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Analysis of π charge distribution in substituted anthraquinones to assess affinity for the Q_B binding site

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In the accompanying paper (Biochim. Biophys. Acta (1990) 1020, 163–168), we have determined the degree of competition between substituted 9,10-anthraquinones for the Q_B binding niche through measurements of the additivity of quinone-quenching effects on chlorophyll fluorescence. Quinones inhibit Q_B function by competitively displacing Q_B through hydrogen-bond formation with the Q_B binding protein. The sign of the net π-charge density on atoms adjacent to the carbonyl moieties is believed to determine the particular hydrogen-bond(s) that result(s). In this study we report CNDO molecular orbital calculations of π electronic charge distribution in substituted 9,10-anthraquinones to explore the relationship of inhibitor activity and competition to sign of net π-charge density. We find that the substitution patterns of 9,10-anthraquinones alter the signs of the net π-charge densities on the carbon atoms adjacent to the carbonyl moieties and thus determine the binding properties of the anthraquinones in the Q_B niche. While most experimentally studied 9,10-anthraquinones use both carbonyl oxygens to hydrogen bond to the histidine-215 and serine-264 regions of the D-1 Q_B binding protein, some quinones appear to hydrogen-bond to only one site. Thus, 9,10-anthraquinones constitute a class of Q_B inhibitors that function as either members of the histidine or serine family of Q_B inhibitors or as simultaneous representatives of both inhibitor groups.

Introduction

The mode of action of exogenous inhibitors of Photosystem II electron transport involves displacement of the native secondary plastoquinone, Q_B, from its binding niche on the D-1 protein [1,2]. Subsequent interaction of the Q_B inhibitor via hydrogen bonding with the D-1 protein blocks the transfer of electrons and alters the photosynthetic efficiency in plant chloroplasts [3]. Numerous quantitative structure-activity studies [4] have investigated the relationship between the molecular structure of these inhibitors and their action on the electron transfer reactions of photosynthesis. These studies have led to a division of Q_B inhibitors into two families, based on a proposal that the two different amino acid side-chains involved in Q_B binding also bind the two classes of inhibitors [5]. An sp^2-hybridized atom attached to a lipophilic substituent and bonded to a nitrogen atom, oxygen atom or =CH moiety [6,7] serves as the site of hydrogen bonding within the Q_B inhibitor. The hydrogen bond is formed via the NH of a peptide bond close to serine-264 in the Q_B-binding protein when a net positive π-charge occurs adjacent to the sp^2 center in the Q_B inhibitor. A net negative π-charge adjacent to the sp^2 center is characteristic of those inhibitors which hydrogen bond to histidine-215 in this same protein [5].

As structural analogs of the Photosystem II electron acceptor Q_B, substituted 9,10-anthraquinones have been extensively studied as inhibitors of Photosystem II electron transport [8–11]. In this manuscript we specifically address how substituents alter the π-charge density of an anthraquinone and thereby enhance or decrease the interaction of the quinone with the Q_B binding site. Comparison of the CNDO [12] calculations of π-electron densities with the results of the competition studies enables a classification of each 9,10-anthraquinone studied as a member of either the histidine or serine family of Q_B inhibitors or as a representative member of both families.

Abbreviations: AQ, 9,10-anthraquinone; CNDO, complete neglect of differential overlap; Q_A and Q_B, the membrane-bound and membrane-exchangeable plastoquinone electron acceptors in Photosystem II, respectively.

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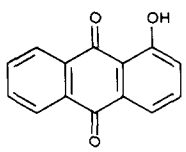
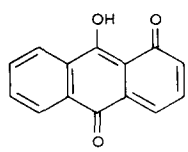
Methods

CNDO molecular orbital calculations [12] were performed to calculate the π-charge distribution in substituted 9,10-anthraquinones. These calculations determined whether the substituted quinones carry net positive or negative π-charges on the carbon atoms of the ring system. As anthraquinone structures are too large to allow geometry optimization through energy minimization, we used the standard molecular bond distances and angles of Pople and Gordon [13]. As required for inhibitory activity [4], a planar ring structure was adopted. The effect of rotation of bonds within ring substituents was considered, but little variation in π-charge density was observed. Generally, the conformations of amino, hydroxy and ethyl groups were fixed in the most stable arrangements as revealed by CNDO-calculated total energies.

A number of additional points were considered for the hydroxy and amino moieties, for there is good evidence that the properties of anthraquinones are markedly affected by the presence of such proton-donor substituents [14]. Infrared [15], phosphorescence [16] and electronic absorption [14,17] studies suggest the presence of intramolecular hydrogen bonding in hydroxy-substituted anthraquinones when the hydroxyl is immediately adjacent to the carbonyl group. CNDO calculations cannot, however, account for such intramolecular bonding except for artificial changes in interatomic distances. Nevertheless, infrared [18,19] studies further suggest that the presence of an intramolecular hydrogen bond stabilizes an altered electron distribution as a consequence of a tautomeric process. CNDO calculations were thus performed on the tautomeric structures of hydroxy-substituted anthraquinones, as in I and II.

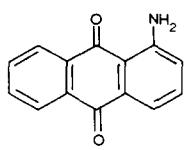


I

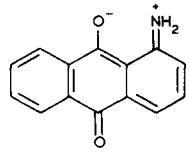


II

There is conflicting evidence, however, from infrared and ultraviolet-visible spectroscopic measurements as to whether aminoanthraquinones exhibit such intramolecular hydrogen bonding [14,15]. Instead, a strong resonance interaction has been shown to exist between the amino and carbonyl groups [15], as illustrated by structures III and IV for 1-amino-9,10-anthraquinone.



III



IV

CNDO calculations for the semiquinone analogs of amino-substituted anthraquinones were also performed.

Results

Table I summarizes the CNDO-calculated net π-charge densities on all ring carbon atoms adjacent to the carbonyl oxygens of those substituted 9,10-anthraquinones included in competition studies. No resonance or tautomeric structures are considered in this table. All quinones examined exhibited a small net negative π-charge density on the carbon atom bonded to C-8 and C-9. The largest magnitude of net π-charge density observed at this carbon occurred with hydroxy substitution at the 8-position (-0.075). Similarly, the carbon atom joining C-5 and C-10 generally exhibited a small net negative π-charge density, except with 1-OH-AQ ($+0.001$) and 1,8-di-OH-AQ ($+0.025$). A net negative π-charge density ranging from -0.002 to -0.075 occurred at the ring carbon atom linking C-9 and C-1, except for three small net positive densities for 2-NH_2-AQ ($+0.013$), 1-Cl-AQ ($+0.007$) and 2-CH_2CH_3-AQ ($+0.007$). At this site linking C9 and C1, net π-charge densities more positive than those observed in the unsubstituted 9,10-anthraquinone result from electron-withdrawing groups (e.g., -Cl) in the 1-position. Electron-releasing substituents at the 1-position (e.g., -OH, -NH_2) result in more negative net π-charge densities at this carbon site than in the parent compound. A more restricted range of net negative π-charge densities was calculated for the carbon site between C-10 and C-4 (-0.002 to -0.048). Four net positive densities at this carbon corresponded to 1-NH_2-AQ ($+0.012$), 1-OH-AQ

TABLE I

CNDO-calculated net π-charge densities of 9,10-anthraquinones

This table summarizes the CNDO-calculated net π-charge densities on all ring carbon atoms adjacent to the carbonyl oxygens of those substituted 9,10-anthraquinones examined in competition studies. Ring carbon atoms not numbered by convention are labeled according to the adjacent carbon atoms (e.g., C9-C1 refers to the carbon connecting C9 and C1).

Quinone	C8-C9	C9-C1	C5-C10	C10-C4
9,10-AQ	−0.002	−0.002	−0.002	−0.002
1-NH_2-AQ	−0.002	−0.039	−0.001	+0.012
2-NH_2-AQ	−0.002	+0.013	−0.001	−0.025
1,2-Di-NH_2-AQ	−0.002	−0.025	0.000	−0.011
1,4-Di-NH_2-AQ	−0.001	−0.024	−0.001	−0.024
1-NH_2-4-OH-AQ	−0.001	−0.015	−0.006	−0.007
1-OH-AQ	−0.006	−0.071	+0.001	+0.022
1,2-Di-OH-AQ	−0.008	−0.048	+0.002	−0.016
1,4-Di-OH-AQ	−0.003	−0.048	−0.003	−0.048
1,8-Di-OH-AQ	−0.075	−0.075	+0.025	+0.025
1-Cl-AQ	−0.004	+0.007	−0.004	−0.002
2-Cl-AQ	−0.004	−0.002	−0.004	+0.011
2-CH_2CH_3-AQ	−0.002	+0.007	−0.001	−0.014

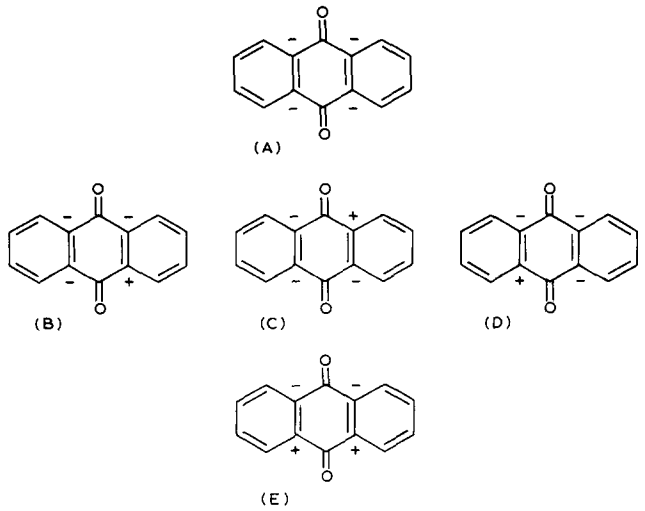


Fig. 1. This figure presents the signs of the CNDO-calculated net π-charge densities on all ring carbon atoms adjacent to the carbonyl oxygens of those substituted 9,10-anthraquinones included in experimental competition studies. No resonance or tautomeric structures are considered here. Structure A is consistent with CNDO calculations for 9,10-AQ, 1,2-di-NH_2-AQ, 1,4-di-NH_2-AQ, 1-NH_2-4-OH-AQ and 1,4-di-OH-AQ. Structure B corresponds to 1-NH_2-AQ and 2-Cl-AQ. Structure C is consistent with 2-NH_2-AQ, 1-Cl-AQ and 2-CH_2CH_3-AQ. Structure D fits 1,2-di-OH-AQ only, and structure E corresponds to 1-OH-AQ and 1,8-di-OH-AQ.

(+0.022), 1,8-di-OH-AQ (+0.025) and 2-Cl-AQ (+0.011). Fig. 1 presents only the associated signs of the CNDO-calculated net π-charge densities in Table I.

For those tautomeric structures involving the 1-hydroxy substituent, Table II presents the CNDO-calculated net π-charge densities on all ring carbon atoms adjacent to the carbonyl functionalities now located at C-1 and C-10. In all cases, net negative π-charge densities resulted at C-2 and at the carbon atom linking C-9 and C-1. Net positive π-charge densities were observed at the carbon atoms connecting C-5 and C-10 and connecting C-10 and C-4, except for 1,4-di-OH-AQ, which exhibited a net negative density at the carbon atom between C-10 and C-4. Also listed in Table II are the net π-charge densities for the hydroxy tautomer of 1-NH_2-4-OH-AQ with the carbonyls at C-9 and C-4. All such calculated densities are negative in sign except at the carbon atom linking C-8 and C-9. Fig. 2 summarizes these results.

Table III compiles the corresponding net π-charge densities for the resonance structures of amino-substituted quinones. Resonance involving the 1-NH_2 substituent produces the requisite sp^2 centers at C-1 and C-10. Net negative π-charge densities are located at both sites adjacent to C-1 and net positive π-charge densities are adjacent to C-2 except when a substituent is located at the 4-position. The resonance structures involving the 2-NH_2 moiety, with sp^2 centers at C-2 and C-9, result in net negative π-charge densities at C-1 and C-3 and net positive π-charge densities at both sites

TABLE II

CNDO-calculated net π-charge densities of tautomeric structures of hydroxy-substituted 9,10-anthraquinones

This table summarizes the CNDO-calculated net π-charge densities on all ring carbon atoms adjacent to the carbonyl oxygens of the tautomeric structures of those hydroxy-substituted 9,10-anthraquinones examined in competition studies. For hydroxy substituents at the 1-position, the net π-charge densities are given for the carbon atoms adjacent to the carbonyl moieties at the C-1 and C-10 positions. For tautomerism involving hydroxy substituents at both the C-1 and C-4 positions, the net π-charge densities are given for the carbon atoms adjacent to the carbonyl moieties at the C-1 and C-4 positions. The hydroxy tautomer of 1-NH_2-4-OH-AQ required calculations of the net π-charge densities at the carbon atoms adjacent to carbonyls at the C-9 and C-4 positions. Ring carbon atoms are labeled according to numbered position in the anthraquinone system (e.g., C2) or according to the adjacent carbon atoms when the ring carbon is not numbered by convention (e.g., C9-C1 refers to the carbon connecting C9 and C1).

Tautomerism involving the 1-OH substituent only

Quinone	C9-C1	C2	C5-C10	C10-C4
1-OH-AQ	−0.150	−0.063	+0.015	+0.032
1,2-Di-OH-AQ	−0.158	−0.001	+0.013	+0.003
1,4-Di-OH-AQ	−0.122	−0.005	0.000	−0.099
1,8-Di-OH-AQ	−0.177	−0.083	+0.044	+0.031

Tautomerism involving the 1-OH and 4-OH substituents

Quinone	C9-C1	C2	C10-C4	C3
1,4-Di-OH-AQ	−0.076	+0.022	−0.076	+0.022

Tautomerism involving the 4-OH substituent only

Quinone	C8-C9	C9-C1	C10-C4	C3
1-NH_2-4-OH-AQ	+0.008	−0.026	−0.140	−0.040

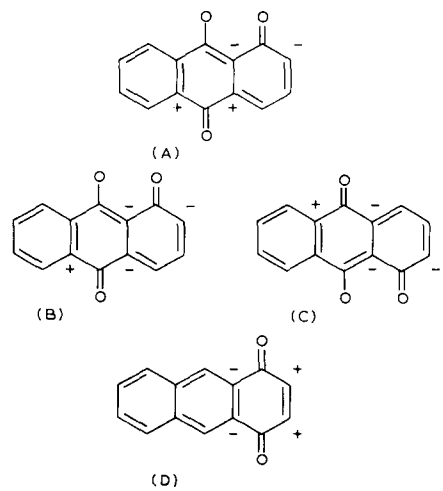


Fig. 2. For tautomeric structures involving the 1-hydroxy substituent, this figure presents the signs of the CNDO-calculated net π-charge densities on all ring carbon atoms adjacent to the carbonyl functionalities now located at the 1- and 10-positions. Structure A is consistent with the tautomer of 1-OH-AQ, 1,2-di-OH-AQ and 1,8-di-OH-AQ. Structure B corresponds to the tautomer of 1,4-di-OH-AQ. Structure C is the tautomeric form of 1-NH_2-4-OH-AQ. Structure D involves tautomerism of the hydroxy group at both positions 1 and 4 in 1,4-di-OH-AQ.

TABLE III

CNDO-calculated net π-charge densities of resonance structures of amino-substituted 9,10-anthraquinones

This table summarizes the CNDO-calculated net π-charge densities on all ring carbon atoms adjacent to the carbonyl oxygens of the resonance structures of those amino-substituted 9,10-anthraquinones examined in competition studies. For amino substituents at the 1-position, the net π-charge densities are given for the carbon atoms adjacent to the sp^2 centers at the C-1 and C-10 positions. For resonance involving amino substituents at C-2, the net π-charge densities are given for the carbon atoms adjacent to the sp^2 centers at the C-2 and C-9 positions. Ring carbon atoms are labeled according to numbered position in the anthraquinone system (e.g., C2) or according to the adjacent carbon atoms when the ring carbon is not numbered by convention (e.g., C9-C1 refers to the carbon connecting C9 and C1).

Resonance involving the 1-NH_2 substituent				
Quinone	C9-C1	C2	C5-C10	C10-C4
1-NH_2-AQ	−0.154	−0.134	+0.006	+0.080
1,2-Di-NH_2-AQ	−0.105	−0.053	+0.008	+0.019
1,4-Di-NH_2-AQ	−0.129	−0.013	+0.008	−0.129
1-NH_2-4-OH-AQ	−0.127	−0.091	+0.003	−0.016
Resonance involving the 2-NH_2 substituent				
Quinone	C1	C3	C8-C9	C9-C1
2-NH_2-AQ	−0.088	−0.092	+0.002	+0.041
1,2-Di-NH_2-AQ	−0.045	−0.081	+0.002	+0.003

adjacent to C-9. Fig. 3 summarizes the signs of these calculated net π-charge densities.

The experimentally studied quinones may be categorized according to the distribution of signs of the net π-charge densities at the four sites adjacent to the sp^2 centers on the anthraquinone rings. (1) All negative signs are observed for 1,4-di-OH-AQ, 1,2-di-NH_2-AQ, 1,4-di-NH_2-AQ, 1-NH_2-4-OH-AQ and the unsubstituted 9,10-AQ. (2) Three negative signs and one positive sign are observed for 9,10-anthraquinones with the following substitution patterns: 2-Cl, 1-NH_2, 1-Cl, 2-NH_2, 2-CH_2CH_3 and 1,2-di-OH-AQ. (3) One carbonyl site with adjacent net negative π-charge densities and one with net positive π-charge densities is observed in 1-OH-AQ, 1,8-di-OH-AQ, the tautomeric forms of 1-OH, 1,2-di-OH, 1,4-di-OH and 1,8-di-OH-AQ, and the resonance structures of 1-NH_2-AQ, 2-NH_2-AQ, 1,2-di-NH_2-AQ and 1-NH_2-4-OH-AQ. (4) Each carbonyl site

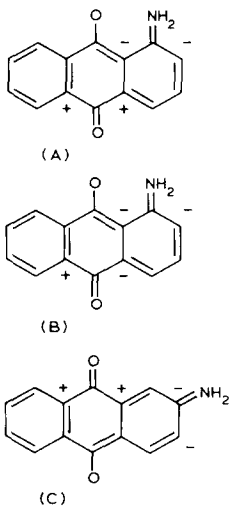


Fig. 3. For resonance structures involving the 1-amino or 2-amino substituent, this figure presents the signs of the CNDO-calculated net π-charge densities on all ring carbon atoms adjacent to the carbonyl functionalities now located at the 1- and 10-positions or the 2- and 9-positions, respectively. For resonance involving the 1-NH_2 substituent, Structure A corresponds to the resonance forms of 1-NH_2-AQ and 1,2-di-NH_2-AQ, while structure B is consistent with the resonance forms of 1,4-di-NH_2-AQ and 1-NH_2-4-OH-AQ. For resonance involving the 2-NH_2 substituent, structure C agrees with both 2-NH_2-AQ and 1,2-di-NH_2-AQ.

TABLE IV

CNDO-calculated net π-charge densities of mono- and di-substituted 9,10-anthraquinones

This table summarizes the CNDO-calculated net π-charge densities on all ring carbon atoms adjacent to the carbonyl oxygens of mono- and di-substituted 9,10-anthraquinones with -Cl, -CH_3, -CH_2OH, -CH_2CH_3 and -OCH_3 groups. Ring carbon atoms not numbered by convention are labeled according to the adjacent carbon atoms (e.g., C9-C1 refers to the carbon connecting C9 and C1).

Quinone	C8-C9	C9-C1	C5-C10	C10-C4
9,10-AQ	−0.002	−0.002	−0.002	−0.002
1-Cl-AQ	−0.004	+0.007	−0.004	−0.002
2-Cl-AQ	−0.004	−0.002	−0.004	+0.011
1,2-Di-Cl-AQ	−0.006	+0.006	−0.005	+0.005
1,4-Di-Cl-AQ	−0.006	+0.006	−0.005	+0.005
1,5-Di-Cl-AQ	−0.004	+0.005	+0.005	−0.004
1,8-Di-Cl-AQ	+0.005	+0.005	−0.004	−0.004
1-CH_3-AQ	−0.003	+0.030	0.000	+0.008
2-CH_3-AQ	−0.002	+0.008	−0.001	−0.016
1,2-Di-CH_3-AQ	−0.003	−0.019	+0.001	−0.006
1,4-Di-CH_3-AQ	−0.002	−0.002	−0.002	−0.002
1,5-Di-CH_3-AQ	+0.007	−0.028	−0.028	+0.007
1,8-Di-CH_3-AQ	−0.031	−0.031	+0.010	+0.010
1-CH_2OH-AQ	−0.003	−0.023	−0.001	+0.005
2-CH_2OH-AQ	−0.002	+0.005	−0.001	−0.011
1,2-Di-CH_2OH-AQ	−0.003	−0.016	−0.001	−0.005
1,4-Di-CH_2OH-AQ	−0.002	−0.016	−0.002	−0.016
1,5-Di-CH_2OH-AQ	+0.005	−0.002	−0.002	+0.005
1,8-Di-CH_2OH-AQ	−0.024	−0.024	+0.006	+0.006
1-CH_2CH_3-AQ	−0.002	−0.023	−0.002	+0.007
2-CH_2CH_3-AQ	−0.002	+0.007	−0.001	−0.014
1,2-Di-CH_2CH_3-AQ	−0.002	−0.014	−0.001	−0.005
1,4-Di-CH_2CH_3-AQ	−0.001	−0.014	−0.001	−0.014
1,5-Di-CH_2CH_3-AQ	+0.007	−0.023	−0.023	+0.007
1,8-Di-CH_2CH_3-AQ	−0.023	−0.023	+0.007	+0.007
1-OCH_3-AQ	−0.001	−0.054	−0.003	+0.018
2-OCH_3-AQ	−0.003	+0.020	−0.001	−0.038
1,2-Di-OCH_3-AQ	−0.001	−0.035	−0.002	−0.015
1,4-Di-OCH_3-AQ	−0.012	−0.001	+0.012	−0.001
1,5-Di-OCH_3-AQ	+0.045	−0.032	−0.032	+0.045
1,8-Di-OCH_3-AQ	−0.053	−0.053	+0.017	+0.017

of the tautomeric form of 1,4-di-OH-AQ has adjacent net π-charge densities with one negative and one positive sign.

We also calculated the net π-charge densities in mono- and di-substituted 9,10-anthraquinones with such common substituents as $-CH_3$, $-CH_2CH_3$, CH_2OH, $-OCH_3$, and -Cl. A compilation of such results appears in Table IV, with the net π-charge densities calculated for the unsubstituted parent compound as reference. From such a general study of anthraquinones with no tautomeric or resonance structures, several observations may be noted. (1) 1,8-disubstitution of all substituents considered leads to one carbonyl site with adjacent net negative π-charge densities and one with net positive π-charge densities. (2) Each carbonyl site of all 1,5-disubstituted compounds and for 1,2-di-Cl-AQ and 1,4-diCl-AQ has adjacent net π-charge densities with one negative and one positive sign. (3) Three negative signs and one positive sign are observed for $-CH_2CH_3$, $-OCH_3$ and $-CH_2OH$ substitution at the 1, 2, 1 and 2, and 1 and 4 positions; with $-CH_3$ at the 2, 1 and 2, and 1 and 4 positions; and with -Cl at the 1 or 2 positions. (4) Three positive signs and one negative sign are observed for 1-CH_3-AQ.

Discussion

We suggest that the competition between quinones as revealed by fluorescence quenching additivity arises from competition for hydrogen bond formation with the Q_B binding protein. As previously hypothesized for other Q_B inhibitors, the sign of the net π-charge density on an atom adjacent to the requisite sp^2 center determines the particular hydrogen bond that results. Quinones have four such atoms adjacent to two carbonyl sp^2 centers which may govern hydrogen bond formation. CNDO molecular orbital calculations of the π-charge distribution in substituted anthraquinones suggest that anthraquinones can be initially classified into three broad categories based on the signs of the net π-charge densities at these sites. Net positive π charge densities at all four carbon atoms suggest that only one carbonyl moiety can be involved in a hydrogen bond with the NH group of the peptide bond near serine-264. Similarly, a single hydrogen bond with histidine-215 is likely for those anthraquinones with net negative π-charge densities at all four carbon atoms. Anthraquinones with different signs for the net π-charge densities at these carbon atoms have the potential for hydrogen bond formation to both sites.

We can combine these results with those of the earlier competition studies using chlorophyll fluorescence and attempt to classify the quinones as to the nature of their binding sites on the Q_B binding protein. We suggest that the competitive action of two quinones based on the fluorescence results arises as a consequence of the same sign of net π-electron density distribution at one or more carbon atoms adjacent to carbonyl groups. For pairs of quinones believed to exhibit distinct binding sites from the absence of competition as monitored by chlorophyll fluorescence levels, different signs of net π-electron density distribution are presumed to exist at all carbon atoms adjacent to carbonyl groups.

We suggest that most of the quinones may potentially hydrogen bond to both the histidine-215 site and the serine-264 site. These quinones include 1-OH, 1-NH_2, 1,2-OH, 1,4-OH and 1,8-OH, all of which competed or partially competed with the other 9,10-anthraquinones examined. Another subset of quinones seem to bind only to or with a preference to the histidine-215 site, as governed by the net negative π-charge density adjacent to one of the carbonyl functionalities. These quinones include 1,4-di-NH_2-AQ, 1-NH_2-4-OH, 1-Cl-AQ, 2-Cl-AQ and 2-CH_2CH_3-AQ. Still other quinones bind only to or with a preference to the serine-264 site, dictated by the net positive π-charge density on a carbon adjacent to the sp^2 carbonyl carbon, including 2-NH_2 and 1,2-di-NH_2-AQ. The assignments of anthraquinones to a single binding site are strongly supported by the lack of competition between both 2-NH_2-AQ and 1,2-di-NH_2-AQ and each of the quinones 1,4-di-NH_2-AQ, 1-NH_2-4-OH, 1-Cl-AQ and 2-CH_2CH_3-AQ. The existence of only partial competition of 2-Cl-AQ with 2-NH_2-AQ and with 1,2-di-NH_2-AQ favors a preference of 2-Cl-AQ for the histidine-215 site.

In order to accommodate these assignments, the resonance forms of 2-NH_2-AQ and 1,2-di-NH_2-AQ (resonance via the 2-NH_2 substituent) are favored, but the resonance structures of 1-NH_2-AQ, 1,4-di-NH_2-AQ and 1-NH_2-4-OH-AQ are not consistent with the observed competition results and are thus not predicted. Tautomerism involving the 1-hydroxy substituent of 1-OH-AQ, 1,4-di-OH-AQ and 1,8-di-OH-AQ is required to provide both net positive and negative π-charge densities adjacent to both carbonyl moieties. The net π-charge densities upon additional tautomerism of 1,4-di-OH-AQ involving both hydroxy moieties is also consistent with the competition results. For 1,2-di-OH-AQ, the regular structure has net π-charge distribution patterns of two negative signs adjacent to one carbonyl and one positive and one negative sign adjacent to the second carbonyl. However, the tautomer of 1,2-di-OH-AQ is more likely to provide direct competition for both the histidine-215 and serine-264 sites with net π-charge densities of only positive and only negative signs at each carbonyl. No tautomerism of the hydroxy substituent in 1-NH_2-4-OH-AQ is predicted; the absence of a tautomeric structure achieves all net negative π-charge densities at the critical ring carbon atoms, as is necessary to be consistent with the competition studies. We note that other

determinations of preferred tautomeric forms of Q_B inhibitors have been similarly accomplished using molecular orbital calculations in conjunction with observed characteristics of inhibitor activity [20].

The binding site predictions may be analyzed in terms of the correspondence between the lipophilicity of the substituted anthraquinone and the proposed binding region of the D-1 protein. Quinones which form hydrogen bonds exclusively or preferentially to histidine-215 would be expected to carry hydrophobic substituents to match the lipophilicity of the transmembrane region of the D-1 protein. The more hydrophilic amino acid loop containing serine-264 on the matrix side of the thylakoid membrane would most likely be favored as the lipophilicity of the anthraquinone ring system is reduced with hydrophilic substituents. Quinones of intermediate lipophilicity could potentially hydrogen bond to both sites. We have previously [8,9,11] used Hansch π values [21,22] to quantify substituent hydrophobicity. Our binding site predictions do assign the anthraquinones with the most hydrophobic substituents (2-CH_2CH_3, 1-Cl and 2-Cl with $\pi = 0.71-1.02$) to the histidine-215 site. Those anthraquinones attributed to the serine-264 site contain hydrophilic substituents (2-NH_2 and 1,2-diNH_2, $\pi = -1.23$ and -2.46, respectively). Intermediate π-values ranging from -0.67 to -1.34 are exhibited by the substituents of those anthraquinones believed to hydrogen bond to both regions. The previously-determined Stern-Volmer f_a parameters [8,9] also show general correlations with binding site. We interpret the f_a value to reflect the ability of the anthraquinone to reach the Q_B binding domain, with f_a values of 0 indicating no affinity for the Q_B site and f_a values of 1 indicating the maximum affinity of the anthraquinone for the Q_B receptor. Quinones predicted to favor the histidine-215 site have average f_a values of 0.6 ± 0.2, those favoring the serine-264 region all have f_a values of 1.0, and those binding to both sites have average f_a values of 0.75 ± 0.15. Thus the proposed binding sites generally reflect lipophilic considerations.

A comparison of the net π-electron charge distributions in substituted 9,10-anthraquinones and other Q_B inhibitors which are plastoquinone analogs reveals a variation with chemical structure. Substituted 9,10-anthraquinones, 4-quinolines, chromones, 1,4-naphthoquinones, triazinones, cyanoacrylates and ureas all presumably hydrogen bond to the Q_B niche using a carbonyl functionality [23]. Only negative π-charges on the atoms adjacent to the carbonyl groups are observed for substituted 4-quinolones [24], chromones [24] and most 1,4-naphthoquinones [5,24,25]. Triazinones [3,25], cyanoacrylates [20] and certain substituted 1,4-naphthoquinones [5,24,25] exhibit both positive and negative π-charges at these sites. Diuron (a urea) exhibits only positive charges adjacent to the carbonyl moiety [3]. That our study of substituted 9,10-anthraquinones reveals all three possible π-charge distributions about a carbonyl moiety in a single class of compounds most likely arises from the consideration of substituents at all eight possible ring positions. Both the number of substituted compounds and the degree of substitution considered in previous studies of other Q_B inhibitors were more limited. Nevertheless, the large number of classes of compounds which are Q_B analogs serve to illustrate that the chemical structure of even related compounds can markedly alter the functional properties of Q_B inhibitors, including their orientation in the Q_B binding niche.

The general study of mono- and di-substituted 9,10-anthraquinones with -CH_3, -CH_2CH_3, -CH_2OH, -OCH_3 and -Cl substituents suggests that most of these compounds belong to the histidine family of inhibitors. With 1,5- and 1,8-disubstitution for all substituents and 1,4-di-Cl-9,10-AQ, net π-charge densities favor hydrogen bonding to both the serine and histidine sites. Only in the case of 1-CH_3–9,10-AQ is hydrogen bonding exclusively to the serine site predicted.

Conclusion

Thus, substituted 9,10-anthraquinones are a single class of compounds for which variations in substitution pattern seem to dictate the binding site on the Q_B protein. In general, most of the 9,10-anthraquinones examined experimentally have net π-electron charge distributions that permit hydrogen bonds to both histidine-215 and serine-264. These 9,10-anthraquinones include the tautomeric forms of 1-OH, 1,2-di-OH, 1,4-di-OH, and 1,8-di-OH-AQ and the non-resonance form of 1-NH_2-AQ. Orientation in the binding niche is consistent with a hydrogen bridge from near serine-264 to C-10 and a hydrogen link from histidine-215 to C-1 in the hydroxy tautomers and to C-9 in 1-NH_2-AQ. For some anthraquinones, however, substituents permit or favor hydrogen bond formation with only one amino acid on the Q_B binding protein. Those members of the histidine-215 family (and the carbonyl site involved in hydrogen bonding) include 1,4-di-NH_2 (C=O at C-9 or C-10), 1-NH_2-4-OH (C=O at C-9 or C-10), 1-Cl (C=O at C-10 only), 2-Cl (C=O at C-9 only), and 2-CH_2CH_3-AQ (C=O at C-10 only). The resonance forms of 2-NH_2 and 1,2-di-NH_2-AQ (with resonance involving the 2-NH_2 group) are representative of the serine-264 family of Q_B inhibitors. Hydrogen bond formation would appear to involve the C-9 carbonyl functionality only.

Thus, chlorophyll fluorescence measurements in conjunction with CNDO calculations reveal that anthraquinones with similar net π-electronic charge distributions can displace one another from their Photosystem II binding site(s). These results also suggest that the chemically related class of substituted 9,10-anth-

raquinones can exhibit the functional properties of either the histidine or serine family of inhibitors or both families of inhibitors. This proposal will be tested in future competition studies of substituted 9,10-anthraquinones with typical representatives of the histidine and serine families of Q_B-inhibitors.

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