Biochimica et Biophysica Acta 932 (1988) 84–90
Elsevier

BBA 42692

Quenching of chlorophyll fluorescence by substituted anthraquinones

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(Received 15 June 1987)

Key words: Chlorophyll fluorescence; Fluorescence quenching; Anthraquinone; (Barley chloroplast)

We have used conventional and modified Stern-Volmer analysis to quantitate the chlorophyll fluorescence quenching abilities of substituted anthraquinones in barley chloroplasts. Anthraquinone molecules quench the singlet photoexcited state of light-harvesting chlorophyll (Chl *), and the reduction in the populations of Chl * lowers the observed chlorophyll fluorescence intensity. As in earlier studies, two parameters characterize the quenching activity of a quinone: the fraction of chlorophyll fluorescence accessible to quinone, f_a, and the Stern-Volmer quenching constant, K_{SV}. The fluorescence data suggest that the hydrophilicity of anthraquinone substituents controls the fraction of chlorophyll fluorescence accessible to quinone. The dependence of f_a on substituent hydrophilicity is quantified to determine the optimal degree of hydrophobic character for maximum affinity between quinone and thylakoid membrane. The magnitudes of the Stern-Volmer quenching constants are found to reflect the electronic characteristics of the anthraquinone substituents.

Introduction

The promotion of organic molecules to electronically excited states occurs upon the absorption of photons of light. Photochemically excited chlorophyll pigments vibrationally relax to the lowest excited electronic state by a rapid thermal equilibration process [1]. A number of alternative pathways permit the subsequent decay of the lowest excited singlet state of chlorophyll to the ground state. In the light-harvesting chlorophyll antennae of photosynthetic organisms, the primary de-excitation pathway is that of efficient excitation transfer to neighboring chlorophyll molecules and ultimately to the primary electron acceptor. Other competing de-excitation mechanisms, including nonradiative decay and fluorescence, lower the quantum yield of photochemistry. The addition of exogenous substances to the chloroplast medium may accelerate the decay of an electronically excited state, and consequently such substances are known as quenchers. The prime method of observing the effect of a quencher on a fluorophore, such as chlorophyll, is by monitoring the effects on the intensity and lifetime of fluorescence emission [2].

Substituted quinones have been observed to dynamically quench the fluorescence of chloro-

Abbreviations: Chl, chlorophyll; DMOE, 1,2-dimethoxyethane; DMSO, dimethyl sulfoxide; f_a, fraction of chlorophyll fluorescence accessible to quencher; F_{max}, maximum chlorophyll fluorescence level with Photosystem II electron acceptor Q reduced; Hepes, 4-(2-hydroxyethyl)-1-piperazinesulfonic acid; I_0 and I, chlorophyll fluorescence intensities in the absence and presence of quinone quencher; K_{SV}, Stern-Volmer quenching constant; [Q], concentration of added quinone quencher.

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phyll and other porphyrin molecules in solution (e.g., Refs. 3–11), presumably via intermolecular electron transfer from the excited singlet state of chlorophyll to quinone [5,9,10].

$$\text{Chl} \xrightarrow{h\nu} \text{Chl}^* \xrightarrow{\text{quinone}} \{\text{Chl}^* \ldots \text{quinone}\}$$

$$\rightarrow \{\text{Chl}^+ + \text{quinone}^-\}$$

The charge transfer complex is assumed to decay to the ground state before separation of the ion pair can occur [5,9].

$$\{\text{Chl}^+ + \text{quinone}^-\} \rightarrow \text{Chl} + \text{quinone}$$

Substituted quinones are often included in plant chloroplast preparations both as artificial electron acceptors and as redox mediators in potentiometric titrations. All too often, the photochemical quenching properties of the quinone molecules, and the subsequent complications, are entirely overlooked. The quenching activity of certain substituted benzoquinones and naphthoquinones on the room-temperature chlorophyll fluorescence of plant chloroplasts has been documented [12–17]. Unlike the uniformly strong quenching by quinones of chlorophyll fluorescence in solution [11–13], the degree of quenching observed in chloroplasts is highly variable. In earlier studies involving barley chloroplasts [12,13], we attributed the variable quenching abilities of substituted benzoquinones and naphthoquinones to contributions from both lipophilic and electronic factors. In general, hydrophobic substituents promote quenching activity. In the case of benzoquinones, the presence of hydrophilic moieties prevents quinone–chlorophyll interaction, while for naphthoquinones, a highly hydrophobic group may counterbalance the ability of a hydrophilic group to limit the solubility of a naphthoquinone in the thylakoid membrane. The electronic character of hydrophobic substituents also influences the degree of quenching. For a benzoquinone, a high degree of quenching requires the presence of both electron-releasing and electron-withdrawing hydrophobic groups. No correlation is observed between quenching effects and quinone reduction potentials or polarographic half-wave reduction potentials. In the case of naphthoquinones, the presence of electron-withdrawing substituents at the 2 and 3 positions leads to significant quenching. Only a qualitative correlation between the extent of quenching and naphthoquinone reduction potentials is observed.

In the current study we present the results of chlorophyll fluorescence quenching studies in barley chloroplasts using various substituted anthraquinones to extend the documentation of quinone quenching effects. An ideal range of substituent hydrophilicity for maximum quenching activity is derived. The data are also consistent with the electronic character of anthraquinone substituents governing the chlorophyll fluorescence quenching activity.

Materials and Methods

As in previous studies [12,13], 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 washing with the same medium and 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 approx. 1 mg Chl per ml. For fluorescence measurements, the chloroplast suspension was diluted with the final buffer to a concentration of 10 μg Chl per ml.

Substituted quinones were purchased from Aldrich Chemical Company unless otherwise noted and included: 9,10-anthraquinone (Alfa); 1-hydroxy-9,10-anthraquinone (ChemService, Westchester, Pa.); 1,2-dihydroxy-9,10-anthraquinone; 1,4-dihydroxy-9,10-anthraquinone (quinizarin); 1,5-dihydroxy-9,10-anthraquinone (anthrarufin); 1,8-dihydroxy-9,10-anthraquinone (chrysazin); 2,6-dihydroxy-9,10-anthraquinone (anthraflavic acid); 1,2,4-trihydroxy-9,10-anthraquinone (Pfaltz and Bauer); 1,2,5,8-tetrahydroxy-9,10-anthraquinone (quinalizarin); 1,4-anthraquinone (Alfa); and 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione; 1-amino-9,10-anthraquinone; 2-amino-9,10-anthraquinone; 1,2-diamino-9,10-anthraquinone; 1,4-diamino-9,10-anthraquinone; 1,5-diamino-

9,10-anthraquinone (Pfaltz and Bauer); 2,6-diamino-9,10-anthraquinone (Pfaltz and Bauer); 1,4,5,8-tetraamino-9,10-anthraquinone; 1-amino-4-hydroxy-9,10-anthraquinone; 1,5-diamino-4,8-dihydroxy-9,10-anthraquinone (Alfa); anthraquinone-1,5-disulfonic acid disodium salt; anthraquinone-2,6-disulfonic acid disodium salt; 3,4-dihydroxyanthraquinone-2-sulfonic acid sodium salt; 2-methoxy-9,10-anthraquinone; 1-amino-2-methyl-3-bromo-9,10-anthraquinone; 2-ethyl-9,10-anthraquinone; anthraquinone-2-carboxylic acid; 1-chloro-9,10-anthraquinone; 2-chloro-9,10-anthraquinone; 3-chloroanthraquinone-2-carboxylic acid (Pfaltz and Bauer). For comparison, the following 1,4-naphthoquinones were also examined: 2-hydroxy-; 5-hydroxy-; 5,8-dihydroxy-; and 2,3-epoxy-2,3-dihydro-1,4-naphthoquinone.

As necessary, quinones were further purified by recrystallization or sublimation. Investigations using 1,5-dihydroxy-9,10-anthraquinone were not possible due to difficulties in obtaining pure crystals, even upon sublimation. Stock solutions of the quinones (10 or 20 mM) were prepared in dimethyl sulfoxide (DMSO) except for 5-hydroxy-1,4-naphthoquinone which required 1,2-dimethoxyethane (DMOE) for solubility. Difficulties in dissolving 2,6-dihydroxy-9,10-anthraquinone in any solvent prevented investigations involving this quinone. Quinone-enriched chloroplast samples were prepared by adding appropriate volumes of quinone stock solutions to diluted chloroplast suspensions to give a final concentration of 2% (v/v) DMOE or DMSO. Neither solvent showed quenching effects on chlorophyll fluorescence at this concentration. Overall quinone concentrations in fluorescence samples ranged from 0 to 200 μM.

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 close Photosystem II reaction centers. Furthermore, fluorescence measurements were made for quinone-chloroplast systems after a 15 min incubation period to maximize quinone–membrane interaction. Thus, Stern-Volmer analysis of the observed chlorophyll fluorescence levels accurately quantifies membrane accessibility and quinone quenching activity, but does not reflect differences in the time-courses of quinone–membrane interactions.

The techniques of Stern-Volmer and modified Stern-Volmer analysis were used as previously described [12,13]. In summary, the Stern-Volmer equation describes both static and dynamic quenching of chlorophyll fluorescence:

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

where I_0 and I are chlorophyll fluorescence intensities in the absence and presence of quinone quencher, respectively, K_{SV} is the Stern-Volmer quenching constant, and [Q] is the concentration of quinone quencher. A linear Stern-Volmer plot of I_0/I vs. [Q] is generally indicative of a single class of chlorophyll fluorophores with equal accessibility to quencher [18,19].

A Stern-Volmer plot may exhibit downward curvature and a limiting I_0/I value when a portion of the chlorophyll population is inaccessible to quencher. A modified Stern-Volmer equation is required to determine the accessible fraction of chlorophyll fluorescence, f_a, and the Stern-Volmer constant for the accessible fraction of chlorophyll fluorescence [18,19]:

$$\frac{I_0}{\Delta I} = \frac{1}{f_a K_{SV}[Q]} + \frac{1}{f_a} \quad (2)$$

where ΔI is the fluorescence intensity difference in the absence and presence of quinone. Heterogeneity in chlorophyll pigment populations may be present within the fluorophores responsible for either the accessible and/or inaccessible fractions of fluorescence. Under such conditions, f_a would represent the total accessible fraction of fluorescence and K_{SV} the average Stern-Volmer quenching constant for all accessible fractions. No measure of differential quinone accessibility to distinct chlorophyll-protein complexes was attempted in this work. Modified Stern-Volmer analyses were performed for all quinones which gave a nonlinear Stern-Volmer plot.

Results

Table I summarizes the results of the Stern-Volmer analysis of the quenching of chlorophyll fluorescence by substituted 9,10-anthraquinones in barley chloroplasts. Data are presented for fluorescence at both 684 nm and 730 nm.

In all cases, hydroxy substitution dramatically increases the fraction of chlorophyll fluorescence that is accessible to and thus affected by quinone. Except for 1,2-dihydroxy-9,10-anthraquinone, the f_a values of hydroxy-substituted 9,10-anthraquinones in Table I are calculated to be in the range of about 0.7–0.9. Variations in the Stern-Volmer quenching constant for hydroxy-substituted 9,10-anthraquinones ranged almost 300-fold. Low K_{SV} values, of $(1.1–7.6)\cdot 10^4$ M^{-1}, were measured for 9,10-anthraquinones with a hydroxy group at the 2-position: 1,2-dihydroxy-; 1,2,4-trihydroxy-; and 1,2,5,8-tetrahydroxy-9,10-anthraquinone. Large K_{SV} values, of $(0.9–2.9)\cdot 10^6$ M^{-1}, were observed for all 9,10-anthraquinones with hydroxy substituents limited to the 1, 4, and/or 8 positions: 1-hydroxy-; 1,4-dihydroxy-; and 1,8-dihydroxy-9,10-anthraquinone.

Linear Stern-Volmer plots were obtained for all 9,10-anthraquinones with amino substituents except 1-amino-4-hydroxy-9,10-anthraquinone. All other substituted 9,10-anthraquinones except 3,4-dihydroxyanthraquinone-2-sulfonic acid required analysis using the modified Stern-Volmer equation (Eqn. 2).

In general, amino substituents decreased the K_{SV} value of 9,10-anthraquinone by 10- to 300-

TABLE I

STERN-VOLMER FLUORESCENCE PARAMETERS FOR 9,10-ANTHRAQUINONES IN BARLEY CHLOROPLASTS

These data present the calculated fraction of chlorophyll fluorescence that is accessible to quinone quencher (f_a) and the corresponding Stern-Volmer quenching constant (K_{SV}) for both the 684 and 730 nm chlorophyll fluorescence of barley chloroplasts incubated with various substituted 9,10-anthraquinones.

Substituents								684 nm fluorescence		730 nm fluorescence	
X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	f_a	K_{SV} (M^{-1})	f_a	K_{SV} (M^{-1})
H	H	H	H	H	H	H	H	0.084	$8.9\cdot10^4$	0.034	$6.0\cdot10^4$
OH	H	H	H	H	H	H	H	0.73	$9.0\cdot10^5$	0.74	$8.3\cdot10^5$
OH	OH	H	H	H	H	H	H	0.34	$2.9\cdot10^4$	0.32	$2.7\cdot10^4$
OH	H	H	OH	H	H	H	H	0.85	$1.9\cdot10^6$	0.86	$1.7\cdot10^6$
OH	OH	H	OH	H	H	H	H	0.93	$1.1\cdot10^4$	0.94	$1.0\cdot10^4$
OH	H	H	H	H	H	H	OH	0.81	$2.9\cdot10^6$	0.80	$2.9\cdot10^6$
OH	OH	H	H	OH	H	H	OH	0.68	$7.6\cdot10^4$	0.67	$8.1\cdot10^4$
NH_2	H	H	H	H	H	H	H	1.00	$6.6\cdot10^2$	1.00	$2.4\cdot10^2$
H	NH_2	H	H	H	H	H	H	1.00	$1.4\cdot10^3$	1.00	$1.2\cdot10^3$
NH_2	NH_2	H	H	H	H	H	H	1.00	$4.9\cdot10^3$	1.00	$3.7\cdot10^3$
NH_2	H	H	NH_2	H	H	H	H	1.00	$1.1\cdot10^4$	1.00	$7.4\cdot10^3$
NH_2	H	H	H	NH_2	H	H	H	1.00	$4.7\cdot10^2$	1.00	$-2.5\cdot10^2$
H	NH_2	H	H	H	NH_2	H	H	1.00	$3.2\cdot10^2$	1.00	$1.3\cdot10^2$
NH_2	H	H	NH_2	NH_2	H	H	NH_2	1.00	$9.4\cdot10^3$	1.00	$3.5\cdot10^3$
NH_2	H	H	OH	H	H	H	H	0.70	$3.0\cdot10^4$	0.62	$3.8\cdot10^4$
NH_2	H	H	OH	NH_2	H	H	OH	1.00	$1.3\cdot10^4$	1.00	$9.8\cdot10^3$
H	SO_3^-	OH	OH	H	H	H	H	1.00	$2.2\cdot10^3$	1.00	$2.5\cdot10^3$
H	SO_3^-	H	H	H	SO_3^-	H	H	0	0	0	0
SO_3^-	H	H	H	SO_3^-	H	H	H	0	0	0	0
H	CH_2OH	H	H	H	H	H	H	0.76	$1.2\cdot10^4$	0.84	$1.4\cdot10^4$
NH_2	CH_3	H	Br	H	H	H	H	0.14	$3.8\cdot10^3$	0	0
H	CH_2CH_3	H	H	H	H	H	H	0.40	$1.8\cdot10^5$	0.41	$1.8\cdot10^5$
H	COOH	H	H	H	H	H	H	0.64	$4.1\cdot10^3$	0.68	$4.2\cdot10^3$
H	Cl	H	H	H	H	H	H	0.41	$2.0\cdot10^6$	0.39	$1.1\cdot10^6$
H	COOH	Cl	H	H	H	H	H	0.64	$3.4\cdot10^4$	0.64	$2.6\cdot10^4$
Cl	H	H	H	H	H	H	H	0.55	$1.8\cdot10^5$	0.46	$3.2\cdot10^5$

fold, while significantly increasing f_a. The strongest quenching induced by quinones with only amino substituents was observed for those with amino groups at positions 1 and 4: 1,4-diamino-9,10-anthraquinone and 1,4,5,8-tetraamino-9,10-anthraquinone. The weakest quenching was observed for 1-amino-; 1,5-diamino-; and 2,6-diamino-9,10-anthraquinone. However, hydroxy substitution of 1-amino-9,10-anthraquinone at the 4-position and dihydroxy substitution of 1,5-diamino-9,10-anthraquinone at the 4- and 8-positions led to increases in the Stern-Volmer quenching constant.

Disubstitution of only sulfonic acid groups (in the form of the sodium salt) resulted in no chlorophyll fluorescence quenching. However, dihydroxy substitution in the presence of a single sulfonic acid group resulted in a small degree of quenching.

The only non-hydroxy-substituted quinones in Table I that promoted a greater extent of fluorescence quenching than the unsubstituted 9,10-anthraquinone were 2-ethyl-9,10-anthraquinone, 1-chloro-9,10-anthraquinone and 2-chloro-9,10-anthraquinone. The placement of the Cl atom in the 2-position resulted in a Stern-Volmer quenching constant 10-times greater than that measured for Cl-substitution at the 1-position. The fraction of chlorophyll fluorescence accessible to quinone was higher for 1-chloro-9,10-anthraquinone, however.

Substitution of 2-chloro-9,10-anthraquinone with a carboxyl group at the 3-position lowers the measured K_{SV} value about 100-fold, while substitution of anthraquinone-2-carboxylic acid with a Cl atom at the 3-position increases K_{SV} by 10-times.

In general, Stern-Volmer parameters measured at 730 nm were comparable to those obtained at 684 nm. For those quinones with linear Stern-Volmer plots, the K_{SV} value at 730 nm was usually slightly lower than that at 684 nm. A slight enhancement of chlorophyll fluorescence was detected at long wavelengths for 1,5-diamino-9,10-anthraquinone.

Table II presents the comparable Stern-Volmer data for 1,4-anthraquinone and 9,10-dihydroxy-1,4-anthraquinone. For comparison, Table II also summarizes the fluorescence quenching data for hydroxy-substituted 1,4-naphthoquinones. It is interesting to note the similar Stern-Volmer quenching constants for 1,4-anthraquinone and 9,10-anthraquinone, but the significantly higher fraction of chlorophyll fluorescence that is quenched by 1,4-anthraquinone. Furthermore, the structurally related 9,10-dihydroxy-1,4-naph-

TABLE II

STERN-VOLMER PARAMETERS FOR FLUORESCENCE QUENCHING BY HYDROXY-SUBSTITUTED 1,4-NAPHTHOQUINONES AND 1,4-ANTHRAQUINONES IN BARLEY CHLOROPLASTS

These data present the calculated fraction of chlorophyll fluorescence that is accessible to quinone quencher (f_a) and the corresponding Stern-Volmer quenching constant (K_{SV}) for both the 684 and 730 nm room-temperature chlorophyll fluorescence of barley chloroplasts incubated with various 1,4-naphthoquinones and 1,4-anthraquinones.

Hydroxy-substituted 1,4-naphthoquinones						684-nm fluorescence		730-nm fluorescence		Ref
Substituent						f_a	K_{SV} (M^{-1})	f_a	K_{SV} (M^{-1})	
X_2	X_3	X_5	X_6	X_7	X_8					
H	H	H	H	H	H	0.66	$1.8 \cdot 10^4$	0.67	$1.8 \cdot 10^4$	[12]
OH	H	H	H	H	H	0	0	0	0	[12]
H	H	OH	H	H	H	1.00	$1.0 \cdot 10^4$	1.00	$1.1 \cdot 10^4$	[12]
O		H	H	H	H	1.00	$1.0 \cdot 10^2$	1.00	$1.0 \cdot 10^2$	[12]
H	H	OH	H	H	OH	0.75	$6.6 \cdot 10^4$	0.70	$6.4 \cdot 10^4$	–
1,4-Anthraquinones										
1,4-Anthraquinone						0.75	$1.4 \cdot 10^5$	0.71	$1.3 \cdot 10^5$	
9,10-Dihydroxy-1,4-anthraquinone						0.75	$5.1 \cdot 10^5$	0.77	$4.7 \cdot 10^5$	

thoquinone, 1-4-dihydroxy-9,10-anthraquinone, and 5,8-dihydroxy-1,4-naphthoquinone compounds have similar f_a values, although the K_{SV} value of the naphthoquinone is 10-times smaller than that of either anthraquinone.

Discussion

In previous studies [12,13] we have characterized the fluorescence quenching capabilities of quinones in terms of the parameters obtained from Stern-Volmer analysis. The f_a value, the fraction of chlorophyll fluorescence accessible to quinone, was regarded as a measure of quinone-membrane affinity. We have suggested [13] that the substituent hydrophobicity as quantified by Hansch π constants [20,21] correlates well with observed f_a values. Furthermore, we have suggested that the Stern-Volmer quenching constant, K_{SV}, reflects the electronic character of quinone substituents. We have proposed [13] that 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.

Conventional and modified Stern-Volmer analysis of the anthraquinone data in the present study also illustrates the correlations suggested above. In particular, we see that the hydrophobic parent 9,10-anthraquinone ($f_a = 0.084$) requires hydrophilic substituents (-OH, $-NH_2$, $-SO_3^-$, -COOH, $-CH_2OH$) to increase the quinone-membrane affinity (as revealed by increased f_a values). Fig. 1 presents the dependence of f_a on substituent hydrophobicity, using Hansch π values [20,21] and assuming π-value additivity for multiple substitution [21]. The 'parabolic' dependence of f_a on π is expected when a large range of π values is considered, suggesting ideal π values for maximum transport of a quinone to its site of action within the chloroplast. Quinone-membrane affinity reaches a maximum value (i.e., $f_a = 1$) at optimal π values in the range of about -1.2 to at least -6.1.

Of particular interest in Fig. 1 are those quinones with hydroxy substituents at both the 1- and 2-positions: 1,2-dihydroxy-; 1,2,4-trihydroxy-; and 1,2,5,8-tetrahydroxy-9,10-anthraquinone. The lack of correlation of f_a with π for these quinones is anticipated. These quinones may show unusual quenching effects as a consequence of intramolecular hydrogen-bonding between the hydroxy substituents [22]. Furthermore, it has been demonstrated that π-value additivity can not be assumed when intramolecular interactions are present [23].

The Stern-Volmer quenching constant appears to reflect the electronic character of the quinone substituents. Electron-releasing groups (-OH, $-CH_2CH_3$) and weakly electron-withdrawing groups (-Cl), as measured by Hammett σ constants [21], lead to K_{SV} values significantly greater than that observed for the parent unsubstituted 9,10-anthraquinone. Thus, substituents which reduce the oxidation-reduction potential of the anthraquinone promote high quenching activity [24]. The low magnitudes of K_{SV} values for amino-substituted quinones may be interpreted to indicate that the amino groups are protonated in the thylakoid membrane and thus, as strongly electron-withdrawing groups, would reduce quenching activity. The additivity of σ constants does not appear to be a reasonable assumption (results not shown), and we are therefore currently

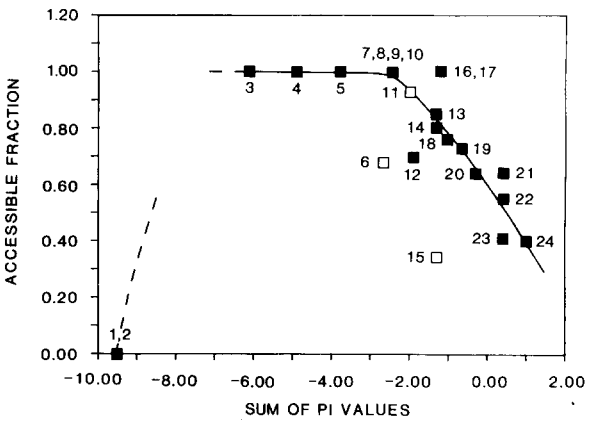

Fig. 1. The fraction of chlorophyll fluorescence accessible to quinone, as measured by the Stern-Volmer parameter, f_a, as a function of the sum of π substituent constants for substituted 9,10-anthraquinones without (■) and with (□) 1,2-dihydroxy substitution. The data points correspond to the following substituents: (1) 1,5-disulfonic acid; (2) 2,6-disulfonic acid; (3) 2-sulfonic acid, 3,4-dihydroxy; (4) 1,4,5,8-tetraamino; (5) 1,5-diamino-4,8-dihydroxy; (6) 1,2,5,8-tetrahydroxy; (7) 1,2-diamino; (8) 1,4-diamino; (9) 1,5-diamino; (10) 2,6-diamino; (11) 1,2,4-trihydroxy; (12) 1-amino-4-hydroxy; (13) 1,4-dihydroxy; (14) 1,8-dihydroxy; (15) 1,2-dihydroxy; (16) 1-amino; (17) 2-amino; (18) 2-methoxy; (19) 1-hydroxy; (20) 2-carboxylic acid; (21) 2-carboxylic acid-3-chloro; (22) 1-chloro; (23) 2-chloro; (24) 2-ethyl.

seeking quantitative structure-activity relationships to define more precisely the role of substituent electronic character in quinone intrinsic quenching activity.

The fluorescence quenching data of 1,4-anthraquinone revealed an increasing trend in f_a values for the series 1,4-benzoquinone ($f_a = 0.21$ [12,13]) < 1,4-naphthoquinone (0.66 [12,13]) < 1,4-anthraquinone (0.75). Each additional ring appears to increase the quinone solubility in the membrane and enhance the chances of fluorescence quenching activity. No simple dependence of K_{SV} value on number of rings is observed, however. The sizable difference in f_a values for 9,10-anthraquinone and 1,4-anthraquinone indicates that the relative position of carbonyl groups and rings is critical for quinone solubility and/or transport to the site of quenching action.

Conclusion

Substituted anthraquinones have been observed to quench the room-temperature chlorophyll fluorescence of barley chloroplasts. The variable degree of quenching has been analyzed using conventional and modified Stern-Volmer techniques. As in our previous studies [12,13], we have assigned the origin of the Stern-Volmer quenching parameters to lipophilic and electronic factors. The substituent hydrophobicity as quantified by Hansch π constants [20,21] led to a parabolic dependence of f_a on π. Furthermore, the Stern-Volmer quenching constant, K_{SV}, reflected the electronic character of quinone substituents. Electron-donating groups and weakly electron-withdrawing groups promoted high quenching activity. These relations of quinone structure and physicochemical properties to quenching activity in plant chloroplasts will be used in further investigations to precisely determine the operative mechanism of fluorescence quenching by extrinsic quinones.

Acknowledgements

This research was supported by an Exxon Education Foundation Grant of Research Corporation, a grant from the California Foundation for Biochemical Research, a Harvey Mudd College Faculty Research Award, and a grant from the Harvey Mudd College Arnold and Mabel Beckman Research Fund. The authors also gratefully acknowledge support from the Chemistry Department of Harvey Mudd College.

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