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LOCALIZATION OF THE SECONDARY QUINONE-BINDING SITE IN REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHAEROIDES* R-26 BY ANTIBODY INHIBITION OF ELECTRON TRANSFER

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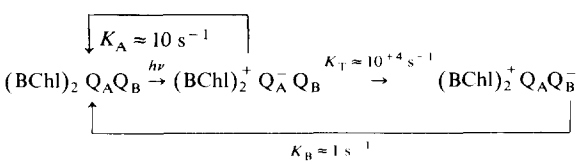
Inhibition of the electron transfer from Q_A to Q_B was measured in the presence of Fab fragments of antibodies directed against the subunits of reaction centers of *Rhodopseudomonas sphaeroides* R-26. Anti-M Fab inhibited the electron transfer, whereas anti-L Fab and anti-H Fab did not. From these experiments, we conclude that the binding site for Q_B is located on the M-subunit.

The photosynthetic reaction center is an integral membrane protein composed of three subunits, L, M, and H, and the cofactors: four bacteriochlorophylls, two bacteriopheophytins, one Fe^{2+} and two ubiquinones. A specialized dimer of bacteriochlorophyll [$(BChl)_2$] functions as a light-induced electron donor and two ubiquinones, Q_A and Q_B, as electron acceptors (for a review, see Ref. 1).

The primary quinone, Q_A, has been shown by photoaffinity labeling to bind to the M-subunit [2]. However, no suitable quinone analog has so far been found for photoaffinity labeling the secondary quinone site. The reason for this failure is believed to be the more stringent structural requirement for functional binding of Q_B at this site [3].

In the presence study we have determined the location of the Q_B-binding site by measuring the inhibition of the electron transfer from Q_A to Q_B in the presence of affinity-purified antibodies directed against the different reaction center subunits. The electron transfer was analyzed by measuring the kinetics of charge recombination between the reduced quinones and $(BChl)_2^+$.

The simplified scheme for the light-induced electron-transfer process is as follows [4,5]:



The rate of charge recombination between Q_A^- and $(BChl)_2^+$ is an order of magnitude faster ($K_A \approx 10\ s^{-1}$) than between Q_B^- and $(BChl)_2^+$ ($K_B \approx 1\ s^{-1}$). Thus, by decomposing the kinetics of charge recombination following a saturating laser flash into a slow and a fast component, the fraction of electrons that recombine from Q_A^- and Q_B^- can be determined [6,7]. The precentage of slow component is, therefore, a measure of the fraction of reaction centers with functional Q_B.

Reaction centers were isolated and depleted of Q_B, as previously described [1,8]. Affinity-purified antibodies against L-, M- and H-subunits were obtained according to published procedures, and were shown by radioimmunoassay to bind to reac-

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tion centers under the conditions used in this study [9]. Fab fragments, prepared from antisera by digestion with papain [10], were used to prevent formation of reaction center-antibody aggregates. Ubiquinone [Q-10 (Sigma Chemical Co.)] was added to reaction centers from a stock solution of 240 μM in 1% lauryl dimethylamine *N*-oxide. The concentrations of quinone, reaction center and Fab were determined according to published procedures [11–13].

Kinetic measurements were performed using a modification of the spectrometer described previously [14]. The pulsed dye laser (Phase-R DL-2100C) operated at $\lambda = 584$ nm with a flash duration of 0.4 μs and energy of 0.2 J/pulse. Absorbance changes were digitized on a Nicolet 1090A digital oscilloscope. The logs of the absorbance changes were obtained and plotted versus time using a Cromemco Z-2 computer. Exponential recovery rates and amplitudes were obtained graphically.

To test whether antibodies block transfer of electrons to Q_B, Fab fragments of twice affinity-purified antibodies against the L-, M- and H-subunits were added together with Q-10 to reaction centers having one quinone per reaction center. The samples were illuminated with a laser flash and the kinetics of charge recombination were monitored (Fig. 1). The samples containing anti-H Fab and anti-L Fab showed the same slow kinetics (characteristic of the charge recombination between Q_B^- and $(BChl)_2^+$) as the control without Fab. Thus, in these samples the electron transfer to Q_B was not inhibited. The sample that contained antibodies against subunit M exhibited fast recovery kinetics (characteristic of the charge recombination between Q_A^- and $(BChl)_2^+$), similar to samples to which *o*-phenanthroline, a known inhibitor of electron transfer to Q_B, had been added. Thus, anti-M inhibited the electron transfer from Q_A to Q_B. A detailed analysis of the kinetics showed that only 8% of the reaction centers exhibited a slow component in the presence of anti-M. Eight different affinity-purified anti-M Fab preparations (each from a separate rabbit) were tested; they all showed similar behavior. Six affinity-purified anti-L Fab and five anti-H Fab were tested; none of these inhibited electron transfer from Q_A to Q_B. The samples containing anti-H

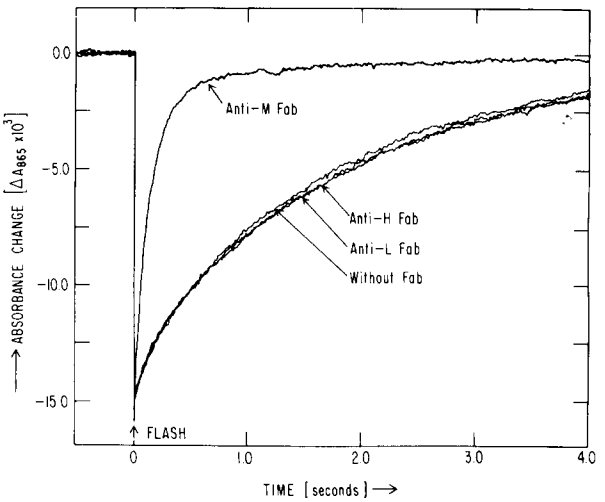

Fig. 1. Recovery kinetics (following a laser flash) of absorbance changes at 865 nm of reaction centers from *Rps. sphaeroides* in the presence of Q-10 and affinity-purified antibodies. Fab (1.4 μM) was added to reaction centers (0.15 μM in 0.1% lauryldimethylamine *N*-oxide, 14 μM EDTA, 10 mM Tris-HCl, pH 8), having one (Q-10) per reaction center. After incubation for 11 h at 4°C, Q-10 (3.4 μM) was added and the sample was incubated for an additional 5 h at 4°C. The samples were flashed at 4°C with a dye laser at $\lambda = 584$ nm; pulse width 0.4 μs, energy 0.2 J/pulse. Each trace represents a computer average of four experiments. A sample containing *o*-phenanthroline (5 mM) gave kinetics similar to those of the one containing anti-M Fab (data not shown). The results show that anti-M inhibits electron from Q_A to Q_B.

Fab from one preparation showed a 30% increase in the rate of charge recombination between Q_B^- and $(BChl)_2^+$. This suggests that the environment of Q_B is sensitive to the presence (or conformation) of the H-subunit. Independent experiments showed that the presence of the H-subunit is required for functional electron transfer to Q_B [15].

To investigate the role of the isoprenoid chain, the above experiments were repeated with Q-2 substituted for Q-10 as the secondary acceptor. The results were qualitatively similar to those shown in Fig. 1, although the kinetics of the slow component of charge recombination could not be fitted well with a single exponential. This suggests that Q-2 does not assume a unique position in the Q_B site and points to the importance of the long isoprenoid chain in anchoring the quinone in a unique position.

In order to determine whether Q_B and anti-M Fab compete for the same binding site on the

reaction center, the effect of the order of addition of the components on the rate at which anti-M inhibits Q_B function was studied. If antibody and quinone bind to the same site, the presence of quinone should delay the inhibitory action of the antibody. For each experiment, two sets of samples were prepared and assayed in parallel. In one set, reaction centers (containing one quinone per reaction center) were incubated with quinone before Fab was added. In the other set, the reaction centers were first incubated with Fab before quinone was added. The kinetics of charge recombination in these samples were then assayed and the amount of slow component (indicative of functional Q_B) determined. The result of one set of experiments using anti-M Fab is shown in Fig. 2a and the control using nonspecific Fab in Fig. 2b. It can be seen that the order of addition of the components makes a large difference (Fig. 2a). The sample that was incubated first with excess quinone shows very little inhibition for the first few hours, whereas the sample incubated first with Fab is almost totally inhibited (15% slow component). Thus, the presence of excess quinone protects (on a time scale of hours) the reaction center from antibody inhibition, indicating competitive binding between anti-M Fab and Q-10. The simplest interpretation of these results is that anti-M and Q-10 compete for the same Q_B-binding site, although a less likely explanation involving a conformational change upon binding cannot be excluded.

Antibody inhibition of Q_B binding indicates that a substantial portion of the Q_B-binding site is accessible to antibodies; its location is presumably near the surface of the reaction center protein. This is not too surprising in view of the observed protonation of Q_B [16,17], a process that requires communication with the outside environment.

To test whether the binding site of Q_A is accessible to antibodies, both quinones were removed from reaction centers prior to incubation with antibody [8]. None of the antibodies tested (four anti-L, five anti-M and five anti-H Fab preparations) prevented subsequently added Q-10 from restoring photochemical activity (measured by the extent of bleaching of the 865-nm peak following a saturating laser flash). These results indicate that the binding site for Q_A is located at a site inacessible to antibodies, presumably in the interior of the protein.

The M-subunit appears to bind both Q_A and

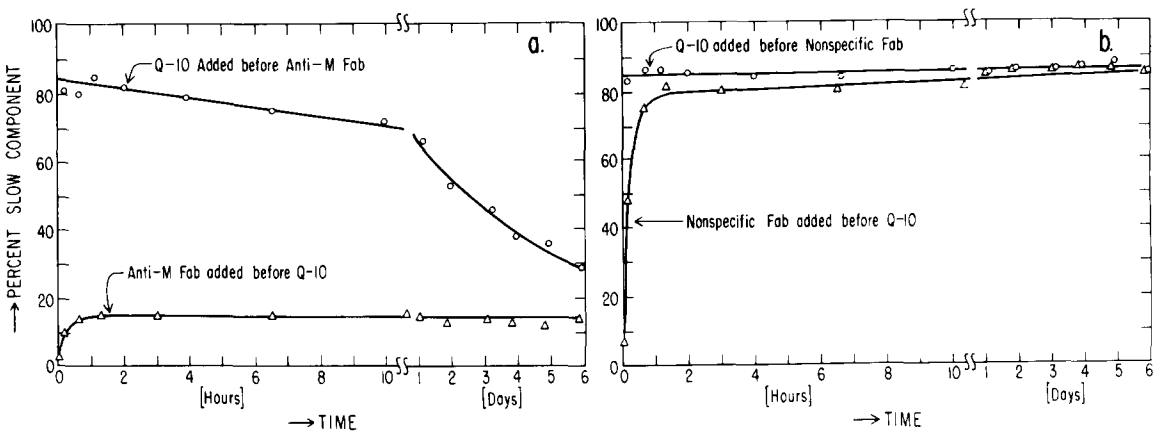

Fig. 2. Percent of slow component of the kinetics of charge recombination (indicative of functional Q_B) versus time of incubation with antibody and quinone. (a) Reaction center samples (0.2 μM, one Q-10 per reaction center, 0.1% lauryldimethylamine *N*-oxide, 10 mM Tris-HCl, pH 8, 10 μM EDTA) were either incubated first with anti-M Fab (1.4 μM) for 3 h at 4°C before adding Q-10 (5 μM) (▲——▲), or first incubated with Q-10 (5 μM) for 15 h at 4°C before adding anti-M Fab (1.4 μM) (○——○). In the latter case, when the quinone concentration was reduced from 5 to 1 μM, the time to reach 50% of slow component decreased from 85 to 20 h (data not shown). The results are characteristic of competitive inhibition indicating that anti-M Fab binds to the same site as Q-10. (b) Control with nonspecific Fab obtained from unimmunized rabbits. Other conditions same as in a.

Q_B and to serve as the quinone-binding protein in the bacterial reaction center. Other quinone-binding proteins associated with the mitochondrial respiratory chain and other photosynthetic electron-transport systems (e.g., Photosystem II of green plants) have been isolated and characterized (for a review, see Ref. 18). The antibody inhibition procedure described here may be useful in localizing the binding site of the quinones in these proteins as well.

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