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BRIEF COMMUNICATION

Characterization of the quenching of chlorophyll *a* fluorescence by β-carotene using the non-linear analysis

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Abstract

Carotenoids (Car) regulate energy flow in photosynthesis by a specific Car-chlorophyll (Chl) interaction in the singlet-excited states, leading to a reduction in Chl fluorescence. We studied quenching of Chl *a*-fluorescence in benzene by *trans*-β-carotene. Non-linear analysis of the quenching process enables to explain the possible molecular mechanism leading to the de-excitation of Chl *a*. The fluorescence intensity was measured at 670 nm for excitation wavelengths of 380, 430, 640, and 650 nm. The β -carotene concentrations ranged from 4×10^{-5} M to 5×10^{-3} M. When the samples were excited at 640 and 650 nm, the Stern-Volmer plots showed that the quenching process has high rate constants, hence β-carotene is a very efficient quencher. Two different types of quenching process could take place.

Additional key words: fluorescence quenching; photosynthesis.

Carotenoids (Car) have multiple functions in photosynthetic systems (Frank and Cogdell 1993, 1996, Frank and Christensen 1995, Koyama 1991). Recently an additional function was proposed which involves a specific Car-chlorophyll (Chl) interaction in the singlet excited states, leading to a quenching of Chl fluorescence (Frackowiak *et al.* 1995, Frank *at al.* 1995). This means that Car may play a role in regulating the energy flow of Chl excited states in the photosynthetic antenna, being used in an energy dissipation route. Earlier reports concerning the quenching of Chl fluorescence by β-carotene (Truscott *et al.* 1965, Singhal *et al.* 1968) have been contradicted. Further studies have shown that β-carotene can quench Chl *a* fluorescence in organic solvents (Beddard *et al.* 1977, Frank and Christensen 1995).

This work presents the results of *in vitro* measurements concerning the quenching of Chl *a*-fluorescence by *trans*-β-carotene. The fluorescence was excited by blue and red radiation. Our values demonstrated a quenching of the first singlet excited state through two ways: complex formation and collision quenching. A significant quenching was observed at a low quencher concentration. The quenching results were obtained using the non-linear analysis.

Chl *a* was extracted from fresh spinach leaves and purified by using column chromatography on sugar, according to a standard procedure (Strain and Svec 1966) with a slight modification. Chl *a* was stored as a stock solution in *n*-hexane at –7 °C. The absorption maxima as well as the band intensity ratios showed the purity of the used Chl (Svec 1978). β-carotene (*Merck*) was used without further purification; its purity was checked by measuring the band intensity ratios in cyclo-hexane. Obtaining of high concentrations of β-carotene in the samples does not raise the solubility difficulties. The spectrum was independent of concentration: for all the concentrations used, there was no apparent deviation from Beer's law. Spectro-grade benzene was used as solvent, without drying. All samples contained the same Chl *a* amount and they were air-saturated. The optical density for Chl *a* was 0.26 (1 cm path cell) at 665 nm. This value corresponds to a concentration of 3.1 μM (Smith and

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Benitez 1955). The samples contained β-carotene at concentrations from 4×10^{-5} to 5×10^{-3} M. The fluorescence intensity was measured at 670 nm for the following excitation wavelengths: 380, 430, 640, and 650 nm. The very low concentration of Chl *a* reduced the effects of possible re-absorption. The absorption spectra, at room temperature, were recorded on a *Lambda 2S Perkin-Elmer* spectrophotometer and the fluorescence spectra were recorded on a steady-state *Aminco-Bowman* fluorescence spectrometer.

The absorption spectrum of Chl *a* in benzene had main absorption maxima at 432 and 665 nm. Fig. 1 shows the absorption spectra of the samples containing 3.1 μM Chl *a* and different concentrations of β-carotene.

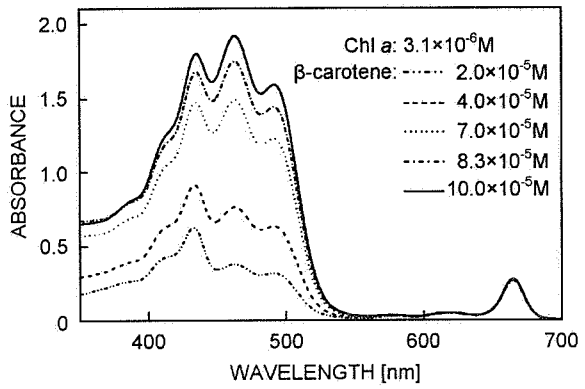

Fig. 1. Absorption spectra of some samples containing Chl *a* and β-carotene in benzene.

For every excitation wavelength the Stern-Volmer type plots showed quenching of the steady-state yield of Chl *a* fluorescence by β-carotene. The quenching results are in accordance with the results of Beddard *et al.* (1977) and Frank *et al.* (1995) for the excitation in red radiation. However, the results are in contrast to those of Singhal *et al.* (1968) for the red and blue excitation radiation. Fig. 2*A,B* shows the plots at two excitation wavelengths from the red spectral range. The ratio Φ_0/Φ contains the corrected values of the Chl *a*-fluorescence in the manner described by Beddard *et al.* (1977). Corrected values of the Chl *a*-fluorescence for the blue excitation wavelength give too large dynamic quenching constants, in the Stern-Volmer type plots. In this case, we also tried a correction in the manner described by Singhal *et al.* (1968), but also without a good result. This thing happens to high concentrations of the quencher, [Q], when its absorption overlaps significantly with the excitation wavelengths of the Chl *a*-fluorescence (plots not shown).

For the 640 and 650 nm excitation wavelengths the Stern-Volmer plots lead to upward curvature and we tried a non-linear least-squares analysis, using the equation of Eftink and Dewey (1991):

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

where K_{SV} is the dynamic quenching constant 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, by the relationship $V = (4/3)\ \pi NR^3$.

Fig. 2*C,D* shows simulated plots of two sets of fluorescence quenching data on the basis of the above equation. For 650 nm excitation wavelength, K_{SV} is 29 M^{-1} and V is 86 M^{-1}. That means a quenching constant of 6.1×10^9 M^{-1} s^{-1}, obtained with Chl *a* fluorescence lifetime of 4.72 ns (D.M. Gazdaru, unpublished) in the absence of the quencher and an active radius of 3.2 nm. For 640 nm excitation wavelength, K_{SV} is 46 M^{-1}, larger than that at 650 nm. That means a very large quenching constant of 9.5×10^9 $M^{-1}s^{-1}$ and an active radius of 4.0 nm.

The obtained results could be explained by two different ways of quenching of Chl-*a* fluorescence. The first way involves the formation of a one-to-one (A...Q) ground-state complex at low [Q] and perhaps higher orders ground-state complexes [*i.e.* $(A...Q_2)$, $(A...Q_3)$] at higher Q concentration. If one or more Q molecules happen to be within a volume element, V, at the instant of photon absorption, then instantaneous (static) quenching occurs. The two active radii values presented above having in view the magnitude of the radii of Chl *a* and β-carotene, are plausible when such complexes are formed. The second way of quenching is related to the probability of Car-to-Chl encounters. Stern-Volmer plot of the lifetime ratios as a function of [Q] is a linear one (Frank and Christensen 1995). We consider that this type of quenching occurs predominantly only in a certain [Q] range and, of course, it has to depend on the spectral overlap between the fluorophore fluorescence and the quencher absorption. One could say that hypothetical $S_0 \rightarrow S_1$ absorption of β-carotene overlaps with the Chl *a* fluorescence spectrum. The values obtained by us for the quenching constants are close to the diffusion controlled limit (Eftink and Dewey 1991) and they depend on the excitation wavelength.

The quenching of the Chl *a*-fluorescence by β-carotene has been indubitably observed under the red excitation radiation, as our previous results have shown (Gazdaru *et al.* 1998). The values obtained could be explained by two different ways of quenching. A significant quenching was put in evidence at lower quencher concentration as compared to other studies (Beddard *et al.* 1977, Frank and Christensen 1995, Frank *et al.* 1995) on air-saturated sample. We think that the values obtained with blue excitation radiation were not correct and hence neither of the two correction manners used (Singhal *et al.* 1968, Beddard *et al.* 1977) is appropriate. However, we suppose that the quenching process takes place also in blue excitation radiation, but it is very difficult to evaluate it.

The quenching of the Chl *a*-fluorescence by β-caro-

tene observed under the red excitation radiation simply suggests that such a direct quenching process may be a possible route of deactivation. In the next future, when Car bound *in situ* in various pigment-protein complexes of higher plants will be examined, the potential roles of the Car in photosynthetic systems may become clearer.

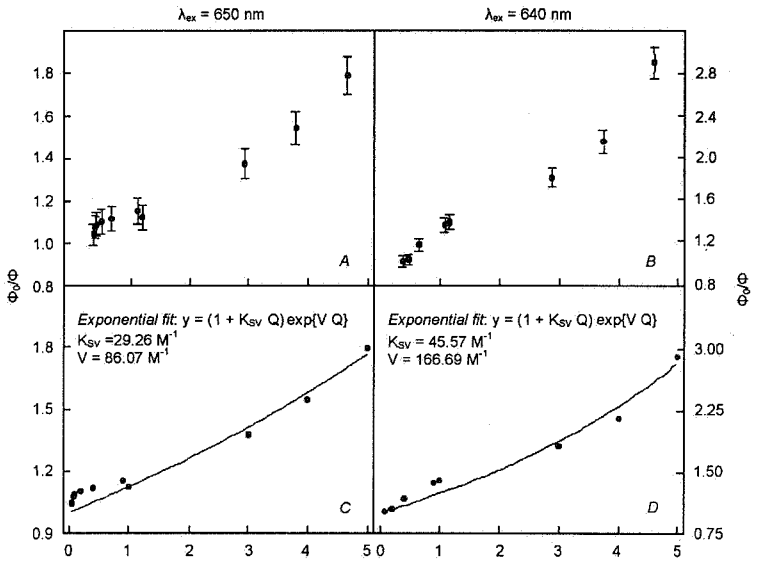

Fig. 2. Stern-Volmer plots illustrating the quenching fluorescence of Chl *a* by β-carotene in benzene (excitation wavelengths *A* 650 nm and *B* 640 nm, emission wavelength 670 nm) and simulated plots of fluorescence quenching data (exponential equation) for the same excitation wavelengths (*C, D*). Φ_0/Φ represents the ratio of the corrected values of the fluorescence in absence and in presence of the quencher; [Q] is the quencher concentration.

References

Beddard, G.S., Davidson, R.S., Trethewey, K.R.: Quenching of chlorophyll fluorescence by β-carotene. – Nature **267**: 373-374, 1977.

Eftink, M.R., Dewey, T.G.: Biophysical and Biochemical Aspects of Fluorescence Spectroscopy. – Pp. 1-41. Plenum Press, New York 1991.

Frackowiak, D., Zelent, B., Malak, H., Cegielski, R., Goc, J., Niedbalska, M., Ptak, A.: Interactions between chlorophyll *a* and β-carotene in nematic liquid crystals. – Biophys. Chem. **54**: 95-107, 1995.

Frank, H.A., Christensen, R.L.: Singlet energy transfer from carotenoids to bacteriochlorophylls. – In: Blankenship, R.E., Madigan, M.T., Bauer, C.E. (ed.): Anoxygenic Photosynthetic Bacteria. Pp. 373-384. Kluwer Academic Publishers, Dordrecht – Boston – London 1995.

Frank, H.A., Cogdell, R.J.: The photochemistry and function of carotenoids in photosynthesis. – In: Young, A., Britton, G. (ed.): Carotenoids in Photosynthesis. Pp. 252-326. Chapman and Hall, London – Glasgow – New York – Tokyo – Melbourne – Madras 1993.

Frank, H.A., Cogdell, R.J.: Carotenoids in photosynthesis. – Photochem. Photobiol. **63**: 257-264, 1996.

Frank, H.A., Cua, A., Chynwat, V., Young, W.J., Zhu, Y., Blankenship, R.E.: Quenching of chlorophyll excited states by carotenoids. – In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. IV. Pp. 3-7. Kluwer Academic Publishers, Dordrecht – Boston – London 1995.

Gazdaru, D.M., Iorga, B., Balan, P.F.: Quenching of chlorophyll *a* fluorescence by β-carotene. – In: Garab, G. (ed.): Photosynthesis. Mechanisms and Effects. Vol. I. Pp. 483-486. Kluwer Academic Publ., Dordrecht – Boston – London 1998.

Koyama, Y.: Structures and functions of carotenoids in photosynthetic systems. – J. Photochem. Photobiol. B **9**: 265-280, 1991.

Singhal, G.S., Hevesi, J., Rabinowitch, E.: Excitation energy migration between chlorophyll and β-carotene. – J. chem. Phys. **49**: 5206-5207, 1968.

Smith, J.H.C., Benitez, A.: Chlorophylls: Analysis in plant materials. - In: Paech, K., Tracey, M.V. (ed.): Modern Methods of Plant Analysis. Vol. IV. Pp. 142-196. Springer-Verlag, Berlin – Göttingen – Heidelberg 1955.

Strain, H.H., Svec, W.A.: Extraction, separation, estimation, and isolation of the chlorophylls. – In: Vernon, L.P., Seely, G.R. (ed.): The Chlorophylls. Pp. 21-66. Academic Press, New York – London 1966.

Svec, W.A.: The isolation, preparation, characterization, and estimation of the chlorophylls and the bacteriochlorophylls. - In: Dolphin, D. (ed.): The Porphyrins. Vol. V. Pp. 341-399. Academic Press, New York 1978.

Truscott, T.G., Chessin, M., Livingston, R.: Intermolecular quenching of a higher excited state of chlorophyll *a* by β-carotene. – J. chem. Phys. **43**: 3409-3410, 1965.