Resonance Raman scattering from *Rhodopseudomonas sphaeroides* reaction centers absorbed on a silver electrode

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1. INTRODUCTION

The reaction center complex from *Rhodopseudomonas sphaeroides* carotenoidless mutant R-26 has been well characterized as to its protein, pigment (4 bacteriochlorophylls (BChl) and 2 bacteriopheophytins (BPh)) and quinone content [1]. The function of 3 or 4 of the pigment molecules as well as the quinones in the reaction center primary photochemistry is also becoming better understood [2,3]. Two of the BChls, the special pair, are believed to function as the primary electron donor on photoexcitation and transfer an electron to a ubiquinone molecule via one of the BPhs, which has been termed the intermediate electron acceptor. In addition, there is evidence that one of the remaining BChls is involved in the initial electron transfer from the special pair to BPh [3]. New spectroscopic methods are needed which can provide additional support to the picosecond optical absorption data, as well as structural information about the bonding interactions of BChl and BPh in the reaction center.

Resonance Raman (RR) spectroscopy is one approach which has been successfully used for molecular identification as well as for the determination of molecular interactions in a large number of biochemical systems [4]. It has already been applied to the study of BChl and BPh both in vitro [5] and in intact reaction center preparations [6]. In addition, it has been used to monitor redox changes in molecules and may provide a more definitive method for identifying the proposed transient intermediates in reaction center photochemistry. The vibrational spectrum of the BChl cation radical differs substantially from the neutral species [7,8].

We have undertaken a RR study of isolated *Rps. sphaeroides* reaction centers spontaneously adsorbed onto a Ag electrode. The advantages of studying the adsorbed complex include increased RR scattering intensity as a result of the surface enhanced effect on Ag [9], quenching of background fluorescence [10], and the possibility of changing the redox state of the reaction center by varying the electrode potential.

2. EXPERIMENTAL

Electrolyte solutions used in these experiments were prepared from distilled and deionized (Milli-Q, Millipore Corp.) water. The electrolyte was 0.1 M Na_2SO_4. The solutions were thoroughly degassed in the electrochemical cell by purging with high purity nitrogen gas for $\geqslant 30$ min. Following the degassing procedure, aliquots of the concentrated reaction center preparation [13] (kindly provided by Dr Mel Okamura) were added to the electrolyte with a Hamilton microliter syringe.

A description of the electrochemical instrumentation and cell used in these studies appeared in [14]. The working electrode consisted of a flattened wire epoxied into 6 mm glass tubing, with exposed dimensions ~2 mm × 8 mm. The electrode surface was mechanically polished prior to immersion in the electrolyte solution containing the reaction

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centers and then electrochemically anodized by oxidation at +0.45 V *vs* SCE followed by reduction at –0.6 V. A total of 25 mC/cm^2 charge was passed in the oxidation step and the charge recovered in the reduction step was within 95% of this value. Following the anodization procedure, RR spectra of the adsorbed reaction centers were taken as a function of electrode potential.

Resonance Raman spectra were acquired by exciting either with the 457.9 nm line of an Ar$^+$ laser (Coherent Radiation Model CR-3) or the 530.9 nm line of a Kr$^+$ laser (Coherent Radiation, Model CR-750K). Other details of the Raman instrumentation were as in [14].

3. RESULTS AND DISCUSSION

Resonance Raman spectra of *Rps. sphaeroides* reaction centers adsorbed on a Ag electrode obtained using 457.9 nm excitation are shown in fig.1 at 2 electrode potentials (A,B) together with the spectrum of the blank (C) (buffer, LDAO, and electrolyte) and a spectrum of BChl in solution (D). Two points should be made prior to discussing the details of the surface spectra:

(1) They were obtained by spontaneous adsorption of the reaction centers onto the Ag electrode from bulk solution at 1 × 10^{-6}M. No RR signals were observable for the reaction centers in solution at this concentration; only an intense, broad fluorescent background was present. Thus, it appears that a considerable increase in the RR scattering intensity occurs for BChl in the surface-bound reaction centers and it is greater than that due to the resonance effect. Moreover, the enhancement cannot be attributed solely to an increase in the reaction center concentration on the electrode surface relative to that in the bulk solution, since the absorbance of the solution was observed to decrease by < 10%. A decrease in absorbance of 10% for 15 ml of a 1 × 10^{-6}M solution can be calculated to result in a factor of ~ 10 increase in the number of reaction center molecules within the laser beam. This is not sufficient to result in observable RR signals at the laser excitation wavelength used here. Hence, it is likely that the signal enhancement arises to a large extent from the proximity of the BChls to the Ag surface.

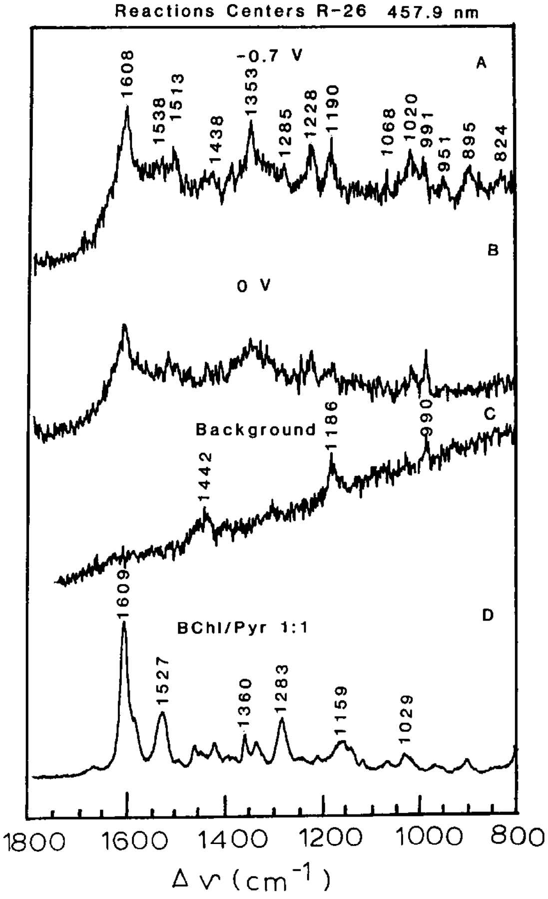

Fig.1. Resonance Raman spectra of *Rps. sphaeroides* reaction centers as a function of potential of the silver electrode: (A) –0.7 V *vs* SCE; (B) 0.0 V *vs* SCE; (C) Blank (buffer, electrolyte, and LDAO); (D) BChl (10^{-2} M) dissolved in CH$_2$Cl$_2$ with sufficient pyridine to form BChl • pyridine; laser excitation wavelength was 457.9 nm; power = 20 mW; monochromator bandpass = 5 cm^{-1}.

(2) They were recorded at room temperature with minimal interference from background fluorescence. These results indicate that the fluorescence is effectively quenched even in large molecular complexes when adsorbed at the metal surface. A similar observation has been made for small molecules at metal and semiconductor surfaces [11].

A comparison of the reaction center spectrum in fig.1A with that of BChl in solution (fig.1D) shows that the most intense peaks in the reaction center

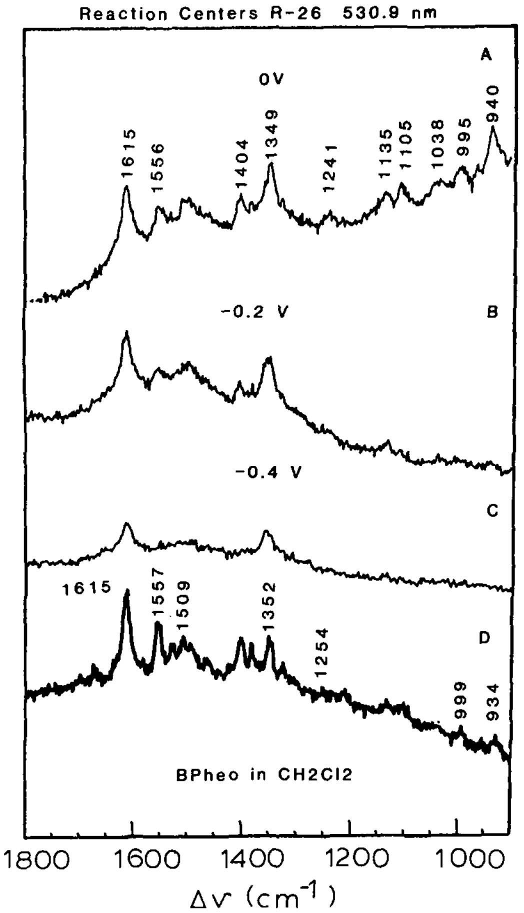

Fig.2. Resonance Raman spectra of *Rps. sphaeroides* reaction centers as a function of potential of the silver electrode: (A) 0.0 V *vs* SCE; (B) −0.2 V *vs* SCE; (C) −0.4 V *vs* SCE; (D) BPh (10^{-2} M) in CH_2Cl_2; laser excitation wavelength was 530.9 nm; other scan parameters were identical to those in fig.1.

spectrum correlate with those of BChl. No prominent BPh bands are observed and this is not unexpected since 457.9 nm excitation produces stronger scattering from BChl in solution as compared to BPh [5]. However, it cannot be said from this data whether the spectrum arises from one or more of the 4 BChl molecules in the reaction center. A detailed comparison of the spectral features of BChl in the surface spectrum with that in solution shows some frequency shifts, especially in the band at 1353 cm^{-1} which is at 1360 cm^{-1} in solution. There are also differences in band intensities; these include bands at 1513, 1392 and 1353 cm^{-1} which are more intense on the surface and that at 1285 cm^{-1} which is less intense.

On examining the signal intensity as a function of electrode potential, it may be seen that the most intense spectrum is that at −0.7 V. There are several possible origins for the potential dependence:

(i) The reaction centers may desorb at the more positive potentials, as observed with other proteins [11,12].

(ii) The reaction centers may reorient at the electrode as a function of potential and thereby change the distance of the BChl(s) with respect to the electrode. An increase in this distance would be expected to have a significant effect on the scattering intensity if the surface enhanced Raman effect is involved [9,14].

(iii) Oxidation of the reaction center BChl may occur at the more positive potential, which would affect the RR intensity since the absorption properties of BChl cation radical are different.

However, based upon solution studies, an enhancement and small shift of the 1608 cm^{-1} band would be expected if oxidation had occured. Since this is not observed, possibility (i) or (ii) appears more probable.

A change in the laser excitation wavelength to 530.9 nm results in the observation of RR scattering from BPh in the reaction centers adsorbed on the Ag electrode (fig.2A–C), as may be seen by comparing the spectra with that of BPh in solution (fig.2D). These spectra were obtained under identical conditions to those in fig.1. It again appears that considerable enhancement of the RR intensity occurs for the reaction centers on the electrode surface. However, in this case the signal-to-noise ratio is greatly improved over that observed for BChl in the reaction center. In addition, the potential dependence is opposite to that described for BChl. The strongest BPh spectra are observed near 0 V, with the signal intensity decreasing as the electrode potential is changed to more negative values. These results suggest that variation in intensity as a function of electrode potential may result from a reorientation or conformational change of the reaction center at the electrode surface rather than desorption. In the spontaneous adsorption process, the interaction between the reaction centers and the electrode may be dynamic and dependent upon the surface charge. If the surface charge varies for different regions of the protein–detergent complex, it may affect the orientation and proximity of BChl and BPh with respect to the electrode as a function of potential.

Here, we demonstrate the feasibility of utilizing surface-enhanced RR spectroscopy to study very large complexes adsorbed on a Ag electrode. The advantages for the reaction center studies include the following:

(i) Highly dilute solutions can be used;
(ii) Fluorescence quenching is observed in room temperature spectra;
(iii) Photodegradation appears to be minimal;
(iv) Potential-dependent and excitation-dependent spectra are observed.

The potential dependence may permit resolution of the RR spectra of the individual BChl or BPh species in the reaction center and may also provide data regarding the surface properties of the complex. Hopefully, it will also be possible to monitor the redox potentials of the reaction center components as a function of the electrode potential, leading possibly to the identification of the vibrational spectra of transient electron acceptors.

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