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Chlorophyll fluorescence measurements to assess the competition of substituted anthraquinones for the Q_B binding site

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As analogs of the Photosystem II plastoquinone electron acceptor, Q_B, substituted quinones compete with Q_B for a common binding domain and thereby inhibit Q_B function. Substituted quinones interact with the Q_B binding niche via hydrogen bonds, and the extent of hydrogen bond formation is determined by quinone structure. We have previously shown that the quinone inhibitory activity can be quantitated using measurements of chlorophyll fluorescence quenching. To assess competition for the Q_B binding site, we report here measurements of the action of various pairs of substituted anthraquinones on the chlorophyll fluorescence emission of barley chloroplasts. The degree of competition between quinones for the Q_B binding site is classified as competition, partial competition, or no competition. Two quinones were classified as undergoing competition, i.e., interacting for the same or overlapping sites, if the chlorophyll fluorescence level in the presence of the two quinones was not as low as that achieved in the presence of either one of the quinones individually. Non-competitive quinones with different binding sites quenched chlorophyll fluorescence to the level expected if the quenching effects of the individual quinones were additive. Partial competition, or some interaction for the same or overlapping sites, was characterized by an extent of fluorescence quenching in the presence of two quinones that was more effective than either quinone alone but not as sizable as that expected when the two quinones act independently. These results reflect an interesting situation whereby substitution patterns can alter the binding characteristics within a single class of inhibitors. In an accompanying manuscript we report the results of CNDO molecular orbital calculations to demonstrate that the π charge distribution in substituted quinones governs their binding properties.

Introduction

The complexity of photosynthetic electron transfer arises from the multi-step nature of the process. One site located on the reducing side of Photosystem II is particularly susceptible to electron transport inhibition. At this site electrons are transferred from a one-electron acceptor, a bound plastoquinone known as Q_A, to a two-electron acceptor, a second plastoquinone known as Q_B. Double reduction of Q_B to the hydroquinone upon two single-electron transfer reactions from Q_A subsequently displaces Q_B from its binding site on the D-1 protein of the Photosystem II reaction center [1]. Diffusion and binding of a new plastoquinone molecule from a pool of plastoquinone molecules normally occur to ensure a high quantum yield of photosynthesis. However, a number of exogenous compounds are known to compete with Q_B for a common binding domain. These compounds thus act as inhibitors of photosynthetic electron transport.

Studies of the topography of the Q_B binding site reveal the stereochemical requirements for the binding of exogenous molecules to inhibit Q_B function. The D-1 protein is a 32 kDa polypeptide that is proposed to span the thylakoid membrane in five hydrophobic α-helices [2]. The 55-amino-acid loop connecting the fourth and fifth transmembrane helices is thought to contain

Abbreviations: AQ, 9,10-anthraquinone; Chl, chlorophyll; CNDO, complete neglect of differential overlap; DMOE, 1,2-dimethoxyethane; DMSO, dimethyl sulfoxide; f_a, Stern-Volmer fraction of chlorophyll fluorescence accessible to quinone; F_{max}, maximum chlorophyll fluorescence level with Photosystem II electron acceptor Q_A reduced; Hepes, 4-(2-hydroxyethyl)-1-piperazinesulfonic acid; I_0, I_Q, chlorophyll fluorescence levels in untreated and quinone-treated chloroplast samples, respectively; K_{SV}, Stern-Volmer quenching constant; Q_A and Q_B, the membrane-bound and membrane-exchangeable plastoquinone electron acceptors in Photosystem II, respectively.

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the Q_B binding site [3,4]. In particular, orientation of Q_B in its binding niche on the D-1 protein appears to involve hydrogen bonds to histidine-215 and to a peptide bond of an amino acid close to serine-264 [2]. Quantitative structure–activity studies have determined the essential element for Q_B inhibition to be an sp^2-hybridized atom bound to N, O, or = CH and attached to a lipophilic substituent [5,6]. A planar conformation is also hypothesized to be a prerequisite for a herbicide to bind to the membrane [7]. Consideration of inhibitory patterns and additional structural specifications have led to a division of Q_B inhibitors into two families, each comprising many compounds [8]. Different π-charge distributions within the two classes of inhibitors suggest the involvement of two different sidechains on the D-1 polypeptide for inhibitor binding [9]. A net positive π charge on the atom adjacent to the sp^2 center in the inhibitor molecule is present in those inhibitors which bind via a hydrogen bond to the NH of a peptide bond close to serine-264. A net negative π-charge adjacent to the requisite sp^2 center is characteristic of those inhibitors which hydrogen bond to histidine-215. Thus, functionally related but chemically different compounds can act as inhibitors at a common binding domain using overlapping binding sites. Stereochemical models [10] further suggest that some Q_B inhibitors not only occupy a different region of the D-1 protein but also do not appear to overlap in space when bound to the Q_B receptor. Additional affinities to different amino acid residues in the Q_B binding niche may contribute to varying binding strengths and/or inhibitory activities of compounds within the serine or histidine 'families' of inhibitors [10,11].

As analogs of Q_B, substituted 1,4-benzoquinones, 1,4-naphthoquinones, and 9,10-anthraquinones possess the required sp^2- structural element for inhibition of Q_B function. Binding experiments with ^{14}C-labeled anthraquinones have demonstrated conclusively that substituted 9,10-anthraquinones bind to the D-1 protein [12]. Inhibitory activity has been measured for these compounds in plant chloroplasts both directly by measurements of electron transport on the reducing side of Photosystem II [12–19] and indirectly by measurements of chlorophyll fluorescence intensity [13,20–22] and flash-induced fluorescence yield [23]. The degree of inhibition varies widely with the particular quinone used. Analyses of fluorescence studies have determined that the variable activities of quinones as inhibitors are consistent with two sources: (1) different affinities of the substituted quinones for the Q_B binding domain attributable to lipophilic factors and (2) different electron-accepting capabilities arising from substituent electronic factors [20–22].

On the basis of structural considerations, substituted 9,10-anthraquinones represent a single class of Q_B inhibitors with the potential for interaction with two sites on the D-1 protein via hydrogen-bond formation. In this manuscript we report on experimental chlorophyll fluorescence investigations to assess the degree of competition between particular 9,10-anthraquinones for binding sites in the Q_B binding niche of barley chloroplasts. In particular, we analyze for the extent of displacement of an anthraquinone from the photosynthetic membrane by another anthraquinone as revealed through variations in chlorophyll fluorescence intensities. We use these results to suggest the presence of common, overlapping, or independent binding sites for various anthraquinone pairs. In the accompanying manuscript, we analyze CNDO molecular orbital calculations [24] to attribute such binding characteristics to particular π charge distributions within the inhibitor molecules.

Materials and Methods

As in previous studies [20–22], chloroplasts were isolated from freshly harvested growth-chamber barley (*Hordeum vulgare*) in a medium containing 0.4 M sucrose/50 mM Hepes-NaOH (pH 7.5)/10 mM NaCl. Centrifugation at $6000 \times g$ for 10 min was followed by resuspension of the chloroplasts in a medium of 0.1 M sucrose/10 mM Hepes-NaOH (pH 7.5)/10 mM NaCl. Following centrifugation at $6000 \times g$ for 10 min, the pellet was resuspended in a medium of 0.1 M sucrose/50 mM Hepes-NaOH (pH 7.5)/5 mM NaCl to give 16.7 μg Chl per ml. For fluorescence measurements the chloroplast suspension was diluted as described below to a concentration of 10 μg chlorophyll (Chl) per ml.

Substituted 9,10-anthraquinones (AQ) were purchased from Aldrich Chemical Company unless otherwise noted and included: 1-hydroxy (ChemService, Westchester, PA); 1,8-dihydroxy (chrysazin); 1,4-dihydroxy (quinizarin); 1,2-dihydroxy; 1-amino-4-hydroxy; 1-amino; 2-amino; 1,2-diamino; 1,4-diamino; 2-ethyl; 1-chloro; 2-chloro. As necessary, quinones were further purified by recrystallization or sublimation. Stock solutions of the quinones (10 or 20 mM) were prepared in dimethyl sulfoxide (DMSO), ethanol, or 1,2-dimethoxyethane (DMOE). As noted in previous studies [20–22,25], these solvents showed no quenching effects on chlorophyll fluorescence at the concentrations employed in diluted chloroplast samples.

Quinone-enriched chloroplast samples were prepared by adding appropriate volumes of quinone stock and buffer solutions to concentrated chloroplast samples to form final samples with [Chl] = 10 μg/ml and [quinone] = 0 to 200 μM. Incubation of chloroplast and quinone solutions for 5 min in the dark at 4°C was sufficient to achieve a time-independent level of chlorophyll fluorescence [20–22].

Room-temperature fluorescence emission spectra were recorded with a Perkin-Elmer LS-5 fluorescence

spectrophotometer interfaced to a Perkin-Elmer Model 3600 Data Station. Chlorophyll fluorescence was induced by excitation at 620 nm with a pulsed xenon lamp and detected over the range of 650 to 760 nm with a Hamamatsu R928 photomultiplier tube. Measurements of chlorophyll fluorescence were made for chloroplasts in the F_{max} state by saturating with high light intensity for 2 min to maximally reduce Q_A. All fluorescence samples contained [Chl] = 10 μg/ml.

Quinone competition studies involved two different experimental protocols. The first method involved an investigation of the additivity of the quenching effects of the two quinones on chlorophyll fluorescence. For each pair of quinones studied, measurements of the chlorophyll fluorescence intensity were made for the following four samples of chloroplasts prepared with [Chl] = 10 μg/ml: (1) chloroplasts with no added quinone; (2) chloroplasts incubated with quinone 1 for 5 min at a concentration of 100 μM; (3) chloroplasts with [quinone 2] = 100 μM for a similar 5 minute incubation period; and (4) chloroplast samples with concentrations of both quinones at 100 μM where both quinones are added 'simultaneously' with a 5 min incubation period before the fluorescence emission spectrum is collected. To ensure that the degree of competition observed arose as a consequence of competition for binding sites and not as a result of different diffusion rates to the Q_B site, we made two additional fluorescence measurements for each quinone pair for samples prepared as follows: (5) chloroplast samples with [quinone 1] = 100 μM and incubated for 5 min before adding quinone 2 at a concentration of 100 μM for a 5 min incubation period before measuring the fluorescence intensity of the sample; and (6) chloroplasts prepared as for sample 5 with the reverse order of the quinones. The extents of competition between anthraquinone pairs as observed for samples 3 and 4 were in agreement with the results obtained for samples 5 and 6. These results support our contention that the differences in inhibitor effectiveness do not arise from differences in rates of diffusion of anthraquinones to the Q_B site. Furthermore, on the timescale of our measurements, no effects of the order of addition of anthraquinones are apparent.

Similar to previous studies [20–22], the second method for assessing the similarity in binding sites of two quinones analyzed the concentration-dependent ability of a quinone to quench chlorophyll fluorescence in plant chloroplasts using conventional and modified Stern-Volmer techniques [26,27]. However, chloroplasts were simultaneously treated with various concentrations of an additional substituted anthraquinone to assess the effectiveness of the second fluorescence quencher in the presence of another inhibitor. (As above, no effects of the order of addition of quinones were observed when fluorescence measurements were obtained for chloroplast samples pretreated with one quinone for 5 min before the addition of the second quinone.) The Stern-Volmer parameters of interest were f_a, which measures the affinity of the quinone for the membrane binding sites, and K_{SV}, the Stern-Volmer quenching constant, which measures the effectiveness of the quinone to quench fluorescence once the quinone has reached its site of action (i.e., independent of membrane accessibility) [20–22,25]. These parameters are related to the chlorophyll fluorescence levels in untreated and quinone-treated chloroplast samples, I_0 and I_Q, respectively, by the equations $I_0/I_Q = 1 + K_{SV}[Q]$ when $f_a = 1$ and $I_0/(I_0-I_Q) = 1/f_a + 1/(f_a \cdot K_{SV} \cdot [Q])$ when $f_a < 1$.

Results

The results of the competition studies are summarized in Table I. The degree of inhibitor competition based on fluorescence measurements was analyzed as follows. Two quinones were classified as undergoing competition, i.e., interacting for the same or overlapping sites, if the chlorophyll fluorescence level in the presence of the two quinones (simultaneous or stacked addition) was not as low as that achieved in the presence of either one of the quinones individually. Non-competitive quinones with different binding sites quenched chlorophyll fluorescence to the level expected if the quenching effects of the individual quinones were additive. Partial competition, or some interaction for the same or overlapping sites, was characterized by an extent of fluorescence quenching in the presence of two quinones (simultaneous or stacked addition) that was more effective than either quinone alone but not as sizable as that expected when the two quinones act independently. As an example of the classification of competing anthraquinones, 100 μM 1-OH-AQ quenches the normal chlorophyll fluorescence level (I_0) to 25% of I_0, 100 μM 2-CH_2CH_3-AQ to 55% of I_0, and the combination of both quinones each at 100 μM to 28% of I_0. The combination of 1,4-di-NH_2-AQ and 2-Cl-AQ results in partial competition: 37% of I_0 at 100 μM 1,4-di-NH_2-AQ; 30% of I_0 at 100 μM 2-Cl-AQ; and 18% of I_0 at 100 μM of each quinone. Non-competition is illustrated by 1,2-di-NH_2-AQ and 1-NH_2-4-OH-AQ with fluorescence levels relative to I_0 of 61% at 100 μM 1,2-di-NH_2-AQ, 33% at 100 μM 1-NH_2-4-OH-AQ, and 21% at 100 μM each of both anthraquinones.

For the 66 possible pairings between quinones given in Table I, 42 combinations resulted in direct competition, 16 in partial competition, and 8 in non-competition. Two quinones, 1-NH_2-AQ and 1,2-di-OH-AQ, competed with all other quinones. 1,4-di-OH-AQ was classified as a competitor with all other quinones except 1,4-di-NH_2-AQ, with which it only partially competed. Similarly, 1-OH-AQ may be regarded as a competitor with all anthraquinones except 1,4-di-NH_2-AQ and 1,8-

TABLE I

Classification using chlorophyll fluorescence measurements of degree of competition between 9,10-anthraquinones for the Q_B-binding site

This table classifies the extent of competition between substituted 9,10-anthraquinones for binding sites in the Q_B binding domain as observed using chlorophyll fluorescence studies. The symbols are defined as follows: C, competitive binding; PC, partial competitive binding for the same or overlapping binding sites; and NC, non-competitive binding of two quinones for non-interacting binding sites. The method by which these classifications were assigned is described in the text. All competition studies were conducted with the concentration of each quinone at 100 μM.

	1-NH_2										
1-NH_2		2-NH_2									
2-NH_2	C		1,2-NH_2								
1,2-NH_2	C	C		1,4-NH_2							
1,4-NH_2	C	NC	NC		1-NH_2-4-OH						
1-NH_2-4-OH	C	NC	NC	PC		1-OH					
1-OH	C	C	C	PC	C		1,2-OH				
1,2-OH	C	C	C	C	C	C		1,4-OH			
1,4-OH	C	C	C	PC	C	C	C		1,8-OH		
1,8-OH	C	C	PC	PC	C	PC	C	C		1-Cl	
1-Cl	C	NC	NC	PC	PC	C	C	C	PC		2-Cl
2-Cl	C	PC	PC	PC	PC	C	C	C	PC	C	
2-CH_2CH_3	C	NC	NC	PC	PC	C	C	C	C	C	C

TABLE II

Stern-Volmer analyses of quinone quenching activity with a second quinone present

These data present the calculated fraction of chlorophyll fluorescence that is accessible to quenching by a 9,10-anthraquinone (f_a) and the corresponding Stern-Volmer quenching constant (K_{SV}) for the 684 nm chlorophyll fluorescence of barley chloroplasts simultaneously incubated with various concentrations of a substituted 9,10-anthraquinone. The f_a and K_{SV} values in the absence of a second quinone are taken from Ref. 18.

Quinone	Background quinone and concentration (μM)		f_a	K_{SV} (M^{-1})
1,4-Di-OH-AQ	1,8-di-OH-AQ	0	0.85	$1.9 \cdot 10^6$
		50	0.34	$1.6 \cdot 10^4$
		100	0.32	$1.3 \cdot 10^4$
1,8-Di-OH-AQ	1,4-di-OH-AQ	0	0.81	$2.9 \cdot 10^6$
		50	0.29	$2.0 \cdot 10^5$
		100	0.23	$1.5 \cdot 10^5$
1,2-Di-NH_2-AQ	1,4-di-NH_2-AQ	0	1.00	$4.9 \cdot 10^3$
		50	1.00	$5.6 \cdot 10^3$
		100	1.00	$5.0 \cdot 10^3$
1,4-Di-NH_2-AQ	1,2-di-NH_2-AQ	0	1.00	$1.1 \cdot 10^4$
		50	1.00	$1.1 \cdot 10^4$
		100	1.00	$1.0 \cdot 10^4$
1,2-Di-NH_2-AQ	2-Cl-AQ	0	1.00	$4.9 \cdot 10^3$
		50	1.00	$3.1 \cdot 10^3$
		100	1.00	$2.7 \cdot 10^3$
2-Cl-AQ	1,2-di-NH_2-AQ	0	0.41	$2.0 \cdot 10^6$
		50	0.41	$1.5 \cdot 10^4$
		100	0.38	$2.2 \cdot 10^4$
1-Cl-AQ	2-CH_2-CH_3-AQ	0	0.55	$1.8 \cdot 10^5$
		50	0.40	$6.3 \cdot 10^4$
		100	0.37	$6.2 \cdot 10^4$
2-CH_2-CH_3	1-Cl-AQ	0	0.40	$1.8 \cdot 10^5$
		50	0.33	$4.1 \cdot 10^3$
		100	0.27	$4.9 \cdot 10^3$

di-OH-AQ which acted as partial competitors. The quinone most limited in ability to compete with other 9,10-anthraquinones is 1,4-di-NH_2-AQ, exhibiting competition with 1-NH_2-AQ and 1,2-di-OH-AQ only. Observation of non-competition was limited to 2-NH_2-AQ and to 1,2-NH_2-AQ each in combination with 1,4-di-NH_2-AQ; 1-NH_2-4-OH-AQ; 1-Cl-AQ; and 2-CH_2CH_3-AQ.

Table II summarizes the results of conventional and modified Stern-Volmer analyses of fluorescence quenching by a subset of representative anthraquinones in the presence of a fixed concentration of a second anthraquinone. Quinones were added simultaneously to chloroplasts and incubated for 5 min prior to recording of the chlorophyll fluorescence spectrum. The calculated fraction of chlorophyll fluorescence that is accessible to quinone quencher (f_a) and the corresponding Stern-Volmer quenching constant (K_{SV}) are reported for the 684 nm chlorophyll fluorescence of the quinone-treated chloroplasts. The f_a value, ranging from 0 to 1, reflects the extent of transport of a quinone to the site of action in the thylakoid membrane as dictated by lipophilic considerations. The K_{SV} value is a reasonable measure of the intrinsic quenching activity of a quinone that has been transported to its site of action.

The fluorescence quenching data of 1,2-di-NH_2-AQ in the presence of 1,4-di-NH_2-AQ and the data of 1,4-di-NH_2-AQ in the presence of 1,2-di-NH_2-AQ give linear Stern-Volmer relations, as found for each quinone alone. The Stern-Volmer quenching constant, K_{SV}, of each quinone is also unaffected by the presence of the second quinone. The Stern-Volmer f_a values for 1,2-di-NH_2-AQ and for 2-Cl-AQ are not affected in the presence of the second quinone, remaining at 1.0 and 0.4, respectively. The K_{SV} value for 1,2-di-NH_2-AQ is decreased 50% by the presence of 100 μM 2-Cl-AQ, while the K_{SV} value for 2-Cl-AQ is decreased 100-fold by 100 μM 1,2-di-NH_2-AQ. Dramatic reductions in f_a and K_{SV} values are observed for both 1-Cl-AQ and 2-CH_2CH_3-AQ in the presence of the second quinone. A lowering of both f_a and K_{SV} values is also noted for the pair of quinones 1,4-di-OH-AQ and 1,8-di-OH-AQ.

Discussion

The Stern-Volmer results in Table II of selected quinone pairings can be analyzed in terms of the competition studies summarized in Table I. For competing quinones, such as 1,4-di-OH-AQ and 1,8-di-OH-AQ, both Stern-Volmer parameters (f_a and K_{SV}) decreased in the presence of the second quinone. The decrease in the f_a value of the principal quinone with increasing concentration of the second quinone is indicative of a loss of binding sites through competition. The lowered K_{SV} values in the presence of a background level of a second quinone suggest that the binding of one quinone alters the binding affinity of the second, perhaps through a change in the D-1 protein conformation in the vicinity of the Q_B binding region. The same analysis may be applied to the simultaneous addition of 1-Cl-AQ and 2-CH_2CH_3-AQ. The K_{SV} parameter of 2-CH_2CH_3-AQ is more markedly affected by 1-Cl-AQ than the reverse.

For those quinone pairings classified as partially competitive, only the K_{SV} parameter was altered. The constant f_a values suggest that the binding sites of the two quinones are independent, although quinone binding affects the binding strength and K_{SV} value of a second quinone. As an example of partially competitive quinones, 1,2-di-NH_2-AQ and 2-Cl-AQ exhibit no changes in f_a in the presence of the second quinone, suggesting independent and non-overlapping binding sites. Variations in K_{SV} are observed, however. The binding of 1,2-di-NH_2-AQ appears to have a much more dramatic effect on the subsequent binding of 2-Cl-AQ than the reverse situation. The 100-fold decrease in the K_{SV} value of 2-Cl-AQ as the second quinone increases in concentration is significant compared to the decrease by less than a factor of two in the K_{SV} value of 1,2-di-NH_2-AQ in the presence of 2-Cl-AQ. Thus, the concentration-dependent variations in the K_{SV} values of partially-competing quinones are probably indicative of slight alterations in the conformation of portions of the Q_B protein as an exogenous quinone binds.

For quinones which did not compete, no changes in the Stern-Volmer parameters were observed, supporting non-interacting binding sites on the D-1 protein. For example, the Stern-Volmer f_a parameters for 1,2-di-NH_2-AQ and for 1,4-di-NH_2-AQ are unaffected by the presence of the other quinone, suggesting that 1,2-di-NH_2-AQ and 1,4-di-NH_2-AQ do not compete for the same or overlapping binding sites on the D-1 protein.

The degree of competition between two quinones is not dependent on the strength of the fluorescence quenching as reflected in the magnitude of the Stern-Volmer K_{SV} parameter. Competition is evident between two quinones with large K_{SV} values (1,4-di-OH-AQ, $K_{SV} = 1.9 \cdot 10^6$ M^{-1} and 1,8-di-OH-AQ, $K_{SV} = 2.9 \cdot 10^6$ M^{-1}); between two quinones with widely different K_{SV} values (1-NH_2-AQ, $K_{SV} = 6.6 \cdot 10^2$ M^{-1} [18] and 2-CH_2CH_3-AQ, $K_{SV} = 1.8 \cdot 10^5$ M^{-1}); and between two quinones with small K_{SV} values (1-NH_2-AQ, $K_{SV} = 6.6 \cdot 10^2$ M^{-1} [18] and 2-Cl-AQ, $K_{SV} = 4.9 \cdot 10^3$ M^{-1}). A similar analysis demonstrates that the magnitude of the f_a parameter does not determine whether competition is observed. Two quinones may compete with f_a values equal to one (1-NH_2-AQ [18] and 1,4-di-NH_2-AQ); with fractional f_a values (1-OH-AQ, $f_a = 0.73$ and 1-Cl-AQ, $f_a = 0.55$); and with fractional and integral f_a values (1-NH_2-AQ, $f_a = 1.00$ and 2-CH_2CH_3, $f_a = 0.40$).

The position of substitution does not determine competition between two quinones for the Q_B binding site.

Competition is observed for the following cases: monosubstitution at the same position (1-Cl-AQ and 1-OH-AQ); monosubstitution at different ring sites (1-NH_2-AQ and 2-CH_2CH_3-AQ); disubstitution (1,4-di-OH-AQ and 1-NH_2-4-OH-AQ); and both mono- and disubstitution (1-NH_2 and 1-NH_2-4-OH-AQ; 2-NH_2-AQ and 1,4-di-OH-AQ; 1,8-di-OH-AQ and 1-NH_2-4-OH-AQ).

Conclusion

The competition patterns reflect the presence of common or overlapping binding sites for most of the 9,10-anthraquinones studied. Such competition could result from binding of each quinone to both the histidine-215 and serine-264 sites or to one of these sites only. However, the observation of no competition between particular anthraquinone pairs is consistent with some quinones binding to only the histidine-215 site and others to only the serine-264 region. The anthraquinones presumably exhibiting single-site binding include 2-NH_2-AQ; 1,2-di-NH_2-AQ; 1,4-di-NH_2-AQ; 1-NH_2-4-OH-AQ; 1-Cl-AQ; and 2-CH_2CH_3-AQ. Thus, 9,10-anthraquinones may constitute a class of compounds for which substitution patterns will substantially alter the binding locations in the Q_B niche. To consider these assignments further, the accompanying paper [28] examines the CNDO-calculated net π-charge density at carbon atoms adjacent to the 9 and 10 positions. From charge distribution considerations in conjunction with the competition studies presented here, assignments of 9,10-anthraquinones to the histidine-215 and serine-264 families of Q_B inhibitors will be possible.

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