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SHORT COMMUNICATION

CHARACTERIZATION OF THE FLUORESCENCE QUENCHING OF CHLOROPHYLL *a* BY 1, 4 BENZOQUINONE USING THE NONLINEAR ANALYSIS

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It has long been known that quinones quench chlorophyll fluorescence. This study reports the parameters of the fluorescence quenching of chlorophyll a by 1,4-benzoquinone, in ethanol, using the nonlinear analysis. The fluorescence intensity was measured at 670 nm for the blue and red excitation wavelengths: 430 nm and 650 nm, respectively. The quinone concentration in the samples ranged from 3.3×10^{-4} M to 10^{-2} M. The modified Stern-Volmer plots of the quenching data have revealed two ways of the quenching mechanism and have offered the posibility to obtain the quenching parameters.

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Keywrds: Chlorophyll *a* fluorescence, 1,4-benzoquinone, Photosynthesis

1. Introduction

In all photosynthetic systems of the higher plants the primary photoreaction consists in an electron transfer from an excited singlet state of a chlorophyll pigment to an electron acceptor. The quenching of chlorophyll *a* (Chl *a*) fluorescence in solution by various oxidizing and reducting agents has been the subject of numerous investigations. Therefore the quenching of Chl *a* fluorescence by quinones has been extensively studied, both *in vivo* and *in vitro* [1–5]. The analysis of the singlet quenching fluorescence of Chl *a* are consistent with the electron-transfer mechanism.

The aim of this work is to present the quenching parameters of the Chl *a* fluorescence by 1,4 – benzoquinone (BQ) in ethanol, on the basis of nonlinear analysis, using the Beddard *et al.* [6] correction for the fluorescence intensities.

2. Procedure

Chl *a* extracted from fresh spinach was cromatographed on the powdered sugar column, according to the method of Strain and Svec [7]. The absorption maxima as well as the band intensity ratios have proved the purity of the Chl *a* [8] used.

BQ obtained from Sigma was used without further purifications. Obtaining of high concentrations of BQ does not raise solubility difficulties. In the concentration range used by us, the spectrum of BQ was independent of the concentration.

Ethanol was used as the solvent of the samples. All the samples contained the same Chl *a* concentration (1.4×10^{-6} M) and they were air-saturated. Our were samples not specially dried. They contain small concentration of Chl *a* dissolved in a polar solvent. Therefore, the chlorophyll aggregates of dimers or oligomers were absent [9]. The samples contained BQ at various concentrations (3.3×10^{-4} M ÷ 10^{-2} M). The fluorescence intensity was measured at 670 nm for the blue and red excitation wavelengths: 430 nm and 650 nm, respectively. The very low concentration of

Chl *a* avoided the effects of self-absorption. The absorption spectra, at room temperature, were recorded on a Lambda 2S Perkin-Elmer spectrophotometer while the fluorescence spectra on a steady-state Aminco-Bowman spectrofluorometer.

The fluorescence data were corrected in the manner described by Beddard *et al.* [6], using the following expression:

$$F_0/F = (\Phi_0/\Phi)\times(1/\alpha)\times(1/\beta)$$

with : $\alpha = 10^{-\varepsilon(1)\,c\,l}$ and $\beta = 10^{-\varepsilon(2)\,c\,l}$.

F_o is the fluorescence intensity in the absence of the quencher and F is the fluorescence intensity after the addition of the quencher;
$\varepsilon(1)$, $\varepsilon(2)$ are extinction coefficients of BQ at excitation and at monitored emission wavelength, respectively; c is quencher concentration;
l is distance from center of cuvette to X and Y direction (normally equal).
The Φ_0/Φ ratios contain the corrected relative intensities of the fluorescence in the absence and the presence of BQ.

3. Experimental results and discussion

For every excitation wavelength the Stern-Volmer-type plots demonstrate the BQ quenching of the steady-state yield of Chl *a* fluorescence.

The Stern-Volmer plots curve upward. In order to fit the experimental data we used a nonlinear least-squares analysis, using the following exponential equation [10]:

$$\Phi_0/\Phi = (1 + K_{SV}[Q])\exp(V[Q])$$

Here K_{SV} is the dynamic quenching constant, $[Q]$ is the quencher concentration and V is the static constant. V can be considered an *active element* surrounding the fluorophore molecule. The magnitude of V can be related to an *active radius*, R, through the relationship $V = (4/3)N\pi R^3$, N being Avogadro number. The Figs. 1 and *2* show the simulated plots of the two fluorescence quenching data on the basis of the above equation.

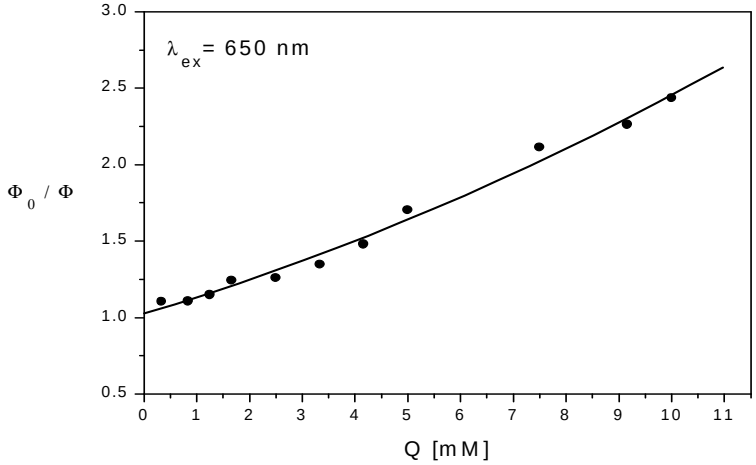

Fig. 1. Simulated plot of fluorescence quenching data (exponential equation) for 650 nm excitation wavelength.

For 650 nm excitation wavelength, K_{SV} = 92.98 M^{-1} and V = 23.64 M^{-1}. That means a bimolecular quenching constant of 1.86 x 10^{10} $M^{-1}s^{-1}$, obtained with Chl *a* fluorescence lifetime of 4.98 ns [D.M. Gazdaru, unpublished results] in the absence of the quencher, and an *active radius* of 21 Å.

For 430 nm excitation wavelength, K_{SV} = 129.17 M^{-1} and V = 45.75 M^{-1}. That means a very large quenching constant of 2.59 x 10^{10} $M^{-1}s^{-1}$ and an *active radius* of 26 Å.

The results presented above could be explained by two different ways of the quenching of Chl *a* fluorescence. The first way involves the formation of a one-to-one (A.....Q) ground-state complex at low [BQ] and perhaps a higher order ground-state complexes [i.e., (A.....BQ_2), (A.....BQ_3)] at higher quencher concentration. If one or more Q molecules happen to be within a *volume element*, *V*, at the instant of photon absorption, then an instantaneous (static) quenching will occur. Having in view the magnitude of the radii of Chl *a* and BQ, the *active radii* values are plausible when such complexes could be formed. The second way of the quenching is related to the probability of quinone-to-chlorophyll encounters. For both modalities, one can assume an electron-transfer quenching mechanism similar to the one proposed by Rehm and Weller [11]. The values for the quenching constants obtained by us are close to the diffusion-controlled limit [10] and they are in agreement with the other results [4].

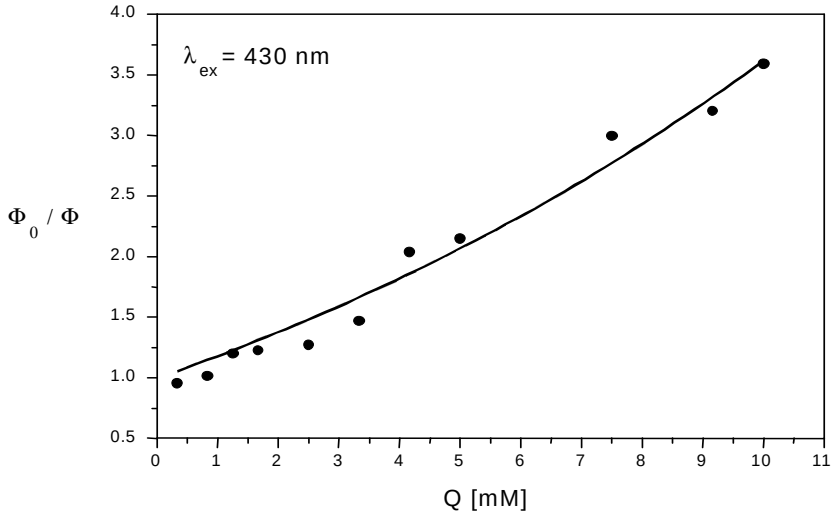

Fig. 2. Simulated plot of fluorescence quenching data (exponential equation) for 430 nm excitation wavelength.

The values obtained for the quenching parameters at 430 nm excitation wavelength are larger than the ones for 650 nm excitation wavelength. We think that the data obtained in blue excitation were not properly corrected and thus the correction manner used is not quite appropriate in this case. This happens at high concentrations of the quencher, [Q], when its absorption overlaps significantly with the excitation wavelength of Chl fluorescence.

4. Conclusion

The analysis of the fluorescence quenching using nonlinear plots has put in evidence the quenching parameters in a good accordance with other studies, revealing the two ways of the quenching process. One of the quenching modalities is that of the static type and the other one is of the dynamic type. It has to be emphasized that the dynamic constant has a higher value than the static one. The quenching process is taking place in the both excitation regions, although the

excitation wavelength in the blue region gives higher values for the analyzed quenching parameters than in red range. In this type of approach it is very important to find the adequate manner for correction of the fluorescence intensities, in order to properly interpret the results.

References

[1] K. K. Karukstis, S. C. Boegeman, S. M. Gruber, C. R. Monell, J. A. Fruetel, M. H. Trris, Progress in Photosynthesis Research (Biggins, J., ed.), Martinus Nijhoff, Dordrecht, Vol. I, 119, 1987.
[2] K. K. Karukstis, S. C. Boegeman, J. A. Fruetel, S. M. Gruber, M. H. Trris, Biochim. Biophys. Acta **891**, 256 (1987).
[3] K. K. Karukstis, S. M. Gruber, C. R. Monell, J. A. Fruetel, S. C. Boegeman, Biochim. Biophys. Acta **932**, 84 (1988).
[4] L. V. Natarajan, J. E. Ricker, R. E. Blankenship, R. Chang, Photochem. Photobiol. **39**, 301 (1984).
[5] G. R. Seely, A. M. Rutkoski, E. R. Shaw, Photochem. Photobiol. **37**, 215 (1983).
[6] G. S. Beddard, R. S. Davidson, K. R. Trethewey, Nature **267**, 373 (1977).
[7] H. H. Strain, W. A. Svec, The Chlorophylls (L. P Vernon, , G. R. Seely, eds.), Academic Press, New York, pp. 21-26, 1966.
[8] W. A. Svec,The Porphyrins (D. Dolphin, ed.) Academic Press, New York, Vol. V, 342, 1978.
[9] J. J. Katz, J. R. Norris, L. L. Schipman, M. C. Thurnauer, M. R. Wasielewski, Annu. Rev. Biophys. Bioener. **7**, 393 (1978).
[10] M. R. Eftink, Biophysical and Biochemical Aspects of Fluorescence Spectroscopy (Dewey, T. D., ed.) Plenum Press, New York, 1, 1991.
[11] D. Rehm, A. Weller, Israel J. Chem. **8**, 259 (1970).