S9-011

Comparison of electron transfer properties of bound iron-sulfur clusters between green sulfur bacterial reaction center and photosystem I

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1. Introduction

Photosynthetic reaction centers (RCs) of anaerobic green sulfur bacteria (PS-C) and PSI are considered to be evolutionarily related. They share many common features, particularly the presence of Fe-S clusters as low potential terminal acceptors that can reduce soluble ferredoxin (see, Sakurai et al., 1996). The two large polypeptides that make up the RC cores show substantial sequence homology with the core of PSI being heterodimeric and that of PS-C being presumably homodimeric (Büttner et al., 1992). In contrast with PSI, PS-C binds two molecules of monoheme cyt *c*, and involvement of quinone in e⁻ transfer chain is controversial. In PS-C, the presence of two Fe-Ss, equivalent to F_A and F_B of PSI and reffered to F_1, F_2 in the following, has been substantiated by several low-temperature EPR studies. Only small signal ascribed to F_X was observed until recently, but a clear F_X^- signal was reported more recently in isolated membranes and in purified PS-C of *C. tepidum* (Vassiliev et al., 2000). In flash-induced absorption change measurements made at room temperature, bleachings due to reduction of a single iron-sulfur cluster has been previously reported (Kusumoto et al., 1995). This paper describes studies of three different Fe-Ss, named F_X, F_1 and F_2 and of electron transfer kinetics involving them in PS-C from *C. tepidum* measured by flash-absorption spectroscopy.

2. Materials and methods

Growth of *C. tepidum*, and preparation of PS-C containing 5 kinds of polypeptide under strictly anaerobic conditions were as described by Kusumoto et al. (1999). The PS-C binds a total of three donors (P840 and two bound cyt *c*). Multiple-flash induced absorption change measurements were as described by Sétif et al. (2001).

3. Results and Discussions

In multiple flash experiments using PS-C that binds two cyt *c*, we provided evidence that only 3 stable acceptors can be photoreduced in the microsecond-millisecond time range. The

chemical identity of these acceptors was not fully assessed, but the relatively slow decay rates ($t_{1/2} > 5$ ms) and the absence of absorbance changes due to menaquinone were taken as evidence that Fe-Ss were involved (Kusumoto et al., 1999). In the present work, we show from measurements of flash-induced absorbance changes between 400 and 480 nm that three different Fe-Ss, named F_X, F_1 and F_2, can be photoreduced at room temperature during a series of consecutive flashes.

As shown in Fig. 1, reoxidation rates of reduced Fe-Ss were relatively slow and must occur through an escape process to some exogenous acceptor as there was neither $P840^+$ nor cyt c^+ available for a recombination reaction 10 ms after flash excitation due to rapid reduction of the oxidized donors by methoxy-PMS (m-PMS). A striking feature of our observations is a great change in the escape rate after reduction of the second Fe-S whereas reduction of the third Fe-S accelerates only marginally the faster phase of escape. When two or three Fe-Ss are reduced, the escape is biphasic, with the faster phase being 12- or 14-fold faster than the slower phase, which is similar to that observed after single reduction.
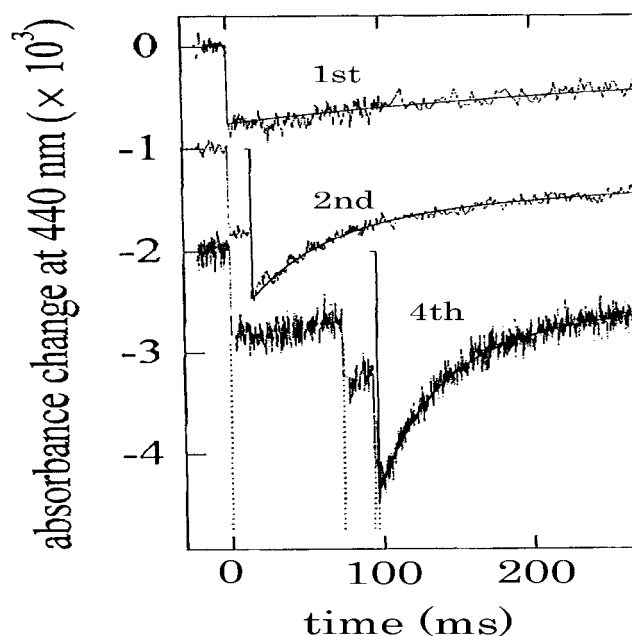
Fig. 1. Flash-induced absorbance changes measured at 440 nm. The standard reaction mixture contained PS-C ($A_{810} = 0.75$), 5 mM sodium ascorbate and 5 μM mPMS. Upper trace: single flash experiment (ruby laser). Middle trace: 2-flashes experiment (ruby lasers, $\Delta t = 15$ ms). Lower trace: 4-flashes experiment (Xe/Xe/ruby/ruby, $\Delta t = 75$ ms/20 ms/2 ms).
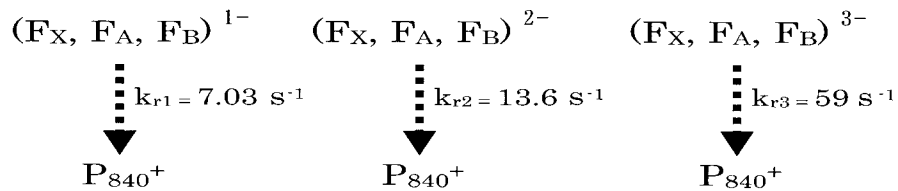
In order to study the charge recombination reactions between $P840^+$ and reduced Fe-Ss, we performed 1, 2 and 3-flashes measurements (for complete reduction of 3 Fe-Ss, the last one was actually 4 flashes) on a slow time scale in the absence of both ascorbate and mPMS in order to minimize processes of donation by exogenous donors and of escape to exogenous acceptors. After completion of the redox equilibrium between P840 and bound cyt c in less than 1 ms, about 6 and 20% of P840 stays oxidized after the first and second flashes, respectively (Kusumoto et al., 1999). This is followed by much slower decays of $P840^+$, from which one could get in principle the apparent rates of charge recombination. However, as these slow phases of $P840^+$ decay are relatively small in absorbance changes, it is more reliable to monitor the decay of cyt c^+ for studying the apparent recombination processes after one or two flashes. Such experiments were performed at 420 nm (maximum of bleaching due to oxidation of cyt c) and 440 nm (in order to correct for the contributions from Fe-Ss by subtracting ΔA_{440} from ΔA_{420}). The intrinsic recombination rates between

P840$^+$ and reduced Fe-Ss were estimated by taking into account of the fraction of P840$^+$ in equilibrium with cyt c. The rate constants thus obtained were 7, 14 and 59 s^{-1} after one, two and three electrons reduction of the Fe-Ss, respectively (Fig. 2). Thus the charge recombination rates were much slower than those found in PSI.

Calculation of the intrinsic rates of charge recombination

We assume that escape is poorly efficient after single charge separation because the firstly reduced Fe-S (F$_1$) is not accessible to exogenous acceptors. As soon as 2 (or 3) Fe-Ss are reduced, escape can occur from the other Fe-S (F$_2$) which is much more accessible to acceptors (Fig.1). F$_2$ is responsible for escape, and the escape rate depends mostly on the population of F$_2^-$ under stationary conditions of equilibrium between Fe-Ss. These assumptions appear to be the most likely way of interpreting our observations. Thus our data provide evidence for a functional asymmetry in accessibility of Fe-Ss to exogenous electron acceptors. This behavior is reminiscent of PSI, in which F$_B$ is the distal cluster which is the direct partner of soluble ferredoxin and is mostly responsible for electron escape to exogenous acceptors (Diaz-Quintana et al., 1998; Vassiliev et al., 1998), and F$_A$ is the proximal cluster which is preferentially reduced at low temperature (Golbeck, 1998). We tentatively associate F$_1$ to F$_A$ and F$_2$ to F$_B$.

For discussing recombination processes, we assume that whatever the number of reduced Fe-S is, recombination occurs from F$_X^-$ by thermal repopulation. This is related to the strong distance dependence of electron transfer, F$_X$ being most probably much closer to P840 than F$_1$ and F$_2$. If one neglects the contribution of the state (F$_X^-$ F$_1$ F$_2$) to the redox equilibrium, the fact that escape is 12-fold faster upon double Fe-S reduction than upon single reduction corresponds to: [F$_2^-$ F$_1$]/[F$_2$ F$_1^-$] = 1/ 12 so that $\Delta E_m = E_m$ (F$_2$) $- E_m$ (F$_1$) = - ln(12) $\times$ RT/F = - 63 mV. If one assumes that F$_X$ and F$_2$ have similar E_ms (see below), one can calculate

$$(F_X, F_A, F_B)^{1-} \qquad (F_X, F_A, F_B)^{2-} \qquad (F_X, F_A, F_B)^{3-}$$

$$\downarrow k_{r1} = 7.03 \text{ s}^{-1} \qquad \downarrow k_{r2} = 13.6 \text{ s}^{-1} \qquad \downarrow k_{r3} = 59 \text{ s}^{-1}$$

$$P_{840}^+ \qquad\qquad P_{840}^+ \qquad\qquad P_{840}^+$$

Fig. 2. Charge recombination rates between P840$^+$ and reduced Fe-Ss following multiple flash excitations. These values were calculated from the apparent rates of charge recombination and from the fractions of P840$^+$ in equilibrium with cyt c-551 after each flashes.
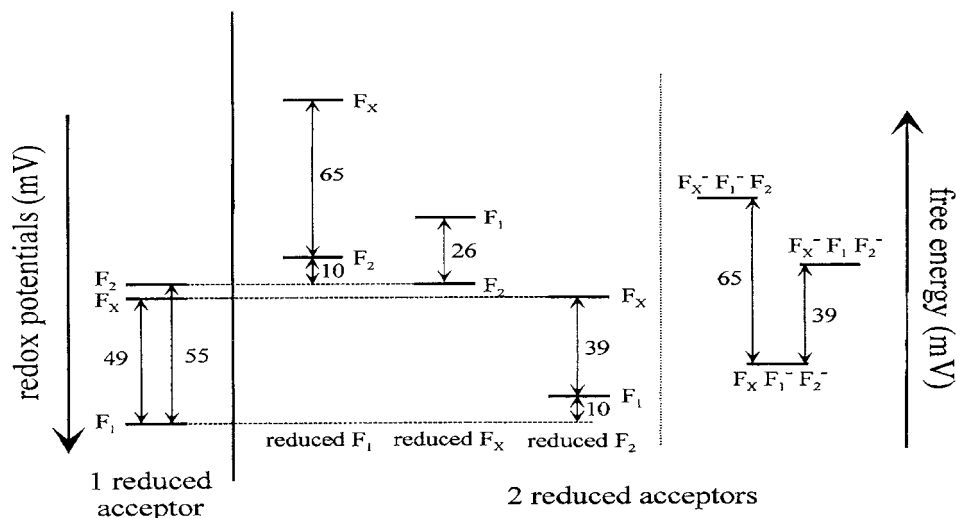
Fig. 3 A characteristic solution of redox potential differences of Fe-Ss in PS-C following multiple flash excitations

$$\Delta E_{\mathrm{m}} = -\ln(12 \times 2) \times RT/F = -81\ \mathrm{mV}.$$

From the preceding assumptions, comparison of the recombination rates after single and triple reduction of Fe-Ss ($k_{r1} = 7\ \mathrm{s}^{-1}$; $k_{r3} = 59\ \mathrm{s}^{-1}$) allows to calculate the amount of F_X^- thermally populated from F_1 after single Fe-S reduction. This amount is $100\ \% \times 7/59 = 12\ \%$ corresponding to a redox gap between F_X and F_1 of less than 60 mV ($-60\ \mathrm{mV} < E_{\mathrm{m}}(F_X)-E_{\mathrm{m}}(F_1) < 0$). One can note that this suggests than E_{m} of F_X is similar to, or even higher than, that of F_2, under single Fe-S reduction, i.e. when F_1 is in an oxidized state. This appears somewhat surprising both by analogy to PSI and by taking into account that no F_X^- is observed during redox titrations of Fe-Ss in PS-C (Scott et al., 1997). However, one must note that our estimates of relative E_{m}s are very crude because electrostatic interactions between Fe-Ss were neglected. One can speculate e.g. that reduction of F_1 may shift the E_{m} of F_X to more negative values due to electrostatic interactions.

Analyses of the rates of electron escape and of charge recombination after the second flash indicate that when F_1 is reduced, the difference $E_{\mathrm{m}}(F_2) - E_{\mathrm{m}}(F_X)$ is always positive, with a value of 45 mV (for $k_{r2} = 14\ \mathrm{s}^{-1}$, observed) and a minimum of 14 mV ($k_{r2} = 25\ \mathrm{s}^{-1}$, assuming an extreme case). Reduction of F_1 thus results in a larger lowering of $E_{\mathrm{m}}(F_X)$ compared to $E_{\mathrm{m}}(F_2)$, suggesting that the electrostatic interaction between F_X and F_1 is larger than that between F_2 and F_1. From the above analyses, we can provide the lower limits but can't provide any upper limit for the relative destabilization of F_X^- (compared to F_2^-) upon F_1 reduction. For illustration of these electrostatic effects, a particular example shown in Fig. 3 corresponds to an electrostatic repulsion between F_1 and F_X which is 71 mV larger than the electrostatic repulsion between F_1 and F_2: $E_{\mathrm{m}}(F_2) - E_{\mathrm{m}}(F_X)$ (F_1 reduced) $= E_{\mathrm{m}}(F_2) - E_{\mathrm{m}}(F_X)$ (F_1 oxidized) $+ 71\ \mathrm{mV}$.

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