Effect of β-Carotene on Delayed Light Emission from Aggregated Chlorophyll

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Z. Naturforsch. **44c,** 132–134 (1990);
received February 6/August 15, 1989

β-Carotene, Delayed Light Emission, Chlorophyll Aggregates, Fluorescence, Phosphorescence

Delayed light emission (DLE) from aggregated chlorophyll is used to probe energy transfer between aggregated chlorophyll and β-carotene. Preilluminated β-carotene when injected into a dark chamber containing aggregated chlorophyll, induces DLE from aggregated chlorophyll. If the dark chamber contains only monomeric chlorophyll, there is no DLE. The intensity of DLE is dependent on the time of illumination of β-carotene. The decay of energy stored by the carotene is not a first order process. The first half life is about 2 min, the next about 10 min.

Introduction

It was reported that there is delayed light emission (DLE) from a solution of aggregated chlorophyll (Chl) following pre-illumination [1]. Previously it was shown that the rate and intensity of the DLE from aggregated Chl depends on several factors (*e.g.* the presence of electron donors and acceptors). The function of carotene as collectors of light energy in photosynthesis and their role in protecting the photosynthetic apparatus against destructive effects of light and singlet oxygen are well established (*e.g.* [2]). The present data shows the effect of β-carotene on DLE from aggregated Chl. The DLE from aggregated Chl is used to probe energy transfer between Chl and carotene. Light energy absorbed by carotene can not only be transferred directly to Chl but, it would appear, that it can also be stored by carotene. These observations, of DLE, cannot be interpreted in terms of the known excitation states of molecular Chl and carotene.

Materials and Methods

The method of measuring DLE was described in detail previously be Yang [1]. In brief, after pre-illumination with white light (3 mW/cm^2) the sample is immediately injected into a dark chamber. The time required for injection is about 0.8 sec. The emitted light is detected by a photomultiplier tube and recorded; the response time of the system is about 0.5 sec.

Chl *a* is prepared from an acetone extract of fresh spinach using a cellulose chromatographic column [3]. The Chl eluate is dried and redissolved in water-saturated petroleum ether. The solution is maintained at 5 °C (over-night) to form aggregated Chl. Aggregate formation is indicated when the color of the solution changes from blue to green. The aggregated Chl, which may be in the form of micro-crystals, are collected by centrifugation and suspended in petroleum ether with a vortex mixer. The sample is again centrifuged and suspended in petroleum ether. All procedures are performed in dim light.

All-trans β-carotene is obtained from Merck Chemical Co., and recrystallized from benzene and methanol. The petroleum ether (60–90 °C) is purified with sulfuric acid. Other chemicals are analytical grade.

Results and Discussion

Illuminating β-carotene induces DLE from aggregated Chl

No DLE is detected from pre-illuminated β-carotene (β-carotene is non-fluorescent) [4] in the absence of aggregated Chl (Fig. 1a). On the other hand, if pre-illuminated β-carotene is injected into a solution of Chl *a* aggregates a DLE signal is detected (Fig. 1b). This observation indicates that excitation energy can be stored in β-carotene. When the pre-illuminated carotene interacts with Chl aggregates the energy stored in the carotene can be transferred to the aggregates and released as DLE of Chl.

Effect of acetone on DLE

When acetone is added to the suspension of aggregated Chl, the aggregates are dissolved as monomeric Chl. Chl in the monomeric form does not exhibit DLE.

Pre-illuminating Chl aggregates results in DLE [1]. If acetone is added to Chl aggregates following pre-illumination, a "burst" of light emission is observed and the DLE is terminated (Fig. 2a). The addition of acetone converts the aggregated Chl to its monomeric form. The monomeric form of Chl does

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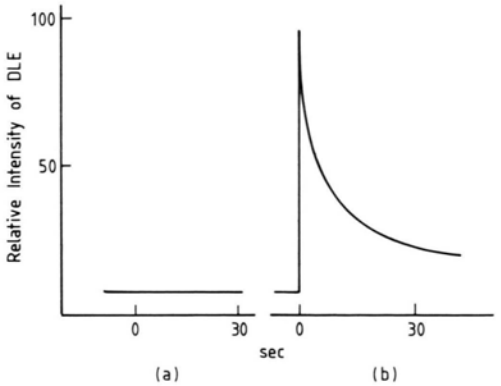

Fig. 1. DLE from a solution of β-carotene and chlorophyll *a* aggregates. A 1 ml sample of β-carotene (2 μg/ml) in petroleum ether is illuminated for 30 sec then, at time zero, injected into a dark chamber containing: (a) 0.5 ml petroleum ether; (b) 0.5 ml petroleum ether plus chlorophyll *a* aggregates (10 μg/ml).

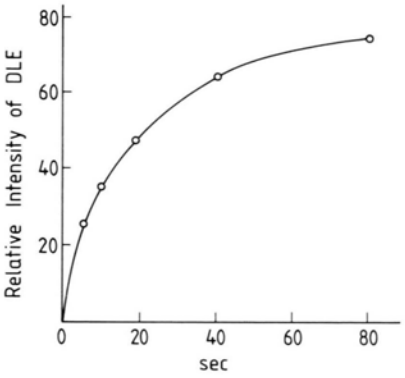

Fig. 3. The relation between the intensity of DLE and time of pre-illumination. A solution of β-carotene was illuminated for various time durations then injected into the sample containing a solution of chlorophyll *a* aggregates.

not exhibit DLE. Only the aggregated form of Chl can store the energy that gives rise to DLE. Since monomeric Chl can not store the "excitation energy", the energy stored in the aggregated Chl is released as a burst of light upon deaggregation.

No "burst" of light is observed when acetone is added to a mixture of Chl aggregates in the presence of β-carotene. Specifically, there is no "burst" in the case of either: Chl aggregates and pre-illuminated carotene, or pre-illuminated Chl aggregates and carotene, or pre-illuminated Chl aggregates and pre-illuminated carotene. In these cases addition of acetone results in an abrupt drop in DLE (Fig. 2b). Phenomenologically, it would appear that in the presence of β-carotene all energy stored in aggregated Chl is transferred to or quenched by carotene, upon deaggregation by acetone; so that, no "burst" of light energy is observed.

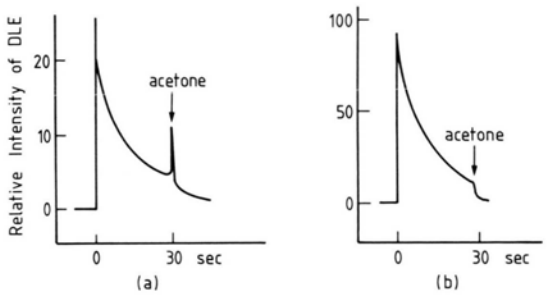

Fig. 2. Effect of deaggregation by acetone on DLE from a solution of β-carotene and chlorophyll *a* aggregates. The relative intensities are all on the same scale. At 30 sec after pre-illumination 0.5 ml of acetone is injected into the sample resulting in deaggregation of chlorophyll. The pigment concentrations are: 10 μg/ml chlorophyll and 2 μg/ml carotene. (a) Pre-illumination of a solution of chlorophyll aggregates. (b) Pre-illumination of a solution containing β-carotene and chlorophyll *a* aggregates.

The time course of the decay of energy stored in β-carotene

The energy stored in β-carotene is indicated as the intensity of DLE from Chl aggregates. The energy stored in the carotene is a function of time of illumination. A maximum is reached after about one 1 min of pre-illumination (Fig. 3).

When pre-illuminated β-carotene solution is injected into a chamber containing aggregated Chl after various dark intervals, the intensity of the DLE decreases as the dark interval lengthens (Fig. 4). This shows that energy stored by β-carotene decays along a non-radiative pathway in the dark. After about a 2 min dark interval, the intensity of DLE decreases to about half of that with zero dark interval. From Fig. 4 it is observed that the decay of energy stored by carotene is not a first order process. While the first half-life of the energy stored by

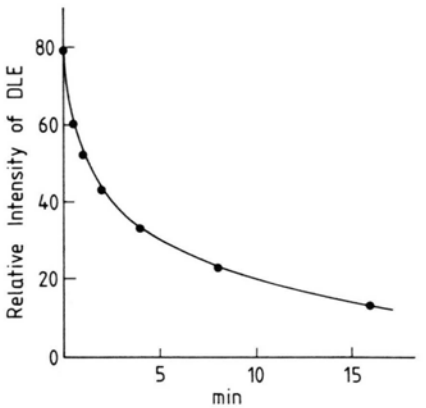

Fig. 4. The intensity of DLE as a function of the dark interval following pre-illumination. After illuminating β-carotene (2 μg/ml) dark interval of various durations are waited before injecting it into the sample containing chlorophyll aggregates (10 μg/ml).

carotene is about 2 min, the next half-life is about 10 min.

The intensity of the DLE is too faint to permit measurement of its emission spectrum.

Delayed fluorescence in vivo has a somewhat similar time course to that reported here for DLE

The model system reported in this work is interesting as it has not been possible hitherto, to consider the possible role of carotene on DLE in chloroplasts. DLE was first reported from chloroplasts by Strehler and Arnold [5]. There have been many studies to uncover the mechanism and parameters controlling DLE (*e.g.* [6]). The exact site of the DLE in the photosynthetic apparatus is not established. Some of the model systems used to investigate the interaction of Chl and carotene include mono and multilayer films of pigments [7], black lipid membranes [8], and mono and multilayer liposomes [9].

The nature of the "energetic states" or modes of energy storage in β-carotene or aggregated Chl is not clear. One can certainly eliminate the possibility of the involvement of singlet or triplet states, simply on the basis that the decay rates of these states are many orders of magnitude faster than the DLE reported in this work. Whether the DLE originates from a peroxide or configurational state or whatever, is the subject of further consideration.

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