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Scanning Electrochemical Microscopy of Living Cells. 3. *Rhodobacter sphaeroides*

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The scanning electrochemical microscope (SECM) was used to probe the redox activity of individual purple bacteria (*Rhodobacter sphaeroides*). The approaches developed in our previous studies of mammalian cells were expanded to measure the rates and investigate the pathway of transmembrane charge transfer in bacteria. The two groups of redox mediators (i.e., hydrophilic and hydrophobic redox species) were used to shuttle the electrons between the SECM tip electrode in solution and the redox centers inside the cell. The analysis of the dependencies of the measured rate constant on formal potential and concentration of mediator species in solution yielded information about the permeability of the outer cell membrane to different ionic species and intracellular redox properties. The maps of redox reactivity of the cell surface were obtained with a micrometer or submicrometer spatial resolution.

Electron transfer (ET) reactions are essential for many cellular functions such as respiration and photosynthesis.[1] Intracellular redox activity can be probed noninvasively by measuring the overall rate of charge transfer (CT) across the membrane. CT measurements were carried out to characterize a cellular enzymatic reaction[2] or to use the microorganism itself as a sensor.[3] Such measurements were performed on many different kinds of biological cells including mammalian cells[4,5] and bacteria. The rate of oxidation/reduction of a redox mediator was used to quantitate respiration activity of various cells,[6] amperometrically detect viable bacteria,[7] and test the antibiotic susceptibility of *Escherichia coli*.[8] Usually, those experiments were performed with large populations of cells. Hence, the time scale was of the order of minutes or hours, and the measured rates were related to metabolic rate and cell propagation. Neither fluorescence nor electrochemical measurements were fast enough to probe kinetics of transmembrane CT.

Scanning electrochemical microscopy (SECM) has previously been used for high-resolution imaging of various cell surfaces.[9] Tsionsky et al. probed photoelectrochemistry of single guard cells.[10] Matsue's group mapped oxygen fluxes at the cell surface associated with cell respiration and photosynthesis.[11] The feedback mode of the SECM operation was recently employed to measure kinetics of the transmembrane charge transfer in normal human breast cells and metastatic breast cells.[5] Topography and redox reactivity of breast cells have been imaged with a submicrometer-scale spatial resolution.[12] Negative SECM feedback was obtained with hydrophilic (e.g., ferrocyanide or ferrocenecarboxylate) mediators, which could not pass through the cell membrane. In contrast, hydrophobic mediators (e.g., menadione or 1,2-naphthoquinone) could enter the cell and be regenerated via bimolecular reaction with intracellular redox centers.

Here we report a SECM study of purple photosynthetic bacteria, *Rhodobacter sphaeroides*, that contain a membrane-bound reaction center protein with a number of bound redox cofactors.[13,14]

One of our objectives is to compare the SECM responses obtained from bacteria to those measured with mammalian cells and to explore the differences in charge-transfer mechanisms. Unlike mammalian cells, *Rb. sphaeroides* has two membranes (outer and cytoplasmic) and no nucleus. Similarly to other Gram-negative bacteria, the outer membrane of *Rb. sphaeroides* is permeable to a number of hydrophilic ions such as ferrocyanide.[2] Three situations encountered in feedback mode SECM experiments with *Rb. sphaeroides* are presented in Figure 1. The tip ultramicroelectrode (UME) is placed in solution containing the

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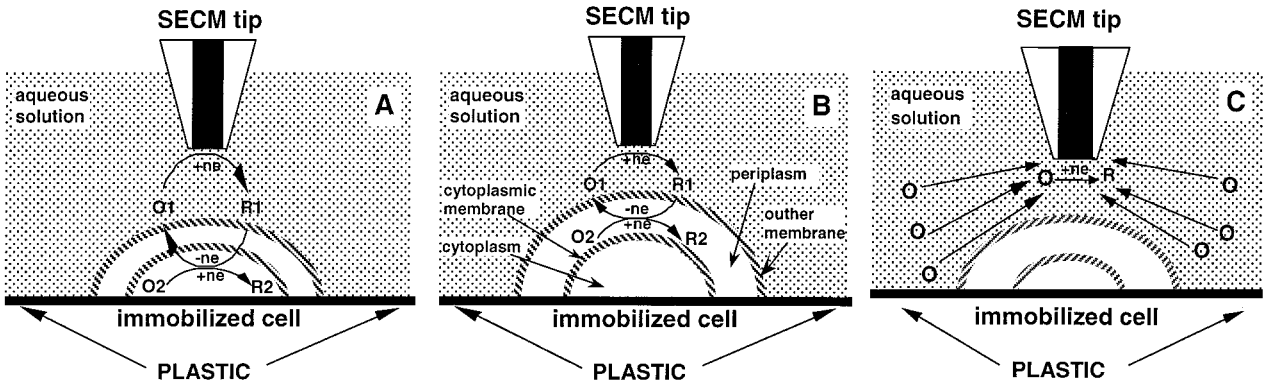

Figure 1. Schematic diagrams of the feedback mode SECM experiments with purple bacteria. (A) The tip is positioned in the solution close to the cell surface. A hydrophobic redox mediator (O1/R1) can cross both the outer cell membrane and the cytoplasmic membrane. Mediator species are regenerated via bimolecular ET reaction with cell-bound redox moieties (O2/R2). (B) Hydrophilic mediator species can permeate the outer membrane and enter the periplasm. (C) Mediator is not regenerated by the cell. Negative feedback is due to the hindered diffusion of redox species to the tip electrode.

oxidized (or reduced) form of a redox mediator. A mediator species is then reduced (or oxidized) at the tip electrode:

$$O1 + e^- = R1 \qquad \text{(at the tip)} \qquad (1)$$

If R1 is a hydrophobic species, it can permeate both membranes and react with oxidized (or reduced) moieties in the cytoplasm (Figure 1A):

$$R1 + O2 = O1 + R2 \qquad \text{(inside the cell)} \qquad (2)$$

The regeneration of the mediator species (reaction 2) produces an enhancement in the faradaic current at the tip electrode (positive feedback) depending on the tip/substrate separation distance (d). In a similar experiment (Figure 1B), a hydrophilic mediator species can only permeate the outer membrane and enter the periplasm. If the periplasm (or the cytoplasmic membrane[2]) contains redox centers capable of reacting with R1, the mediator regeneration occurs as described above. Finally, if the formal potential of the mediator is too positive (or too negative) for its reoxidation (or rereduction) by *Rb. sphaeroides*, the cell acts as an insulator, and negative feedback is observed (Figure 1C).

The apparent rate constant of the mediator regeneration process (k) can be evaluated by fitting the experimental tip current vs tip/cell distance (i_T vs d) curves to the theory.[15] The analysis of the dependencies of k on the concentration and formal potential of the redox mediator yields information on the pathway and kinetics of the multistep transmembrane CT reaction.[5,16] This experimental approach can also be used to measure kinetics of photochemical ET in the reaction center without extracting it from the cell. Recently, electron-transfer cofactors in the membrane-bound reaction center (RC) protein of *Rb. sphaeroides* were probed by direct voltammetry in lipid films, and peaks attributable to the reduction of the quinone A cofactor and the oxidation of the primary electron acceptor were identified.[17] By using the SECM to probe the ET kinetics in intact bacteria, one can avoid complicated and time-consuming purification of membrane-bound proteins and study them in their natural, intracellular environment. Matsue's group showed recently that photosynthetic activity of chloroplasts can be monitored by a microscopic amperometric sensor.[18] Although our primary goal here is to characterize the *Rb. sphaeroides* redox reactivity in the dark, we will also check how the irradiation modifies the redox response of the bacteria.

EXPERIMENTAL SECTION

Chemicals. Hexammineruthenium(III) chloride was obtained from Strem Chemicals. Potassium ferricyanide and benzoquinone were from Sigma. 1,2-Naphthoquinone, ferrocenecarboxylic acid, 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO; free radical, 98%), and 1,1′-ferrocenedicarboxylic acid (all from Aldrich), menadione (General Biochemicals), 1,4-naphthoquinone and N,N,N′,N′-tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD, from Eastman Kodak Co.), sodium ferrocyanide (Fisher Scientific), and all other chemicals were reagent grade. All aqueous solutions were prepared from deionized water (Mill-Q, Millipore).

Electrodes. A two-electrode setup was used for SECM experiments with either a 5.5-μm-radius carbon fiber UME tip, a 5.0-μm-radius Pt tip, or a 2.5-μm-radius Pt tip as a working electrode and a 0.25-mm Ag/AgCl electrode as a reference electrode. The tips were prepared as described previously[19] and polished with 0.05-μm alumina before each experiment. The current vs distance curves obtained with those tips at conductive and insulating substrates fit well the theory for RG = 10.

Cell Culture. Cells of the purple photosynthetic bacterium (*Rb. sphaeroides* wild-type strain 2.4.1) were grown anaerobically in modified Hutners media.[20] The cells were harvested by centrifugation at 7500*g* for 10 min and stored as frozen pellets at −20 °C. Prior to each experiment, adherent cells that had been plated at low density in a 60-mm plastic culture dish were washed with phosphate-buffered saline (PBS; 153 mM Na^+, 4 mM K^+, 1 mM Ca^{2+}, 1 mM Mg^{2+}, 144 mM Cl^-, and 10 mM phosphate, pH 7.4).

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Instrumentation and Procedures. Voltammetry of different redox mediators was performed in the same phosphate buffer solution in which the experiments with bacteria were carried out. Steady-state voltammograms were obtained in a two-electrode regime using a BAS 100B electrochemical workstation (Bioanalytical Systems, West Lafayette, IN) with either a 25-μm Pt or an 11-μm C working electrode. The half-wave potentials of redox mediators were measured with respect to a homemade Ag/AgCl reference (+60 mV vs commercial Ag/AgCl in PBS), which was also used in SECM experiments.

All SECM measurements were performed at ambient temperature (23 ± 2 °C) in a plastic culture dish mounted on a horizontal stage of the SECM. The SECM apparatus and procedures were as described previously.[12,21] Three types of experiments were performed: (i) the i_T was recorded as a function of the tip position as the tip was moved laterally in a horizontal ($x-y$) plane a few micrometers above the bacterium surface; (ii) similarly, a gray scale image of the bacterium was obtained by recording variations in tip current while the probe was scanned in the $x-y$ plane above the bacterium; and (iii) i_T vs d curves were obtained by positioning the tip above the bacterium and slowly moving it vertically down to the bacterium surface (typically at a rate of 0.5 μm/s). The analysis of i_T-d curves was based on an earlier model.[12,15]

Before each experiment, a pellet of bacteria was dispersed in 5 mL of PBS buffer and was centrifuged at 4500 rpm for 10 min. The supernatant was removed, and the pellet was reconstituted by the addition of 5 mL of PBS buffer and centrifuged again as above. This step was repeated 4 times. Finally, the pellet was dispersed in a plastic culture dish with a final volume of 5 mL of PBS buffer containing the redox mediator at the specified concentration and bacteria were allowed to deposit on the bottom of the plastic dish.

With air-sensitive redox mediators (e.g., menadione and naphthoquinone), both voltammetry and SECM experiments were performed in deaerated solutions. To prevent damage to the bacteria, oxygen was removed from the medium only for the brief period of time required for actual measurements. After positioning the tip in solution, a flow of nitrogen was passed through a small volume (~5 mL) of aqueous solution covering the bacteria. The nearly complete removed of O_2 was evident from cyclic voltammograms.

A halogen projector lamp (EVW, 250W, Ushio) was used as a light resource when the cells were illuminated. The lamp was kept 10 in. away from the bacteria, and a flat bottle of water was put between them to filter out infrared light. After finding an immobilized bacterium and positioning the tip above it, the lamp was turned on, and the current−distance curve was obtained. The lamp was turned off immediately after the data were recorded to avoid the increase in temperature in the culture dish.

RESULTS AND DISCUSSION

Four SECM current−distance curves are shown in Figure 2. Curve 1, which was obtained with a tip approaching the plastic surface of the culture dish, fits the theory for a diffusion-controlled process with an insulating substrate (solid line). In three other curves (2−4), the same tip approached *Rb. sphaeroides* cells in solutions containing different hydrophobic redox species, i.e., 1,2-naphthoquinone (curve 2), 1,4-naphthoquinone (curve 3), and menadione (curve 4). In each case, a quinone species was reduced to a diol at the tip at a diffusion-controlled rate:

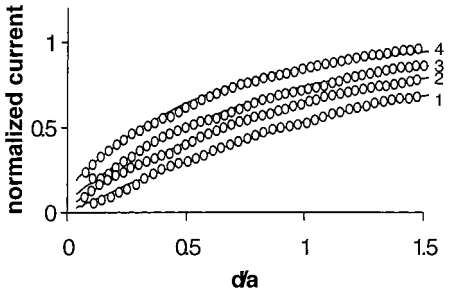

Figure 2. Approach of the carbon tip to the plastic surface (1) and bacteria cells (2−4). Phosphate buffer (pH 7.4) contained 30 μM 1,4-naphthoquinone (1, 3), 30 μM 1,2-naphthoquinone (2), and 30 μM menadione (4). Solid lines are theoretical curves for an insulating substrate (1) and finite heterogeneous kinetics (2−4). The tip current is normalized by the $i_{T,\infty}$ value measured in the bulk solution. The tip−substrate separation distance is normalized by the tip radius, a = 5.5 μm.

$$RO_2 + 2e^- + 2H^+ = R(OH)_2 \qquad \text{(at the tip)} \qquad (3a)$$

Similarly to our earlier studies of mammalian cells,[5,12] the reduced form of a hydrophobic quinone mediator penetrated into the cell and was reoxidized via a bimolecular reaction with some intracellular redox moieties (Figure 1A)

$$R(OH)_2 - 2e^- = RO_2 + 2H^+ \qquad \text{(inside the cell)} \qquad (3b)$$

The I_T in curves 2−4 (Figure 2) was higher than in curve 1, indicating that the mediator was regenerated by the cell at a measurable rate. The effective heterogeneous rate constants of reaction 3b (k) obtained by fitting a large set of current−distance curves to the SECM theory are (0.7 ± 0.2) × 10^{-3} (1,2-naphthoquinone), (1.4 ± 0.2) × 10^{-3} (1,4-naphthoquinone), and (2.6 ± 0.2) × 10^{-3} (menadione) cm/s. One should notice that the first two rate constants are much lower than the k values measured for the same mediators in our previous experiments with breast cells (i.e., $k \geq 10^{-2}$ cm/s for both 1,2-naphthoquinone and 1,4-naphthoquinone). Based on our previous analysis of cellular responses,[5] this difference can be attributed either to (i) a slower rate of generation of redox components (which can oxidize the quinone species) in purple bacteria compared to that in breast cells, (ii) a more negative intracellular redox potential in *Rb. sphaeroides* cells, or (iii) a slower bacterial membrane permeation by mediator species. The first explanation may appear plausible because *Rb. sphaeroides* is significantly smaller than a breast cell (though the *Rb. sphaeroides* cell radius, ≤10 μm, is still larger than the tip radius). However, the rate constant of menadione regeneration by *Rb. sphaeroides* is within the range of k values measured for different breast cell lines (1.5 × 10^{-3}−3.8 × 10^{-3} cm/s[5]). This is not consistent with an assumption of slower generation of redox centers in *Rb. sphaeroides* cells. The intracellular redox potential of *Rb. sphaeroides* cells may be only slightly more negative than that in breast cells (see below). Thus,

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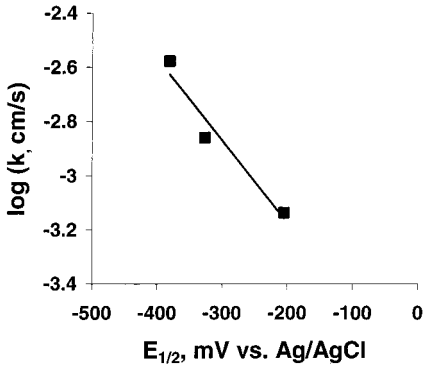

Figure 3. Dependence of the effective heterogeneous rate constant of oxidation of quinone mediators by purple bacteria on redox potential of mediator species. For parameters, see Figure 2.

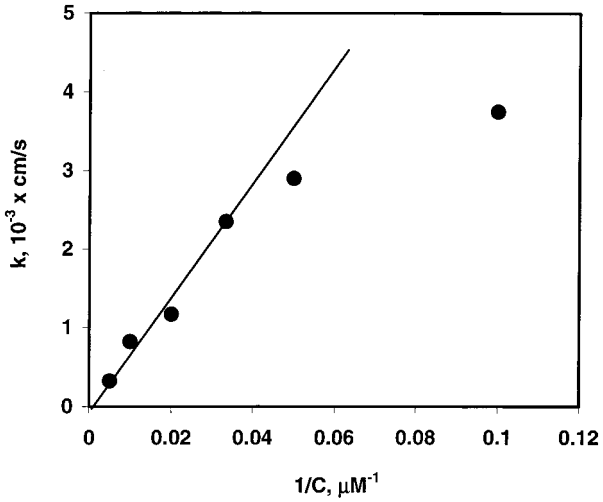

Figure 4. Dependence of the effective heterogeneous rate constant on concentration of menadione in PBS. The tip was a 5.5-μm-radius carbon fiber.

membrane permeability seems to be an important factor limiting the overall CT rate in *Rb. sphaeroides* cells.

Because the driving force for reaction 3b is determined by the difference between the mixed intracellular potential and the formal potential of the redox mediator (the latter can be approximated by the reversible half-wave potential of the quinone species, $E_{1/2}$), the linear dependence of log k vs $E_{1/2}$ can be expected. The linear dependence of log k on $E_{1/2}$ observed in purple bacteria (Figure 3) is in sharp contrast with the rates of oxidation of the same quinone mediators measured at mammalian cells.[5] No correlation between the formal potential of a redox mediator and the oxidation rate constant was found in those experiments.

The slope of a straight line in Figure 3 corresponds to the effective transfer coefficient, $\alpha = 0.18$. This number is significantly lower than the α value of ~0.5 expected from the Marcus theory for bimolecular ET reactions.[22]

This is another indication that there are other factors besides the mediator potential (e.g., intracellular concentration of redox centers, membrane permeability, etc.) that determine the overall rate of transmembrane CT process. The role of steady-state intracellular concentration of redox centers can be evaluated from Figure 4, which shows the dependence of the effective rate constant of menadiol oxidation by bacteria on concentration of menadione in PBS. According to earlier analysis,[5,16,23] the measured k value should be independent of $C_{\text{menadione}}$ as long as the intracellular concentration of redox centers is much higher (i.e., at least by the factor of 10) than $C_{\text{menadione}}$ in solution. Conversely, when the intracellular concentration of redox species capable of oxidizing menadiol is lower than the concentration of a mediator in solution, k is expected to be inversely proportional to $C_{\text{menadione}}$. From Figure 4, the effective concentration of redox centers in *Rb. sphaeroides* is of the order of 50−100 μM, i.e., similar to the values found in breast cells.[16]

The results of kinetic experiments with several hydrophobic redox mediators presented in Table 1 can be analyzed to evaluate the intracellular redox potential. Clearly, the effective rate constant of mediator oxidation by *Rb. sphaeroides* cells decreases as the formal potential becomes more positive. For benzoquinone mediator ($E_{1/2} = -150$ mV vs Ag/AgCl at pH 7.4), the oxidation rate constant is too low to measure under our experimental conditions (i.e., $k \lesssim 3 \times 10^{-4}$ cm/s). The reduction of a somewhat more positive redox species ($TMPD^+$) was also immeasurably slow. However, more positive species such as $TMPD^{2+}$ or TEMPO were readily reduced. These observations suggest that the potential value encountered by hydrophobic mediator species inside a *Rb. sphaeroides* cell is within the range −110 ± 90 mV vs Ag/AgCl (+160 mV vs NHE). Of the cofactors in the RC protein, this value is closest to that of quinone A in solution, ~−50 mV vs NHE. at pH 8.[24] It is possible that our value reflects reactions of the quinone pool that resides in hydrophobic regions of the cytoplasmic membrane.[24b,25] The relatively low rate constants measured for the reductions of two rather positive species point to additional limitations, e.g., slow membrane permeation.

Table 1. Dependence of Heterogeneous Rate Constant on Redox Potential of Hydrophobic Mediators

redox species in solution (30 μM)	$E_{1/2}$, mV vs Ag/AgCl	cell reaction	k, 10^{-3} × cm/s
menadione	−81	oxidation	2.6 ± 0.2
1,4-naphthoquinone	−327	oxidation	1.4 ± 0.2
1,2-naphthoquinone	−205	oxidation	0.7 ± 0.2
benzquinone	−150	oxidation	negative feedback
TMPD (first wave)	−29	reduction	negative feedback
TMPD (second wave)	402	reduction	2.6 ± 0.1
TEMPO	444	reduction	1.2 ± 0.1

Another family of current−distance curves was obtained for several hydrophilic redox mediators (Figure 5). Such mediators do not penetrate the membranes of mammalian cells. Accordingly, only negative feedback responses were obtained when an SECM tip approached a breast cell in solution containing a hydrophilic redox species (e.g., ferrocyanide or ferrocenecarboxylate).[5] In contrast, *Rb. sphaeroides* cells can reduce (or oxidize) hydrophilic mediators at a measurable rate. Although the reduction rate of the more positive ferrocenedicarboxylic acid is significantly faster

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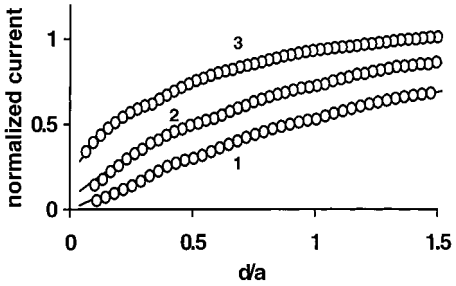

Figure 5. Approach of a 5-μm-radius tip to the plastic surface (1) and bacteria cells (2, 3) in phosphate buffer solution containing hydrophilic redox species. The redox mediator was 30 μM FcCOONa (1, 2) and 30 μM $Fc(COOH)_2$ (3). Solid lines are the theoretical curves for an insulating substrate (1) and finite heterogeneous kinetics (2, 3).

Table 2. Dependence of Heterogeneous Rate Constant on Redox Potential of Hydrophilic Mediators

redox species in solution (30 μM)	$E_{1/2}$, mV vs Ag/AgCl	cell reaction	k, 10^{-3} × cm/s
$Ru(NH_3)_6^{3+}$	-226	oxidation	1.9 ± 0.3
$Fe(CN)_6^{3-}$	113	oxidation	1.3 ± 0.3
$Fe(CN)_6^{4-}$	113	reduction	negative feedback
$FcCOO^-$	246	reduction	0.9 ± 0.1
$Fc(COO)_2^{2-}$	367	reduction	2.4 ± 0.4
$Ru(CN)_6^{4-}$	653	reduction	1.6 ± 0.2

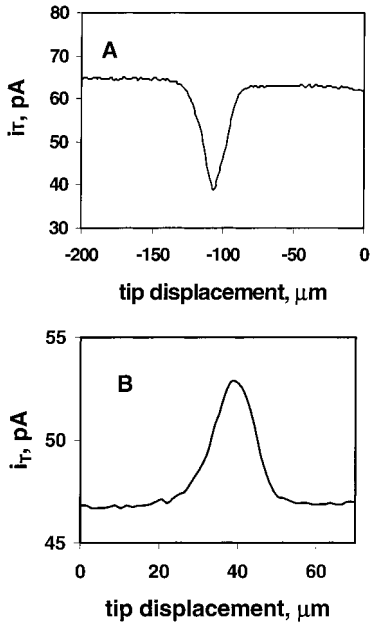

Figure 6. Current vs tip position dependencies for an SECM tip scanned laterally over a *Rb. sphaeroides* cell in solution containing (A) 30 μM *p*-benzoquinone and (B) 30 μM $Ru(NH_3)_6Cl_3$. The tip was a 5.5-μm-radius carbon fiber (A) and a 5.0-μm-radius Pt (B). The tip potential was −350 (A) and −450 mV (B).

than that for a less positive ferrocenecarboxylate, a more extensive data set (Table 2) does not reveal linear log k vs $E_{1/2}$ dependence. This indicates that membrane permeation is an important rate-limiting factor for charged hydrophilic species. Hence, relatively slow reduction of very positive $Ru(CN)_6^{3-}$ species by the cells.

One should notice that the intracellular potential value (∼−110 mV) extracted from Table 1 is inconsistent with the results obtained for hydrophilic mediators (Table 2). If the intracellular potential were that negative, the reduction of $Fe(CN)_6^{3-}$ rather than oxidation of $Fe(CN)_6^{4-}$ would be observed. From the data presented in Table 2, the intracellular potential appears to be 180 ± 70 mV vs Ag/AgCl (+450 mV vs NHE). This is closest to values of 453−505 V vs NHE reported for the primary electron acceptor of the RC protein in pH 7.4 to pH 8 solutions.[26−28] RC is a transmembrane protein, and the primary acceptor residence sites are probably amphiphilic in nature.[25,29,30] The difference in apparent potential values obtained with two types of mediators indicates that hydrophilic and hydrophobic mediators react with different types of redox centers. This may happen because hydrophilic ions can only cross the outer cell membrane of a Gram-negative bacterium and enter its periplasm, while hydrophobic redox species are also capable of permeating the cytoplasmic membrane (Figure 1). Another possibility is that some intracellular redox centers may be accessible only to hydrophilic (or hydrophobic) species.

When the tip was scanned horizontally above the cells in a plane parallel to the bottom of the dish and benzoquinone was employed as a redox mediator, the tip current above the cell was significantly lower than that above the plastic surface (Figure 6A). This result is in agreement with an immeasurably low rate constant of benzoquinone regeneration (Table 1). Accordingly, a gray scale image of a *Rb. sphaeroides* cell shows negative SECM feedback for the entire cell surface (Figure 7A). By contrast, in a solution containing 30 μM $Ru(NH_3)_6Cl_3$, the i_T increased when the tip was scanned horizontally above the cell (Figure 6B). In this case, positive feedback could be expected because of a fairly high k value of $Ru(NH_3)_6^{2+}$ oxidation (Table 2). Unlike mammalian cells for which two closely spaced current maximums are typically observed when the tip is scanned over the center of the cell,[12] only one peak was obtained during the tip scan above a bacterium. The difference is due to the absence of a nucleus in *Rb. sphaeroides* cells. This point is further illustrated by the map of redox reactivity in two individual bacteria (Figure 7B). In breast cells, the bright halo over the cell cytoplasm signified the region of high redox activity, and a dark, redox inactive area in the center of the cell was attributed to a nucleus that is impenetrable to the mediator species.[5] In contrast, the tip current is higher over the center of the *Rb. sphaeroides* cell (because these bacteria are shaped like a spherical cap, and the central part gets closer to the tip during the constant-height scan) and lower over the periphery of the cell.

To check the effect of light on the cellular redox response, the current−distance curves were obtained with a SECM tip approaching irradiated *Rb. sphaeroides* cells. The rate constants of regeneration of TMPD and $Fc(COOH)_2$ by illuminated cells were significantly lower than the k values in the dark (Table 3). This observation agrees well with the report by Yasukawa et al.,[18] who showed that the rate of mediator reduction by an algal protoplast decreased under illumination while the oxidation rate

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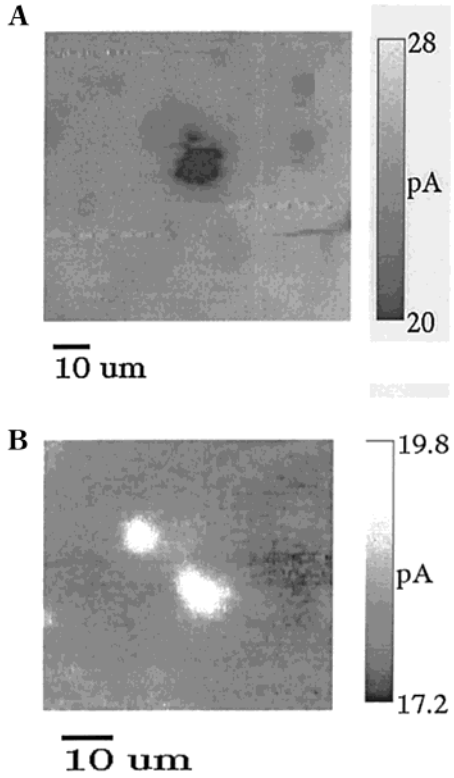

Figure 7. *Rb. sphaeroides* cells imaged with a 2.5-μm-radius Pt tip in pH 7.4 PBS containing 30 μM (A) benzoquinone and (B) $Ru(NH_3)_6Cl_3$.

Table 3. Effect of Illumination on Heterogeneous Rate Constants Measured with Different Mediators[a]

mediator (30 μM)	cell reaction	rate constant, cm/s $\times 10^{-3}$ [a] without illumination	rate constant, cm/s $\times 10^{-3}$ [a] illuminated
TMPD	reduction	2.6 ± 0.1 (5)	2.0 ± 0.2 (9)
$Fc(COOH)_2$	reduction	2.4 ± 0.4 (6)	0.8 ± 0.1 (14)
menadione	oxidation	2.6 ± 0.2 (9)	1.6 ± 0.5 (15)

[a] The number of experiments with different cells of the same type is indicated in parentheses.

increased. Both effects were attributed to photosynthetic activity in the protoplast. It is possible that light converts the primary donor P to P^+, so that the rate of reduction of mediators oxidizing P decreases since its concentration would be smaller under illumination. However, the oxidation of menadiol also became slower under illumination (Table 3). Thus, the observed behavior is rather complicated, and the interplay of photosynthesis and other cellular redox reactions determining the SECM response has yet to be clarified.

CONCLUSIONS

Redox reactivity of *Rb. sphaeroides* cells has been probed by the scanning electrochemical microscope using a number of hydrophobic and hydrophilic redox mediators**.** Hydrophobic redox species are capable of permeating both the outer cell membrane and cytoplasmic membrane. The effective rate constants of redox reactions of such mediators (e.g., different quinones) with intracellular redox moieties have been measured and shown to correlate well with the mediator formal potential. From kinetic analysis, the effective concentration of intracellular redox centers capable of oxidizing menadiol was found to be of the order of 50−100 μM, and the intracellular potential encountered by hydrophobic redox species was evaluated as −110 ± 90 mV vs Ag/AgCl (+160 mV vs NHE). Hydrophilic ionic species can only cross the outer membrane of a bacterium and react with redox centers in its periplasm. This may be the reason for a different apparent value of intracellular potential (180 ± 70 mV vs AgCl; 450 mV vs NHE) found from regeneration rates of hydrophilic mediators. An alternative explanation is that hydrophobic and hydrophilic species may react with different intracellular redox centers.

Topography and redox reactivity of *Rb. sphaeroides* cells were imaged by SECM with a micrometer spatial resolution. The maps of redox reactivity in *Rb. sphaeroides* look strikingly different from those obtained for breast cells in refs 5 and 12. The higher redox response in the center of a *Rb. sphaeroides* cell (as opposed to the lower redox activity in the center of a breast cell) can be explained by the absence of a nucleus in bacteria.

Although the irradiation of purple bacteria with visible light affects their redox responses, further efforts are needed to prove the possibility of probing photochemistry of the reaction center in those cells by SECM.

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