MANGANESE CATALYST AS A POSSIBLE CATION CARRIER IN THERMOLUMINESCENCE FROM GREEN PLANTS

Yorinao INOUE

Laboratory of Plant Physiology, The Institute of Physical and Chemical Research, Wako-shi, Saitama, 351, Japan

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1. Introduction

Thermoluminescence is the light emitted upon heating materials in darkness which had been irradiated at low temperature. The thermoluminescence profile from green plants shows four luminescence peaks at different temperatures of 118, 267, 303°K and 325°K which have been denoted by Arnold and Sherwood [1] as Z, A, B and C bands, respectively. The A, B and C bands were related to photosynthetic activities whereas the Z band, the emission from a triplet state [2] of chlorophylls, was not related to photosynthesis. It is generally accepted that these emission bands result from recombination of electrons and holes generated by chlorophyll photoreactions and stabilized in frozen states. Most of these glow peaks are emitted from photosystem II (PS II) particles [3] and have been considered to originate from back reactions of electron carriers between PS-I and PS-II [4], although Desai et al. [5] reported that not only PS-II but also PS-I photoreactions are involved in the energy storage. Arnold and Azzi [6] stated that two quanta are required for charging the A or B band and suggested that the emission originates from the holes in the oxygen-evolution center.

Despite these observations on the site responsible for leaf thermoluminescence, the chemical nature of the reaction partners responsible for the thermoluminescence emission is unknown.

A different approach to this problem was made in previous studies [7,8]. We have measured the glow curves of angiosperm leaves greened under intermittent flashes of long intervals (5 min) or gymnosperm leaves and some green algal cells greened in darkness. These two types of leaves and algal cells were specifically devoid of the water-splitting activity, although both PS-I and PS-II had been developed almost completely. The glow curves of such leaves were greatly different from those of mature leaves, being devoid of the major bands (Arnold's A and B bands). On exposure of such leaves to continuous light or to flashes of short intervals (2 s), these bands were developed rapidly, being accompanied by generation of the Hill activity with water as electron donor [9,10]. This process was driven by the light absorbed by chlorophylls and involved two or more than two consecutive photoreactions with an appropriate dark interval. The fact that the light condition required for this activation of leaves were similar to those for photoreactivation of the oxygen-evolving system of Mn-deficient algal cells [11,12] strongly suggested that the Mn-catalyst in the oxygen-evolving system plays an important role in storing energy for thermoluminescence. The present study was undertaken under these circumstances to measure the glow curve of Mn-deficient algal cells and to see the effect of continuous or intermittent illumination after Mn^{2+} addition. It was shown that the cation of the Mn catalyst is a probable reaction partner in thermoluminescence emission from photosynthetic apparatus of green plants.

2. Experimental

Scenedesmus obliquus cells were cultured autotrophically in a Mn-free medium. The Mn content in the Mn-deficient cells determined by atomic absorption was 1/35 of that in normal cells. The cells after

cultivation were placed uniformly on a membrane filter, cooled to −55°C, illuminated with red light for excitation of thermoluminescence and, then, warmed slowly in darkness for measurement. The light emitted from the sample was measured by photoelectron counting and recorded on an X-Y recorder against temperature, as described previously [8].

3. Results and discussion

The broken curve on the bottom in fig.1a shows the result obtained for normal *Scenedesmus* cells, which indicates two bands emitted at −30°C and +35°C, respectively. The former weak band is the Z_v band denoted after its variable nature of emission temperature depending on the excitation temperature [7], and the latter strong band is the B band of Arnold [1]. The dotted curve on the bottom is the result obtained for Mn-deficient cells, in which the B band is extremely low as compared with that of normal cells. Four solid curves in fig.1a are the data obtained after 12 h of incubation of such Mn-deficient cells in the presence or absence of 50 μM of Mn^{2+} in the dark or under continuous illumination with white light (600 $\mu W/cm^2$). As seen from the solid curve on the bottom, the B band was greatly enhanced by the illumination in the presence of Mn^{2+}, while the incubation in other conditions did not alter the profile at all. It is evident from these data that photoreactions are involved in the development of the B band in the presence of Mn^{2+}.

In the next experiment, the Mn-deficient cells were pre-incubated in darkness for 2 h in a medium containing 50 μM of Mn^{2+} and, then, exposed to Xe flashes (duration of each flash = 10 μs) repeated 300 times at uniform dark intervals (Td). The four solid curves in fig.1b show the effect of dark interval on the appearance of the B band. The flashes at the interval of Td = 2 s enhanced the B band remarkably, whereas the same number of flashes at longer intervals (upper two curves, Td = 8 and 30 s) or shorter intervals (bottom curve, Td = 0.1 s) were not as effective in enhancing the band as the flashes at Td = 2 s. This is more clearly demonstrated by the data shown in fig.2, in which the height of the B band thus developed by 300 flashes was plotted as a function of dark interval. The height was maximal around Td = 2–4 s, and dropped sharply at shorter intervals and gradually at

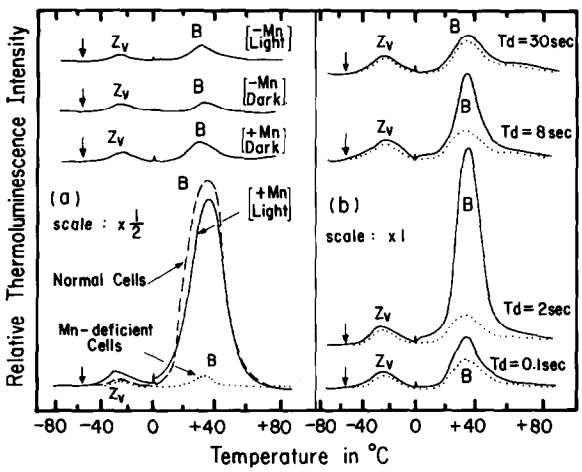

Fig.1a. Thermoluminescence profiles of Mn-deficient *Scenedesmus* cells; dotted and broken curves on the bottom are the profiles of Mn-deficient and Mn-sufficient cells, respectively. Four solid curves are the profiles of Mn-deficient cells measured after 12 h incubation in the presence or absence of 50 μM $MnCl_2$ in the light or dark: the incubation conditions are specified on each profile. The excitation was made by 1 min illumination with red light (≧ 630 nm, 600 $\mu W/cm^2$) at −55°C, as indicated by arrows. Fig.1b. Enhancement of thermoluminescence band induced by flashes at varying intervals. Mn-deficient cells were incubated in darkness at room temperature for 2 h in a culture medium containing 50 μM $MnCl_2$ and then exposed to 300 flashes (10 μs in duration, 2×10^2 ergs/cm^2/flash) repeated at uniform interval. The interval indicated as Td on each profile was varied between 100 ms and 30 s.

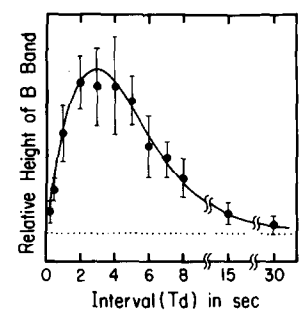

Fig.2. Effects of dark interval on the enhancement of thermoluminescence bands by flashes. The relative height of the B band enhanced by exposure to 300 flashes at varied intervals were estimated on the profiles as shown in fig.1b. The bars on solid circles show the fluctuation of the measurement including errors. The dotted line indicates the original height of the B band observed for Mn-deficient cells.

longer intervals. This indicates that the development is a multi-quantum process which involves more than two consecutive photoevents with a dark reaction between them. The steep drop at shorter intervals implies that a dark step between the photoreactions is limiting the rate in such conditions, and the gradual drop at longer intervals indicates that an intermediate generated by the first photoreaction decays during the dark interval unless it is further converted by the next flash to the final state active to emit the B band. This dependency on dark interval is in good agreement with the dependency found for the multi-quantum photoactivation of the oxygen-evolving system analyzed by Radmer and Cheniae [13] for Mn-deficient algal cells and by us [14] for intermittently flashed wheat leaves.

It was thus demonstrated that at least two sequential photoreactions are necessary for activation of the Mn-deficient cells in the presence of Mn to be able to emit the B band strongly. This indicates that a Mn complex formed from Mn^{2+} by photoreactions is involved directly as the center of emission of the B band or indirectly to activate a neighbouring emission center to emit the B band. The deduction in previous papers based on the data on angiosperm and gymnosperm leaves that the B band is emitted from the structure responsible for or closely related to oxygen evolution was further confirmed by the close resemblance of the dependency on the dark interval for activation of the B band to that for the oxygen-evolving activity. It seems very probable that the positive holes generated by PS-II photoreaction at low temperature are stabilized in the oxygen-evolving system as cations of an activated Mn-catalyst.

The valency change of a Mn atom during either charge-collection for oxidation of water [15–17] or photoactivation of the latent oxygen-evolving system [13,17] has been postulated by several investigators. Based on the redox potential necessary to oxidize water [18], the valency of the Mn atom of the Mn-catalyst cation produced by illumination at low temperature is assumed to be 4. Previous observations on the role of Mn in photosynthetic oxygen evolution together with the present observation that the activation of Mn-catalyst to make it operative for energy storage in thermoluminescence requires more than two consecutive light reactions, suggest a binuclear complex, (Mn^{2+}–Mn^{2+}), as the latent Mn catalyst formed in the Mn-deficient cells with added Mn^{2+} ions in the dark. Two sequential photoreactions will convert this complex to a (Mn^{3+}–Mn^{3+}) complex via an intermediate, (Mn^{2+}–Mn^{3+}) or (Mn^{3+}–Mn^{2+}), and this (Mn^{3+}–Mn^{3+}) complex will be the active form of the Mn-catalyst capable of being excited to a (Mn^{4+}–Mn^{4+}) complex which may be frozen at low temperature, storing energy for thermoluminescence or releasing energy at room temperature to oxidize water to oxygen.

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