In photosynthetic reaction centers, the free energy difference for electron transfer between quinones bound at the primary and secondary quinone-binding sites governs the observed secondary site specificity

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ABSTRACT The secondary quinone-binding site (Q_B site) of bacterial reaction centers from *Rhodobacter sphaeroides* is generally regarded to be highly specific for its native ubiquinone-10 molecule. We demonstrate here that this is a misconception rooted in the kinetic methods used to assay for occupancy of a quinone in the Q_B site. We show that observance of occupancy of the Q_B site, revealed by kinetic assay, is sensitive to the free-energy difference for electron transfer between the quinone at the primary quinone-binding site (Q_A site) and the Q_B site ($-\Delta G_e^0$). For many of the compounds previously tested for binding at the Q_B site, the $-\Delta G_e^0$ between Q_A and Q_B is too small to permit detection of the functional quinone in the Q_B site. With an increased $-\Delta G_e^0$ achieved by replacing the native ubiquinone-10 at the Q_A site with lower-potential quinones or by testing higher-potential Q_B candidates, it is shown that the Q_B site binds and functions with the unsubstituted 1,4-benzoquinone, 1,4-naphthoquinone, and 9,10-phenanthraquinone, as well as with their various substituted forms. Moreover, quinones with the *ortho*-carbonyl configuration appear to function in a similar manner to quinones with the *para*-carbonyl configuration.

The photochemical reaction center of photosynthetic bacteria is an integral membrane protein that contains four bacteriochlorophylls (BChls), two bacteriopheophytins (BPhs), and two quinones associated with discrete catalytic sites, designated the primary and secondary quinone-binding sites (Q_A and Q_B , respectively; for review, see ref. 1). After light excitation of a special pair of bacteriochlorophylls ($BChl_2$), an electron is transferred by way of BPh to the quinone at the Q_A site to form the semiquinone (Q_A^-) and the state $BChl_2^+ - Q_A^- Q_B$, where $BChl_2^+$ is the oxidized cation radical of $BChl_2$ (2). The Q_A^- then reduces the quinone bound at the Q_B site to form $BChl_2^+ Q_A Q_B^-$ (3, 4).

In the bacterial reaction centers from *Rhodobacter sphaeroides* the native ubiquinone-10 (UQ-10) molecules can be reversibly removed from both the Q_A and Q_B sites (5, 6). Moreover, the Q_A site can be functionally reconstituted with a wide variety of other natural and synthetic quinone structures (6–8). In contrast, with the exception of one observation (9), parallel efforts to reconstitute Q_B activity have been successful only with quinones containing the native ubiquinone (UQ) configuration (10–13). These negative findings have led to the sentiment that the Q_B site, unlike the Q_A site, is designed to provide stringent binding requirements for the UQ structure (10, 12, 13). However, such a view is not entirely consistent with the well-known, broad specificity of the Q_B site for a variety of herbicide structures (14–16). These apparently unusual characteristics have prompted us to

enquire further into the specificity of the Q_B site for quinone structures. We have questioned in particular the methods of assay used to establish the binding strength of a quinone to the Q_B site and have drawn upon the detailed general description for Q_B function that has been developed by Wraight and Stein (11).

The methods used to assay for the occupancy of a quinone in the Q_B site rely entirely on kinetic analysis of various flash-activated electron-transfer reactions within the reaction center that are altered by the presence of a functional quinone in the Q_B site. In these methods, an essential requirement for the recognition of a functional occupant of the Q_B site is electron transfer from the light-generated Q_A^- to Q_B . Wraight's model, which is summarized in Fig. 1, introduces other parameters that describe the interaction and reduction of quinones at the Q_B site. This model includes not only the rates of quinone interaction with the Q_B site (k_{on} and k_{off}), which define the dissociation constant ($K_d = k_{off}/k_{on}$), but also the rates of electron transfer between quinones occupying the Q_A and Q_B sites (k_{ab} and k_{ba}), which define the free energy of the reaction ($-\Delta G_e^0 = RT \ln k_{ab}/k_{ba}$). It is clear from Fig. 1 that, under certain conditions, the dissociation constant measured is not the true K_d but is an apparent value (K'_d). This situation is evident when equilibrium among the three charge-separated states (upper line of the scheme in Fig. 1) is attained under conditions when:

$$k_{ab} + k_{ba} \gg k_{ad} \quad \text{and} \quad k_{on}[Q] + k_{off} \gg k_{ad}. \quad [1]$$

Under these conditions, the K'_d is dependent on both the K_d and the $-\Delta G_e^0$ for electron transfer from Q_A to Q_B in the following way:

$$K'_d = K_d/[1 + \exp(-\Delta G_e^0/RT)]. \quad [2]$$

Eq. 2 shows that as $-\Delta G_e^0$ increases the K'_d for quinone at the Q_B site decreases and hence will increase the possibility of detecting and measuring the functional presence of a quinone in the Q_B site. We have used this device to reevaluate the specificity of the Q_B site.

Abbreviations: BPh, bacteriopheophytin; Q_A , primary quinone-binding site; Q_B , secondary quinone-binding site; $BChl_2$, special pair of bacteriochlorophyll molecules; Q_A^- , semiquinone formed in the Q_A site; Q_B^- , semiquinone formed in the Q_B site; UQ, UQ-10, and UQ-0, ubiquinone, -10, and -0, respectively; $-\Delta G_e^0$, free energy difference for electron transfer from Q_A to Q_B ; E_m , *in situ* oxidation-reduction potential; AQ, 9,10-anthraquinone; MeAQ, 1-methyl-9,10-anthraquinone; $BChl_2^+$, oxidized cation radical of $BChl_2$; cyt c, cytochrome c; BQ, 1,4-benzoquinone.

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FIG. 1. Reaction scheme for electron transfer from Q_A to Q_B and quinone binding at the Q_B site.

MATERIALS AND METHODS

Reaction Center Preparation. Reaction centers were isolated from *Rb. sphaeroides* strain R-26 (17). Extraction of UQ-10 from both the Q_A and Q_B sites, or the Q_B site only, was performed (6) with slight modifications (18).

Q_A Replacement. The procedure of Gunner *et al.* (8, 18, 19) was used for the reconstitution of Q_A activity with quinones other than the native UQ. All procedures were carried out in 10 mM Tris-HCl (pH 8.0) under aerobic conditions at $23 \pm 1^\circ\text{C}$. Detergent concentrations were maintained well below the critical micelle concentration and sufficiently constant to have no significant effect on our results (for discussion, see ref. 8).

Quinones chosen as Q_A replacements had *in situ* oxidation-reduction potential (E_m) values lower than UQ-10 (18, 19) so that the $-\Delta G_e^0$ for electron transfer from a quinone at the Q_A site to a particular quinone at the Q_B site would be increased.

Displacement of quinone in the Q_A site by a test quinone in the Q_B site was minimized by choosing Q_A replacements whose K_d values at the Q_A site were at least 60-fold tighter than the K_d values at the Q_B site for quinones chosen to reconstitute the Q_B site. The concentrations used to reconstitute the two quinones at the Q_A and Q_B sites were chosen so that occupancy of the Q_A site by the designated Q_A replacement was always $>90\%$. Conversely, under the conditions reported here, interference of the Q_A replacements with the Q_B site was not significant; Q_A replacements themselves neither displayed functional Q_B activity nor acted as competitive inhibitors of electron transfer from UQ-10 at the Q_A site to ubiquinone-2 at the Q_B site (unpublished results).

Spectroscopic Measurements. Reaction center protein was activated by a 10- μsec xenon flash (full width at half-maximum light intensity) that activated $\geq 85\%$ of the reaction centers.

In the visible region, the flash-induced absorbance changes in the oxidation state of BChl_2^+ were measured at its Q_X band using 605–540 nm while those of cytochrome *c* (cyt *c*) were monitored at its α -band by using 550–540 nm (20). In the near-infrared region, the kinetics of the flash-induced shift of the Q_Y band of BPh that reports electron transfer from Q_A to Q_B were monitored in the single-beam mode at 750 nm (3, 4).

Data Analysis. Kinetic traces were analyzed as described (19). K_d values for quinones at the Q_B site and the rate constant for quinone binding (k_{on}) at the Q_B site were determined with a nonlinear least squares analysis routine from the Asystant+ software, version 1.0 (MacMillan Software, New York).

Methods To Examine for Q_B Reconstitution. 1. Reduction kinetics of flash-generated BChl_2^+ . When quinone occupies only the Q_A site, the kinetics of charge recombination from $\text{BChl}_2^+ \text{Q}_A^-$ to $\text{BChl}_2 \text{Q}_A$ occur as a nearly single exponential

(see ref. 8) with a characteristic rate constant (k_{ad}); for example, the k_{ad} values for UQ-10 and 9,10-anthraquinone (AQ) are close to 7 and 70 sec^{-1} , respectively (19). With UQ-10 at both the Q_A and Q_B sites, the observed rate constant for reduction of BChl_2^+ is 0.7 sec^{-1} (21, 22). Under these conditions, the fractional occupancy of quinone at the Q_B site is proportional to the fraction of slow phase of the BChl_2^+ reduction kinetics and is readily observed (10, 11).

2. Ratio of cyt *c* oxidized on the second and first flashes. With Q_A present and Q_B absent in the reaction center, cyt *c* oxidation occurs only on the first flash (23). However, with Q_B functionally present, the oxidation of Q_A^- by Q_B permits cyt *c* oxidation on a second flash. Assuming the Q_A is kinetically capable of reducing Q_B (see method 3), the second/first flash ratio of cyt *c* oxidation is governed by Q_B occupancy and the $-\Delta G_e^0$ between Q_A and Q_B (22, 24).

3. Q_A^- to Q_B electron transfer observed by accompanying electrochromic shifts of BPh. The formation of Q_A^- induces a shift in the Q_Y transition of BPh that is distinct from that accompanying the formation of Q_B^- (3, 4). This assay is useful in that it distinguishes between the states $\text{BChl}_2^+ \text{Q}_A^- \text{Q}_B$ and $\text{BChl}_2^+ \text{Q}_A \text{Q}_B^-$ and permits the time resolution of electron transfer between Q_A and Q_B .

Chemicals. The quinones used in this work were obtained from several sources as follows: 1-methyl-9,10-anthraquinone (MeAQ) and 2,3-dimethyl-1,4-benzoquinone from J. Malcolm Bruce, University of Manchester; the latter was also obtained from Chang-An Yu, Oklahoma State University; we gratefully acknowledge the compounds provided by our colleagues. 1,4-Benzoquinone (BQ); 1,4-naphthoquinone; 9,10-anthraquinone (AQ); 9,10-phenanthraquinone; 3,5-di-*tert*-butyl-1,2-benzoquinone; and 2,3-dichloro-1,4-naphthoquinone were purchased from Aldrich. When necessary, these compounds were purified by recrystallization from petroleum ether (50–110°C). The purity of all quinones was established by reverse-phase HPLC.

The ferrous form of cyt *c* was prepared by reducing 1 ml of 10 mM cyt *c* (ferric form) (horse heart, type VI from Sigma) with 400 μl of freshly prepared well-buffered 100 mM sodium dithionite followed by removal of the excess dithionite on a Sephadex G-50 gel filtration column. The cyt *c* was 98% reduced after this treatment.

RESULTS

Q_B Site Reconstitution with BQ. As stated above, functional reconstitution of the Q_B site with quinones possessing the native UQ headgroup configuration is well-established. The tailless ubiquinone-0 (UQ-0) representing the minimal UQ structure, also functions in the Q_B site (11). In our investigations, the unsubstituted BQ was chosen as a candidate to test for binding at the Q_B site not only because it is the simplest quinone but also because its *in vitro* oxidation-reduction potential ($E_{1/2}$) value in dimethylformamide is substantially higher (≈ 0.14 V) than that of UQ-0 (25). Thus, if these dimethylformamide values are a reasonable guide to the values exhibited in the Q_B site (see ref. 19), then the $-\Delta G_e^0$ between UQ-10 at the Q_A site and BQ at the Q_B site should be favorable and the function and occupancy of BQ in the Q_B site should be detectable. However, if this is not the case, then the use of AQ or MeAQ to increase the $-\Delta G_e^0$ will improve the chance of detecting the reaction.

Fig. 2A shows that when the native UQ-10 occupies the Q_A site the activity of BQ at the Q_B site is only marginal in all three of the standard Q_B assays; at 20 μM BQ, Q_B activity is $<18\%$. However, when UQ-10 at the Q_A site is replaced with AQ (Fig. 2B) to increase the $-\Delta G_e^0$ by 0.15 eV, the activity of BQ at the Q_B site is increased substantially.

In Fig. 3, again with AQ in the Q_A site and BQ in the Q_B site, the kinetics of electron transfer from Q_A to Q_B were examined

FIG. 2. In reaction centers from *Rb. sphaeroides* R-26, the function of BQ at the Q_B site when the native UQ-10 at the Q_A site (A) is replaced with AQ (B). The reaction solution contained $0.15 \mu\text{M}$ reaction centers in 10 mM Tris-HCl/ 0.001% lauryldimethylamine oxide, pH 8.0, at 23°C in the absence (curve a) and presence (curve b) of $30 \mu\text{M}$ BQ. Conditions for A and B were the same except that in B reaction centers depleted of their native UQ-10 were reconstituted with $15 \mu\text{M}$ AQ. BChl $_2^+$ reduction kinetics were monitored at 605–540 nm. Cyt *c* oxidation on the first and second flash was monitored at 550–540 nm with a 272-msec flash interval. Conditions were as for the BChl $_2^+$ kinetics except that $2.03 \mu\text{M}$ cyt *c* (ferrous form) and $30 \mu\text{M}$ BQ were present. Cyt *c* oxidation in the absence of added Q_B was similar to the traces shown in Fig. 5B. Electron transfer from Q_A to Q_B was assayed at 750 nm in a reaction solution containing $10 \mu\text{M}$ reaction centers in the presence of $30 \mu\text{M}$ BQ. The scale for absorbance changes indicated on the right applies to all traces presented.

by the absorbance change at 750 nm (ΔA_{750}). Fig. 3A shows that the amplitude of ΔA_{750} , interpreted as the generation of

FIG. 3. Use of the BPh absorption shift to observe the specific binding and formation of BQ^- at the Q_B site in reaction centers of *Rb. sphaeroides* R-26. (A) Flash-activated formation of BQ^- in the Q_B site was determined by monitoring absorbance changes at 750 nm at the indicated concentrations of BQ using a reaction center sample reconstituted at $10 \mu\text{M}$ as described in Fig. 2B. (B) Changes in the amplitude of ΔA_{750} as a function of the concentration of BQ, taken from the traces shown in A, were fit to the full quadratic binding equation, $K_d = 38 \mu\text{M}$. (C) Binding rate constant for BQ at Q_B was determined from the slope of a linear fit to k_{750} versus the concentration of BQ. Abbreviations: 9,10-AQ, AQ; 1,4-BQ, BQ.

BQ^- in the Q_B site, is proportional to the concentration of BQ between 20 and $200 \mu\text{M}$. A plot of the flash-induced ΔA_{750} amplitude versus $\log[\text{BQ}]$ reveals that apparent binding of BQ to the Q_B site is well described by the theoretical fit to the full quadratic equation for ligand binding at a site with a K_d value of $38 \mu\text{M}$ (Fig. 3B). In addition, at the maximum BQ concentration, the kinetics of ΔA_{750} are rapid. Although not shown in Fig. 3A, time resolution of the 2.17 mM trace revealed a half-time of $145 \mu\text{sec}$. This half-time is similar to that found for electron transfer from UQ-10 in the Q_A site to UQ-0 (11) and UQ-10 (3, 4, 22) in the Q_B site. Fig. 3C describes the concentration dependence of electron-transfer kinetics from AQ at the Q_A site to BQ at the Q_B site and demonstrates that the binding reaction between BQ and the Q_B site occurs during the lifetime of Q_A^- and that the binding rate ($k_{\text{on}}[\text{BQ}] + k_{\text{off}}$) is rate-limiting relative to the electron-transfer reaction ($k_{\text{ab}} + k_{\text{ba}}$). Under these conditions, the rate of binding for BQ at the Q_B site can be determined from the slope of the observed binding rate (k_{750}) versus the concentration of BQ. This rate constant is $10^6 \text{ M}^{-1}\text{sec}^{-1}$ and is similar to that observed for UQ-0 (11) and other small nonquinone molecules that bind at the Q_B site (14). The fact that BQ exhibits concentration-dependent electron-transfer kinetics from AQ at the Q_A site to BQ at the Q_B site suggests that, at micromolar concentrations of BQ, the Q_B site is primarily unoccupied prior to the flash. This observation agrees with previous observations made for the binding of UQ-10 to the Q_B site in reaction center containing vesicles (26).

Dependence of the Apparent Quinone Affinity on $-\Delta G_{\text{e}}^0$. Using the cyt *c* oxidation assay, Fig. 4 confirms quantitatively that variation of the $-\Delta G_{\text{e}}^0$ between Q_A and Q_B substantially alters the apparent affinity of the Q_B site for quinone. The figure shows that by extending the BQ concentration to higher values (cf. Fig. 2A) that the measurement of a K_d value ($200 \mu\text{M}$) is achievable for BQ even with UQ-10 at the Q_A site. When UQ-10 at the Q_A site is replaced with AQ to increase $-\Delta G_{\text{e}}^0$ by 0.15 eV , the Q_B site exhibits a K_d value of $38 \mu\text{M}$ for BQ. Similarly, when MeAQ is at the Q_A site to increase the $-\Delta G_{\text{e}}^0$ by 0.22 eV , a K_d value of $14 \mu\text{M}$ for BQ is observed.

Thus, although this work warrants further quantitative examination, it is clear that the reaction $-\Delta G_{\text{e}}^0$ can strongly influence the K_d value of the quinone in the Q_B site. These results also reveal that the E_m of BQ in the Q_B site is just sufficiently favorable to reconstitute Q_B activity when UQ-10 is at Q_A site.

FIG. 4. Dependence of apparent quinone binding at the Q_B site on the $-\Delta G_{\text{e}}^0$ for Q_A to Q_B electron transfer in reaction center of *Rb. sphaeroides* R-26. The $-\Delta G_{\text{e}}^0$ was varied by replacing the native UQ-10 at the Q_A site ($-E_m = 0.07 \text{ V}$) with $15 \mu\text{M}$ AQ ($-E_m = 0.22 \text{ V}$) or with $15 \mu\text{M}$ MeAQ ($-E_m = 0.29 \text{ V}$). The theoretical lines for K_d were obtained as described in Fig. 3B. Reaction and conditions for measuring cyt *c* oxidation were as in Fig. 2 except that the concentration of BQ was varied.

FIG. 5. Function of methyl- and ring-substituted quinones at the Q_B site with UQ-10 (Left) and AQ (Right) at the Q_A site of reaction centers from *Rb. sphaeroides* R-26. (A) Conditions for monitoring $BChl_2^+$ reduction kinetics were as in Fig. 2 in the absence (curve a) and the presence (curve b) of $25 \pm 5 \mu M$ of each Q_B candidate. The indicated absorbance scale is the same for both panels. (B) Conditions for monitoring cytochrome c oxidation were carried out as in Fig. 2. The top two traces were typical of cytochrome c oxidation in the absence of added Q_B ; all other cytochrome c oxidation traces were obtained from samples containing $25 \pm 5 \mu M$ of the indicated Q_B candidates. The time and absorbance scales are the same for both panels.

Q_B Site Reconstitution with Low-Potential Quinones. In Fig. 5, we take advantage of the large $-\Delta G_{e-}^0$ provided by AQ at the Q_A site to demonstrate that the Q_B site will function with quinones that, like BQ, were believed to be nonfunctional at the Q_B site. Thus, when the native UQ-10 occupies the Q_A site, reconstitution of the Q_B site with 2,3-dimethyl-1,4-benzoquinone or 1,4-naphthoquinone yields marginal Q_B activity, whether using either the $BChl_2^+$ reduction kinetics assay (Fig. 5A Left) or the cytochrome c oxidation assay (Fig. 5B Left). However, when AQ is incorporated into the Q_A site, substantial Q_B activity is exhibited. The amount of cytochrome c oxidized on the second flash relative to the first flash ranges from 40 to 70% for all the quinones tested (Fig. 5B Right) while the slow phase of the $BChl_2^+$ reduction kinetics constitutes >35% of the total $BChl_2^+$ (Fig. 5A Right). The observed variation in Q_B activity for these quinones probably reflects differences in their K_d values. These experiments demonstrate clearly that the Q_B site will bind and function with the methyl-substituted and ring-extended BQs that resemble the head groups of the naturally occurring quinones, plastoquinone and menaquinone.

Q_B Site Reconstitution with High-Potential Quinones. The other way to generate a more favorable $-\Delta G_{e-}^0$ is to use higher-potential Q_B candidates while retaining the native UQ-10 at the Q_A site. In Fig. 6 we show that the high-potential 2,3-dichloro-1,4-naphthoquinone and the *ortho*-quinones, 3,5-di-*tert*-butyl-1,2-benzoquinone and 9,10-phenanthraquinone, all exhibit substantial Q_B activity when monitored by the cytochrome c oxidation and by the $BChl_2^+$ kinetics assays.

We have also examined the amplitude and kinetics of the BPh electrochromic shift at 750 nm to eliminate the possibility that these quinones may be functioning merely as nonspecific acceptors that oxidize Q_A without actually entering the Q_B site. The results from these experiments (data not shown) are very similar to those observed for BQ in Fig. 3, thereby demonstrating that these high-potential quinones are indeed functioning in the Q_B site. The experiments strengthen considerably our assertion that provision of a sufficiently large $-\Delta G_{e-}^0$ leads to easy detection of quinone occupancy at the Q_B site.

DISCUSSION

We have demonstrated that previous efforts to reconstitute the Q_B site of bacterial reaction centers with quinone analogues other than the native UQ were unsuccessful because of the small $-\Delta G_{e-}^0$ between tested quinones at the Q_B site and the native UQ-10 at the Q_A site. The relatively high *in situ* E_m value that prevails for the Q_A/Q_A^- couple of UQ-10 restricts the examination of quinones for binding and function at the Q_B site to those that also have as high or higher E_m values. Unfortunately, *in vitro* $E_{1/2}$ measurements for quinones in dimethylformamide (25) and *in situ* E_m measure-

FIG. 6. Function of high-potential *ortho*- and halogenated *para*-quinones at the Q_B site with the native UQ-10 at the Q_A site. (A) $BChl_2^+$ reduction kinetics in the absence (curve a) and presence (curve b) of $35 \mu M$ Q_B . (B) Cyt c oxidation on the first and second flash in the presence of $35 \mu M$ Q_B . Cyt c oxidation in the absence of added Q_B was similar to the trace in Fig. 5B. The conditions were as in Fig. 2.

ments for quinones at the Q_A site (18) reveal that the effects of ring extension and most other substitutions tend to lower the oxidation-reduction potential values relative to BQ. However, we have diminished this restriction by either deliberately choosing Q_A replacements with *in situ* E_m values that are much lower than UQ-10 or by choosing Q_B candidates with *in vitro* $E_{1/2}$ values very much higher than UQ-10.

From this work, another view of the Q_B site emerges. Rather than being specific for a single-ringed UQ, it is clear that the site will bind and support the function of other naturally occurring benzo- and naphthoquinone structures that are found in higher plants and other bacteria. In addition, the Q_B site will function with synthetic quinone analogues containing up to three rings, as well as with quinones that have their carbonyls arranged in the *ortho* rather than the more familiar *para* configuration.

These results are at least qualitatively similar to those found for the Q_A site (8). Work with the Q_A site has further demonstrated not only that there is no requirement for a *para*-carbonyl structure but also that the contribution to binding free energy from the "second" carbonyl is small (8). From the work presented here, it is reasonable to consider that the Q_A and Q_B sites may exhibit the same minimal requirements for binding. That is, binding to both the Q_A and Q_B sites may require only a partially unsaturated ring that is substituted with one carbonyl. This model is remarkably similar to that deduced by Trebst *et al.* (27) from inhibition studies done with the urea/triazine class of herbicides on the Q_B site of the reaction center of photosystem II of green plants. The binding requirements of the Q_B site for triazines and, by implication, for plastoquinone revealed from this study are given as an aromatic ring substituted with an sp^2 hybridized group with a lone electron pair and substituted with a lipophilic moiety (27).

The significant influence of $-\Delta G_e^0$ on the apparent affinity of the Q_B site for quinonoid compounds that we have described is equally important for studies into herbicide sensitivity and site-directed mutagenesis at the Q_A and Q_B sites. Although it is obvious that a changed amino acid residue can impart changes in the strength of interaction of a native quinone or herbicide at the Q_B site, it is also the case, as we have shown, that large increases or decreases in quinone K_d , and hence herbicide activity (IC_{50} values), can arise from an altered $-\Delta G_e^0$. Further, since the basic electrochemical properties of both the Q_A and Q_B sites contribute to $-\Delta G_e^0$, it follows that the source of mutation-induced alterations in either the apparent affinity for quinone or in the herbicide susceptibility at the Q_B site is just as likely to reside in the Q_A site as in the Q_B site. Thus a change in the amino acid complement to alter the *in situ* E_m values for quinone in the Q_A site can have major effects that are observed in the reactions at the Q_B site. This prediction is supported by our preliminary unpublished experiments in which AQ replacement at the Q_A site results in not only a decreased K_d for UQ-0 at the Q_B site, but also an increase in the amount of Ametryne, a Q_B site inhibitor, needed to effect inhibition. It follows that site-directed mutagenesis at the Q_A site could also be useful for either increasing or decreasing the observed herbicide sensitivity of the Q_B site. Thus, mutations deliberately directed to lower or raise the E_m value of quinone in the Q_A site will confer upon the Q_B site a controlled degree of resistance or susceptibility to herbicides. Clearly, recognizing the influence of $-\Delta G_e^0$ on quinone specificity is vital not only for obtaining a comprehensive understanding of the functional and structural properties of

the Q_B site in both plants and bacteria, but also perhaps as an important parameter in devising biotechnological strategies in the field.

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