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Multivariate analysis of Photosystem II fluorescence quenching by substituted benzoquinones and naphthoquinones

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The present investigation examines the action of substituted benzoquinones and naphthoquinones as inhibitors of chlorophyll fluorescence in barley chloroplasts. Stern–Volmer analyses have been used to determine the fractions of chlorophyll fluorescence accessible to quinone as a measure of quinone-membrane solubility and the Stern–Volmer quenching constant as a measure of quinone intrinsic activity. Correlations between quinone structural properties and experimentally measured chlorophyll fluorescence parameters have been made to determine the contribution of lipophilic and electronic factors to quinone solubility and intrinsic activity. In particular, 1,4-benzoquinones must have hydrophobic substituents or hydrogen atoms at positions 2, 3, 5 and 6 for fluorescence quenching activity. The magnitude of the Stern–Volmer quenching constant is affected by the electronic character of hydrophobic substituents, with a maximum value observed in the presence of both electron-releasing and electron-withdrawing moieties. Similarly, the lipophilic character of 2,3-substituents of 1,4-naphthoquinones determines the fraction of chlorophyll fluorescence accessible to quinone, with a maximum accessibility observed for hydrophobic substituents. Naphthoquinone intrinsic activity is also correlated with the electronic character of the substituents and is observed to be at a maximum value with only electron-withdrawing substituents.

Introduction

The efficiency of the photosynthetic process in plants is dependent upon the transfer of absorbed light to photochemical reaction centers and the subsequent electron transport along a specialized pathway of electron donors and acceptors. One indirect measure of photosynthetic efficiency is the level of chlorophyll fluorescence emission upon light absorption, that is, the amount of excitation that is lost from the photochemical path in the form of chlorophyll fluorescence. Certain substituted quinones, when added to the suspending medium of plant chloroplasts, quench the level of

Abbreviations: Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DMSO, dimethylsulfoxide; f_a, fraction of chlorophyll fluorescence accessible to quencher; F_{max}, maximum fluorescence, level of chlorophyll fluorescence with Photosystem II electron acceptor Q reduced; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic 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; $[Q]_{50}$, quinone concentration necessary to induce a decrease in chlorophyll fluorescence intensity to 50% of the observed level in the absence of quinone; TMBQ, 2,3,5,6-tetramethyl-1,4-benzoquinone; TCBQ, 2,3,5,6-tetrachloro-1,4-benzoquinone; QSAR, quantitative structure-activity relationships.

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room-temperature chlorophyll fluorescence [1–3]. The mode of action of these substituted quinones as fluorescence quenchers is not clearly known, but studies suggest that they function to dissipate excitation energy by interaction with Photosystem II light-harvesting chlorophyll-protein complexes or by interaction at or near the Photosystem II reaction-center chlorophyll [1,4–6]. The objective of this study is to determine those structural and physico-chemical parameters of substituted benzoquinones and naphthoquinones that enhance interaction with photosynthetic membranes as evidenced by chlorophyll fluorescence quenching in barley chloroplasts.

In particular, the present investigation uses Stern–Volmer analysis methods [7,8] to compare the fluorescence quenching abilities of extrinsic benzoquinones and naphthoquinones on the chlorophyll emission spectra of barley chloroplasts. Both classical and modified Stern–Volmer equations enable the determination of fluorescence quenching constants and corresponding fractions of chlorophyll fluorescence accessible to extrinsic quinones. Correlations between quinone structural properties and experimentally measured chlorophyll fluorescence parameters are made to determine the relation of chemico-physical properties to inhibitory action and to provide a means toward the elucidation of the operable mechanism of fluorescence quenching.

Materials and Methods

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. After centrifugation at 6000 × *g* for 10 min and washing with the same medium, the chloroplasts were resuspended in a medium of 0.1 M sucrose/10 mM Hepes-NaOH (pH 7.5)/10 mM NaCl. Following centrifugation at 6000 × *g* for 10 min, the pellet was resuspended in the same medium and kept in the dark for 15 min at approx. 4 °C. Following a final centrifugation at 6000 × *g* for 10 min, the pellet was resuspended in a medium of 0.1 M sucrose/10 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: 1,4-benzoquinone (Eastman Organic Chemicals); 2,5-dihydroxy-1,4-benzoquinone; 2,3,5,6-tetrachloro-1,4-benzoquinone; 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (Alfa Products); 2,3,5,6-tetramethyl-1,4-benzoquinone; tetrahydroxy-1,4-quinone hydrate; 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; 1,4-naphthoquinone; 2-hydroxy-1,4-naphthoquinone; 2-methoxy-1,4-naphthoquinone; 2-methyl-1,4-naphthoquinone; 2-amino-1,4-naphthoquinone; 2-bromo-1,4-naphthoquinone; 2,3-dichloro-1,4-naphthoquinone (Alfa); 2-chloro-3-morpholino-1,4-naphthoquinone; 2-amino-3-chloro-1,4-naphthoquinone; 2-chloro-3-pyrrolidino-1,4-naphthoquinone; 2,3,5,6-tetrahydroxy-1,4-naphthoquinone; 5-hydroxy-1,4-naphthoquinone; 2,3-epoxy-2,3-dihydro-1,4-naphthoquinone. As necessary, quinones were further purified by recrystallization or sublimation. Stock solutions of the quinones (20 mM) were prepared in ethanol or DMSO. The quinone-enriched chloroplast samples were prepared by adding appropriate volumes of quinone stock solutions to diluted chloroplast suspensions to give a final concentration of 1% (v/v) ethanol or DMSO. Both solvents showed no quenching effects on chlorophyll fluorescence at concentrations less than or equal to 2% (v/v) in aqueous solution. Quinone concentrations in chloroplast samples ranged from 0 to 200 μM.

Room-temperature fluorescence emission spectra were measured 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 650–740 nm with a Hamamatsu R928 photomultiplier tube. Measurements of chlorophyll fluorescence were made for chloroplasts in the F_{max} state, that is, with saturating light intensity to close Photosystem II reaction centers and induce maximal chlorophyll fluorescence levels.

For experiments involving the quenching effect of quinones on chlorophyll in organic solution,

chlorophyll was extracted from isolated pea chloroplasts using an 80% (v/v) acetone-water mixture. Fluorescence measurements were made with chlorophyll samples of 5 μg/ml and quinone concentrations ranging from 0 to 1600 μM. Excitation and emission parameters were identical to those employed for fluorescence measurements involving barley chloroplasts.

Both static and dynamic quenching of chlorophyll fluorescence can be described by the Stern–Volmer equation:

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

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 Stern–Volmer plot of I_0/I vs. [Q] has a y-intercept of 1 and a slope equal to K_{SV}. A linear Stern–Volmer plot is generally indicative of a single class of chlorophyll fluorophores with equal accessibility to quencher [7,8].

In situations where a chlorophyll population exists which is inaccessible to quencher, a Stern–Volmer plot exhibits downward curvature. As fluorescence arising from accessible chlorophyll molecules is quenched at increasingly higher quencher concentrations, the origin of the remaining fluorescence is from inaccessible fluorophores. The total chlorophyll fluorescence in the absence of quinone quenchers, I_0, may be described by:

$$I_0 = I_{0,a} + I_{0,i} \quad (2)$$

where the subscripts a and i refer to the accessible and inaccessible chlorophyll fluorescence, respectively. In the presence of quencher the intensity of the accessible fraction of fluorescence is decreased according to the Stern–Volmer equation (Eqn. 1), whereas the inaccessible fraction is not quenched. Therefore,

$$I = \frac{I_{0,a}}{1 + K_{SV}[Q]} + I_{0,i} \quad (3)$$

where K_{SV} is the Stern–Volmer constant for the accessible fraction of chlorophyll fluorescence. Combination of Eqns. 2 and 3 results in a modified Stern–Volmer equation [7,8]:

$$\frac{I_0}{\Delta I} = \frac{1}{f_a K_{SV}[Q]} + \frac{1}{f_a} \quad (4)$$

where ΔI is the fluorescence intensity difference in the absence and presence of quinone and f_a is the fraction of the initial fluorescence which is accessible to quencher. Eqn. 4 is also valid for a situation with more than one class of accessible chlorophyll fluorophores, where f_a simply represents the sum of accessible fractions of fluorescence and K_{SV} is an average Stern–Volmer quenching constant [8].

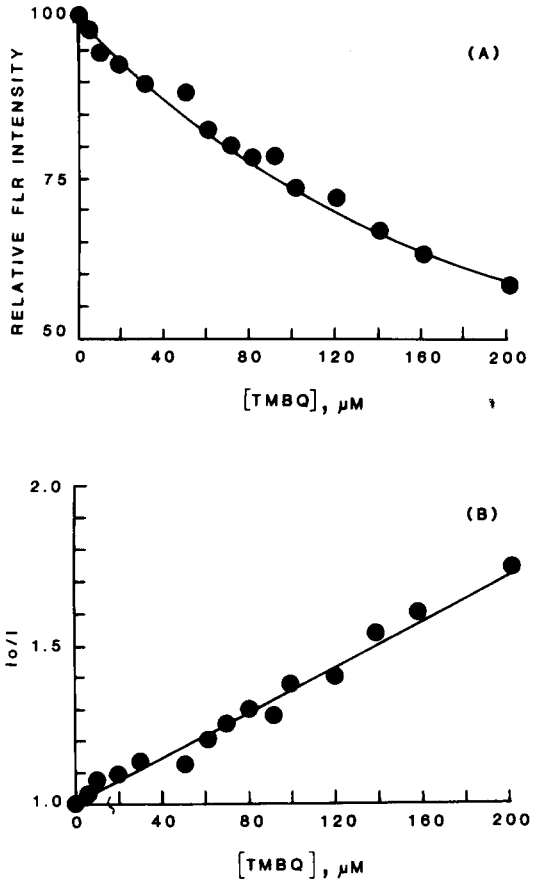


Fig. 1. (A) Chlorophyll fluorescence emission levels of barley chloroplasts at F_{max} as a function of added 2,3,5,6-tetramethyl-1,4-benzoquinone (TMBQ). (B) Stern–Volmer plot for barley chloroplasts with added TMBQ.

Results

Fluorescence quenching effects of benzoquinones in chloroplasts

The F_{max} chlorophyll emission spectra of barley chloroplasts incubated in the presence of variable concentrations of substituted quinones were recorded. A quenching of the chlorophyll fluorescence level occurs at all wavelengths, with the chlorophyll emission maximum remaining at 684 nm with added quinone. Fig. 1A presents the relative F_{max} chlorophyll fluorescence level at 684 nm of barley chloroplasts as a function of concentration of added 2,3,5,6-tetramethyl-1,4-benzoquinone (TMBQ). The linearity of the Stern–Volmer plot of Fig. 1B indicates that, for the given quinone concentration range, all F_{max} chlorophyll fluorescence is accessible to TMBQ with a Stern–Volmer quenching constant of $3.5 \cdot 10^3$ M^{-1}.

In contrast, Fig. 2A and B present the dependence of in vivo chlorophyll fluorescence intensity on the concentration of added 2,3,5,6-tetrachloro-1,4-benzoquinone (TCBQ) and the corresponding Stern–Volmer plot. The non-linearity of the Stern–Volmer plot in Fig. 2B suggests the presence of chlorophyll fluorescence that is 'inaccessible' to TCBQ, and the modified Stern–Vomer plot of Fig. 2C reveals that the fractional accessible fluorescence is 0.51 with a Stern–Volmer quenching constant of $3.7 \cdot 10^4$ M^{-1}.

Table I summarizes the fluorescence quenching parameters of Stern–Volmer quenching constant, K_{SV}, and fractional accessible fluorescence, f_a, for all benzoquinones examined. Data are presented for both 684-nm fluorescence intensities and 730-nm emission levels. A number of substituted benzoquinones exhibited no quenching effects on chlorophyll fluorescence and are classified with Stern–Volmer quenching constants of 0 M^{-1} and $f_a = 0$. The quinone 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone (DBMIB), a known inhibitor of photosynthetic electron transport, exhibited two distinct Stern–Volmer quenching constants over the concentration ranges of 0–70 μM and 100–200 μM. No variation in the wavelength of maximum emission was observed for any quinone exhibiting quenching effects.

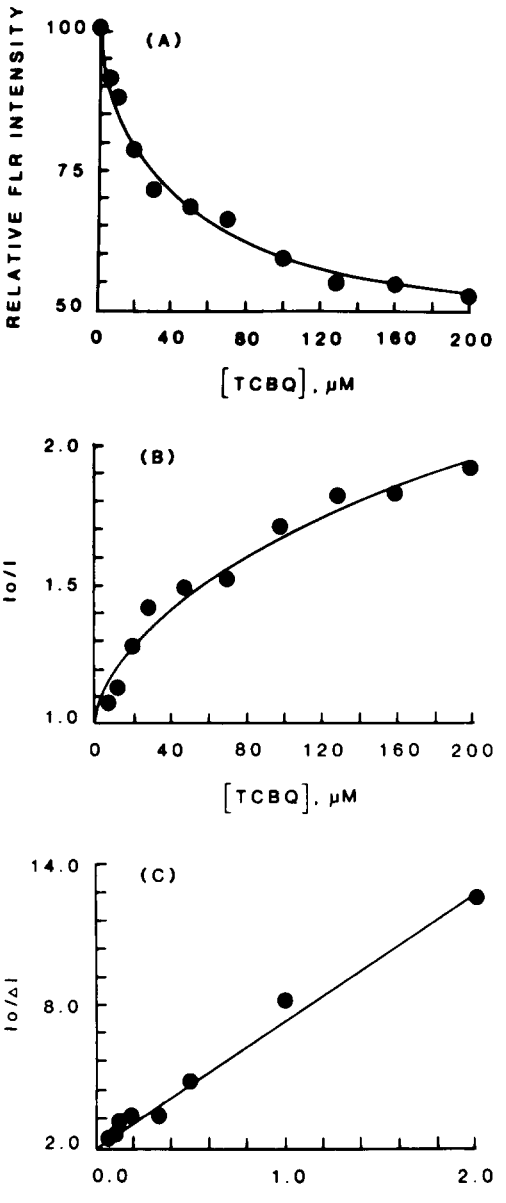


Fig. 2. (A) Chlorophyll fluorescence emission levels of barley chloroplasts at F_{max} as a function of added 2,3,5,6-tetrachloro-1,4-benzoquinone (TCBQ). (B) Stern–Volmer plot for barley chloroplasts with added TCBQ. (C) Modified Stern–Volmer plot for barley chloroplasts with added TCBQ.

Fluorescence quenching effects of naphthoquinones in chloroplasts

Fig. 3A presents the Stern–Volmer plots for the quenching activity of the extrinsic naphthoquinones 1,4-naphthoquinone and 2,3-dichloro-1,4-

TABLE I

STERN–VOLMER PARAMETERS FOR FLUORESCENCE QUENCHING BY SUBSTITUTED BENZOQUINONES IN BARLEY CHLOROPLASTS

These data present the calculated fraction of in vivo 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 substituted benzoquinones.

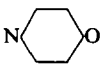
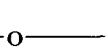
Substituents				684-nm Fluorescence		730-nm Fluorescence	
X_2	X_3	X_5	X_6	f_a	K_{SV} (M^{-1})	f_a	K_{SV} (M^{-1})
H	H	H	H	0.21	$4.0 \cdot 10^4$	0.20	$4.2 \cdot 10^4$
Cl	Cl	Cl	Cl	0.51	$3.7 \cdot 10^4$	0.50	$2.8 \cdot 10^4$
Cl	Cl	CN	CN	0	0	0	0
H	OH	H	OH	0	0	0	0
Cl	OH	Cl	OH	0	0	0	0
OH	OH	OH	OH	0	0	0	0
CH_3	CH_3	CH_3	CH_3	1.00	$3.5 \cdot 10^3$	1.00	$3.5 \cdot 10^3$
Br	CH_3	Br	$CH(CH_3)_2$	0.88 [a]	$1.9 \cdot 10^5$	0.88 [a]	$1.4 \cdot 10^5$
				0.92 [b]	$1.3 \cdot 10^5$	0.92 [b]	$1.4 \cdot 10^5$

[a] Parameters for the quinone concentration range of 0–70 μM.
[b] Parameters for the quinone concentration range of 100–200 μM.

TABLE II

STERN–VOLMER PARAMETERS FOR FLUORESCENCE QUENCHING BY SUBSTITUTED NAPHTHOQUINONES IN BARLEY CHLOROPLASTS

These data present the calculated fraction of in vivo 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 substituted naphthoquinones.

Substituents				684-nm Fluorescence		730-nm Fluorescence	
X_2	X_3	X_5	X_6–X_8	f_a	K_{SV} (M^{-1})	f_a	K_{SV} (M^{-1})
H	H	H	H	0.66	$1.8 \cdot 10^4$	0.67	$1.8 \cdot 10^4$
OH	H	H	H	0	0	0	0
OCH_3	H	H	H	0.34	$6.4 \cdot 10^3$	0.33	$6.7 \cdot 10^3$
CH_3	H	H	H	1.00	$7.5 \cdot 10^3$	1.00	$7.5 \cdot 10^3$
NH_2	H	H	H	1.00	$1.6 \cdot 10^3$	1.00	$1.5 \cdot 10^3$
Br	H	H	H	0.91 [a]	$6.7 \cdot 10^4$	0.96 [a]	$5.5 \cdot 10^4$
				0.98 [b]	$8.1 \cdot 10^4$	0.99 [b]	$9.8 \cdot 10^4$
Cl	Cl	H	H	0.86	$3.8 \cdot 10^5$	0.87	$2.7 \cdot 10^5$
NH_2	Cl	H	H	0.32	$3.1 \cdot 10^4$	0.35	$2.1 \cdot 10^4$
Cl		H	H	0.45	$3.2 \cdot 10^4$	0.40	$3.0 \cdot 10^4$
Cl		H	H	0.51	$2.3 \cdot 10^4$	0.62	$2.1 \cdot 10^4$
H	H	OH	H	1.00	$1.0 \cdot 10^4$	1.00	$1.1 \cdot 10^4$
		H	H	1.00	$1.0 \cdot 10^2$	1.00	$1.0 \cdot 10^2$

[a] Parameters for the quinone concentration range of 0–70 μM.
[b] Parameters for the quinone concentration range of 100–200 μM.

TABLE III

STERN–VOLMER PARAMETERS FOR FLUORESCENCE QUENCHING BY SUBSTITUTED QUINONES IN EXTRACTED CHLOROPHYLL

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 the 684-nm room temperature fluorescence of extracted chlorophyll in the presence of various substituted quinones.

Quinone	f_a	K_{SV} (M^{-1})
1,4-benzoquinone	1.00	87
2,5-dihydroxy-1,4-benzoquinone	1.00	91
1,4-naphthoquinone	1.00	81
2-hydroxy-1,4-naphthoquinone	1.00	63
2-methyl-1,4-naphthoquinone	1.00	89
2-methoy-1,4-naphthoquinone	1.00	71
2-bromo-1,4-naphthoquinone	1.00	110

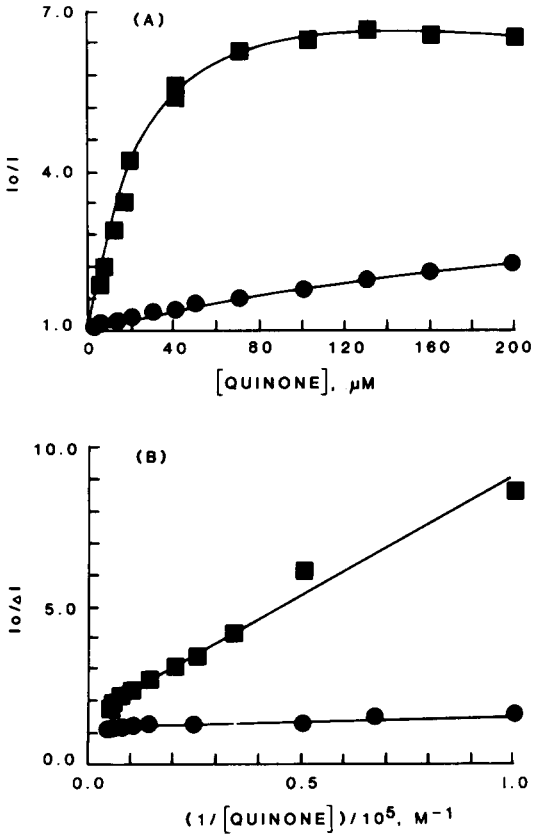


Fig. 3. (A) Stern–Volmer plots for barley chloroplasts in the presence of added 1,4-naphthoquinone (●) and 2,3-dichloro-1,4-naphthoquinone (■). (B) Modified Stern–Volmer plots for barley chloroplasts in the presence of added 1,4-naphthoquinone (■) and 2,3-dichloro-1,4-naphthoquinone (●).

naphthoquinone. The modified Stern–Volmer plots in Fig. 3B result in $f_a = 0.66$ and $K_{SV} = 1.8 \cdot 10^4$ M^{-1} for 1,4-naphthoquinone and $f_a = 0.86$ and $K_{SV} = 3.8 \cdot 10^5$ M^{-1} for 2,3-dichloro-1,4-naphthoquinone.

Table II summarizes the 684- and 730-nm fluorescence quenching parameters calculated for all substituted naphthoquinones examined. Only 2-hydroxy-1,4-naphthoquinone failed to exhibit a measurable chlorophyll fluorescence quenching. 2-Bromo-1,4-naphthoquinone exhibited two distinct Stern–Volmer quenching constants over the concentration ranges of 0–70 μM and 100–200 μM. Emission spectra exhibited a fluorescence maximum at 684 nm in the presence of all naphthoquinones.

Fluorescence quenching effects of selected quinones on chlorophyll in organic solution

The effects of a number of substituted benzoquinones and naphthoquinones on the fluorescence of chlorophyll in organic solution were measured. Each quinone induced a quenching of chlorophyll emission to give a linear Stern–Volmer relationship. Table III lists the Stern–Volmer quenching constants obtained at the wavelength of maximum emission, 671.5 nm, for chlorophyll in an 80% (v/v) acetone-water mixture. All quinones induced a comparable degree of quenching of chlorophyll fluorescence in organic solution. Furthermore, the extent of quenching observed in acetone solution was much weaker than the significant quenching effects often observed in isolated organelles. This observation is in agreement with a previously reported [1] higher quinone quenching efficiency in chloroplasts than in organic solution.

Discussion

Many substituted quinones are potent inhibitors of photosynthetic electron transport in chloroplasts (e.g., Refs. 4 and 9–11). Most studies locate the site of electron-transport inhibition at the reducing side of Photosystem II, either (a) before the plastoquinone pool at the 32–34-kDa herbicide-binding protein which mediates electron flow between Q_A and Q_B [4] or (b) after the plastoquinone pool in the region of the Rieske

iron-sulfur center [12]. Changes in ring substituents or in quinone concentration can shift the site of inhibition [13]. Inhibition of electron flow is sensitive to low quinone concentrations, requiring one inhibitor molecule per Photosystem II electron-transport chain [13,14].

A second mode of quinone inhibition has been postulated to explain the observed quenching of chlorophyll fluorescence by certain substituted benzoquinones and naphthoquinones. Higher quinone concentrations are needed for fluorescence quenching than for electron transport inhibition, typically on the order of one quinone molecule per light-harvesting chlorophyll molecule. Both steady-state and time-resolved fluorescence measurements support a quenching mechanism involving the dissipation of excitation energy via an interaction of quinone with light-harvesting antenna chlorophyll molecules or an interaction at or near the reaction center chlorophyll of Photosystem II [1,4–6]. The present results are consistent with such a mechanism of fluorescence quenching involving an interaction of extrinsic quinones with Photosystem II light-harvesting chlorophyll-protein complexes and a subsequent alteration of the excitation-transfer process among Photosystem II chlorophyll antennae.

Analysis of the present data further suggests that the origin of the observed fluorescence quenching capabilities of substituted benzoquinones and naphthoquinones involves contributions from two distinct factors: (1) the solubility of the quinone in the membrane and (2) the intrinsic activity of a quinone to induce fluorescence quenching. Stern–Volmer analyses provide the fraction of chlorophyll fluorescence accessible to quinone as a measure of quinone solubility and the Stern–Volmer quenching constant as a measure of quinone-intrinsic activity. It is important to differentiate between these two parameters in order to interpret correctly the fluorescence quenching data and to facilitate an understanding of the quenching mechanism.

The benzoquinone data of Table I may be analyzed in terms of the contributions of lipophilic and electronic factors to quinone solubility and intrinsic activity. In particular, benzoquinones must have hydrophobic substituents or hydrogen atoms at positions 2, 3, 5 and 6 for fluorescence quenching activity. The presence of hydrophilic groups (e.g., –OH, –CN) prevents the interaction of quinone with the receptor site on the thylakoid membrane. Quenching activity can not be promoted by the localization of hydrophilic groups on a single 'side' of the benzoquinone ring (i.e., at positions 2 and/or 3 or at positions 5 and/or 6) nor by the presence of hydrophobic groups in addition to hydrophilic substituents. With respect to quinone intrinsic activity, the electronic character of hydrophobic substituents affects the magnitude of the Stern–Volmer quenching constant. A benzoquinone with only electron-withdrawing hydrophobic substituents has a similar K_{SV} value to that of 1,4-benzoquinone, while one with electron-releasing hydrophobic substituents exhibits a lower K_{SV} value. The presence of both electron-releasing and electron-withdrawing hydrophobic groups increases K_{SV} above the value for 1,4-benzoquinone.

The naphthoquinone data of Table II may be similarly analyzed with respect to solubility and intrinsic activity parameters. The lipophilic character of the substituents on the α-ring of 1,4-naphthoquinone (i.e., at positions 2 and 3) determines the fraction of chlorophyll fluorescence accessible to naphthoquinone. Hydrophobic substituents increase f_a to a maximum value of 1, while hydrophilic substituents lower f_a to a minimum value of 0. In the case of 2,3-substitution of a hydrophilic and a hydrophobic substituent, the effects on f_a are 'additive' and therefore often cancel each other. An exception to these generalizations on f_a effects is observed for 2-amino-1,4-naphthoquinone, where the highly hydrophilic amino group (or its protonated form, $-NH_3^+$) results in a linear Stern–Volmer plot with $f_a = 1.00$. Charge effects may play a role in the increased interaction of 2-amino-1,4-naphthoquinone with the membrane. Substitution of a hydrophilic group on the β-ring of 1,4-naphthoquinone and the observed linear Stern–Volmer plot suggest that the above generalizations as to the dependence of quinone-membrane solubility on substituent hydrophobicity are limited to 2,3-substituents.

For those mono-substituted naphthoquinones examined, a general correlation of f_a with Hansch hydrophobicity constants (π) [15] is found (results not shown). However, correlations for disubstitu-

tion based on π value additivity are not as favorable. A linear relationship between f_a and lipophilicity over a certain range of π values is reasonable, but a 'parabolic' dependence of f_a on π with increasingly higher lipophilic values is likely to arise from limited quinone solubility. An optimal π value for maximum solubility may then be determined [16]. We are currently seeking quantitative structure-activity relationships (QSAR) to define more precisely the role of hydrophobic character on fluorescence-quenching activity.

As revealed by alterations in the magnitude of the Stern–Volmer quenching constant, naphthoquinone intrinsic activity appears to be correlated with the electronic character of the 2,3-substituents. The presence of one or two electron-releasing substituents at these positions lowers K_{SV} below the 1,4-naphthoquinone value, while the presence of one or two electron-withdrawing substituents increases K_{SV}. The K_{SV} value in the presence of one electron-releasing and one electron-withdrawing group remains approximately at the 1,4-naphthoquinone value; i.e., the electronic effects of substituents on the capacity of a naphthoquinone to induce fluorescence quenching are 'additive'.

Both parameters of solubility and intrinsic activity are important for comparisons of quinone quenching action. At a given concentration, a quinone with a relatively low solubility yet high intrinsic activity may act as an equally strong quencher of chlorophyll fluorescence as a high-affinity quinone with low intrinsic activity, as in the case of TMBQ and TCBQ, each at a concentration of 200 μM. However, at a lower quinone concentration level of 100 μM, the higher K_{SV} value of TCBQ results in a larger degree of chlorophyll fluorescence quenching than observed for the more 'accessible' TMBQ. A single $[Q]_{50}$ parameter, indicating the quinone concentration necessary to induce a decrease in chlorophyll fluorescence intensity to 50% of the observed level in the absence of extrinsic quinones, is a misleading indicator of an inhibitor's effects. This measure does not distinguish between solubility and intrinsic activity contributions to the origin of fluorescence quenching and does not permit predictions of quinone quenching effects at concentrations other than the $[Q]_{50}$ value. Comparisons of inhibitor potency should be made with caution when $[Q]_{50}$ values (or analogous measures of potency) are the only available parameters.

In conclusion, we have used Stern–Volmer analyses of chlorophyll fluorescence quenching in barley chloroplasts by added benzo- and naphthoquinones to assess those physico-chemical factors of quinones that govern the quenching process. Hydrophobic moieties promote the partitioning of quinones between the lipophilic thylakoid membrane and the surrounding aqueous environment. Furthermore, the fluorescence quenching activity of a quinone is sensitive to the charge distribution on the quinone ring. It should be noted that those substituents that promote fluorescence quenching do not necessarily parallel those groups which lead to electron-transport inhibition [4,9–11]. Although the present data do not enable a determination of the operative quenching mechanism, the Stern–Volmer analyses of the data are consistent with the proposal that a receptor site for chlorophyll fluorescence quenching may be located on a surface-exposed polypeptide of the Photosystem II light-harvesting chlorophyll-protein complexes [1,4–6]. However, the nature of the quinone-membrane interaction or the presence of quinone-protein binding sites cannot be conclusively determined from the present data. A multiparameter approach to structure-activity relationships is currently in progress to gain additional insight into the mechanism of chlorophyll fluorescence quenching by extrinsic quinones in chloroplasts.

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