–203.
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.
Norris, J. R., Ed. (1988) *Isr. J. Chem. 28*, 57–224.
Parson, W. W., & Monger, T. G. (1976) *Brookhaven Symp. Biol. 28*, 196–212.
Peloquin, J. M., Bylina, E. J., Youvan, D. C., & Bocian, D. F. (1989) *Biophys. J. 55*, 182a.
Peloquin, J. M., Violette, C. A., Frank, H. A., & Bocian, D. F. (1990) *Biochemistry 29*, 4892–4898.
Prince, R. C., & Youvan, D. C. (1987) *Biochim. Biophys. Acta 890*, 286–291.
Robert, B., & Lutz, M. (1986) *Biochemistry 25*, 2303–2309.
Robert, B., & Lutz, M. (1988) *Biochemistry 27*, 5108–5114.
Schick, G. A., & Bocian, D. F. (1987) *Biochim. Biophys. Acta 895*, 127–154.
Scolnik, P. A., & Marrs, B. L. (1987) *Annu. Rev. Microbiol. 41*, 703–726.
Wasielewski, M., & Tiede, D. (1986) *FEBS Lett. 204*, 368–372.
Williams, J. C., Steiner, L. A., Ogden, R. C., Simon, M. I., & Feher, G. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 6505–6509.

Williams, J. C., Steiner, L. A., Feher, G., & Simon, M. I. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7303-7307.
Williams, J. C., Steiner, L. A., & Feher, G. (1986) *Proteins: Struct., Funct., Genet. 1*, 312-325.
Woodbury, N., Becker, M., Middenforf, D., & Parson, W. W. (1985) *Biochemistry 24*, 7516-7521.
Youvan, D. C., Bylina, E. J., Alberti, M., Begusch, H., & Hearst, J. E. (1984) *Cell 37*, 949-957.
Zhou, Q., Robert, B., & Lutz, M. (1985) *Biochim. Biophys. Acta 890*, 368-376.

Pulmonary Surfactant-Associated Protein A Enhances the Surface Activity of Lipid Extract Surfactant and Reverses Inhibition by Blood Proteins in Vitro†

Amanda M. Cockshutt,*,‡ Jeffrey Weitz,§ and Fred Possmayer‡,∥

Department of Biochemistry and Department of Obstetrics and Gynaecology, MRC Group in Fetal and Neonatal Health and Development, The University of Western Ontario, London, Ontario N6A 5A5, Canada, and Department of Medicine, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

Received March 22, 1990; Revised Manuscript Received May 22, 1990

ABSTRACT: Although a monolayer of dipalmitoylphosphatidylcholine, the major component of pulmonary surfactant, is thought to be responsible for the reduction of the surface tension at the air-liquid interface of the alveolus, the participation of unsaturated and anionic phospholipids and the three surfactant-associated proteins is suggested in the generation and maintenance of this surface-active monolayer. We have examined the effects of surfactant-associated protein A (SP-A) purified from bovine lavage material on the surface activity of lipid extract surfactant (LES), an organic extract of pulmonary surfactant containing all of the phospholipids and SP-B and SP-C, but lacking SP-A. Measurements of the surface tension during dynamic compression were made on a pulsating bubble surfactometer. Addition of SP-A to LES reduces the number of pulsations required to attain surface tensions near zero at minimum bubble radius. This increase in surface activity is dependent upon the presence of Ca^{2+} in the assay mixture. Maximal enhancement is observed at or below 1% of the lipid concentration (w/w). The addition of two blood proteins, fibrinogen and albumin, at physiological concentrations to LES causes severe inhibition of surface activity. Addition of SP-A in the presence of Ca^{2+} completely counteracts the inhibition by fibrinogen. The amount of SP-A required for full reversal of this inhibition was less than 0.5% of the lipid concentration. Complete reversal of inhibition by albumin was also observed, even though there was a ~5000-fold molar excess of inhibitor. Addition of lysophosphatidylcholine also inhibits LES; however, SP-A has no effect on this inhibition.

The type II epithelial cell produces and secretes pulmonary surfactant which serves to reduce the surface tension across the air-liquid interface of the alveolus, and hence facilitates breathing. This substance, composed of approximately 90% lipids and 10% protein, reduces surface tension during breathing by generating a monolayer at the interface enriched in the saturated phospholipid dipalmitoylphosphatidylcholine (DPPC)[1] (Goerke, 1974; King, 1984; Notter et al., 1984; Possmayer et al., 1985; Van Golde et al., 1988). DPPC constitutes only ~40% of the surfactant; the remainder is mainly unsaturated phosphatidylcholines, anionic phospholipids such as phosphatidylglycerol (PG) and phosphatidylinositol (PI), and the three surfactant-associated proteins SP-A, SP-B, and SP-C (Possmayer, 1988). It has recently been demonstrated both in vitro and in vivo that many of the surface tension reducing properties of surfactant lipids are potentiated by interactions with the small, hydrophobic proteins SP-B and SP-C which make up only ~1% of surfactant (Hawgood et al., 1987; Revak et al., 1988; Suzuki et al., 1986; Takahashi & Fujiwara, 1986; Yu et al., 1987, 1988).

SP-A is an abundant protein representing 5-10% of bovine surfactant (Yu et al., 1983). The structure elucidation of this highly conserved protein has revealed some very interesting features (Hawgood, 1989). The primary translation product is a ~28K protein that is posttranslationally modified to generate a glycoprotein with a molecular weight of ~36K that is acetylated, sulfated, and proline hydroxylated. The protein can be divided into 3 functional regions: the N-terminal 7 amino acids form a short tail containing a cysteine which participates in an interchain disulfide bridge, a collagen-like stretch consisting of 23 repeats of the Gly-X-Y triplet, and a glycosylated carboxy-terminal region which has considerable homology with several Ca^{2+}-dependent lectins. The oligomeric organization of SP-A has been elucidated (Voss et al., 1988) and appears to form an octadecameric structure very similar to the complement component C1q.

† Supported by grants from the Medical Research Council of Canada. A.M.C. is the recipient of an MRC Studentship. J.W. is a Scholar of the Heart and Stroke Foundation of Ontario.

* Address correspondence to this author at University Hospital, Room 8L-15, 339 Windermere Rd., London, Ontario N6A 5A5, Canada.
‡ Department of Biochemistry, The University of Western Ontario.
§ Department of Medicine, McMaster University.
∥ Department of Obstetrics and Gynaecology, The University of Western Ontario.

[1] Abbreviations: PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; LES, lipid extract surfactant; SP, surfactant-associated protein; lyso-PC, lysophosphatidylcholine; R_{min}, minimum bubble radius; R_{max}, maximum bubble radius; PG, phosphatidylglycerol; BSA, bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NRDS, neonatal respiratory distress syndrome; ARDS, adult respiratory distress syndrome.

0006-2960/90/0429-8424$02.50/0