THE TEMPERATURE DEPENDENCE OF CHLOROPHYLL FLUORESCENCE YIELD IN PHOTOSYSTEM 2

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1. Introduction

Fluorescence yield changes of chlorophyll *a* have been extensively utilized as a monitor of primary photochemistry in Photosystem 2 of green plants and algae [1]. Investigations of Photosystem 2 at low temperatures have become quite popular in recent years and have considerably expanded our knowledge of the primary photochemical reactions [2]. Low temperature experiments have been utilized predominantly to isolate 'primary' photochemical reactions from secondary 'dark' events, since at reduced temperatures (e.g. 77°K) most secondary reactions are frozen out. However since samples held at low temperatures cannot technically be regarded as physiologically competent, the investigator must be wary of drawing analogies to room temperature experiments. For example, measurements of the increase of fluorescence yield in dark-frozen spinach chloroplasts and *Chlorella pyrenoidosa* following a saturating flash at 77°K have yielded the unexpected observation that only 15–20% of the maximal fluorescence is obtained after a single flash [3, 4]. Although den Haan et al. attribute this minimal increase to a decreased quantum efficiency for charge separation at 77°K [4], rather than a backreaction between the reduced primary acceptor and the oxidized donor [5, 6], a plausible explanation at the molecular level for this diminished quantum efficiency has not been advanced.

In this communication the temperature dependence of fluorescence yield monitored after a single flash or during continuous illumination is examined. It is proposed that the low fluorescence yield obtained by den Haan et al. [4] reflects structural changes of water associated with the photosynthetic membrane.

2. Methods

Spinach chloroplast or *Chlorella pyrenoidosa* samples were prepared and maintained at low temperatures as previously described. Measurements of the fluorescence yield during the transient excitation from a GE FT230 xenon flashlamp (Energy/flash ~250 μJ/cm^2; halfwidth, 16 μsec) were performed as described by den Haan et al. [4]. Fluorescence yield changes (at 680–690 nm) following a saturating flash ($\phi_{300\mu sec} = F_{300\mu sec}/F_0$) were monitored by means of a small detecting xenon flash (EGG FX76) ignited 300 μsec after application of the photochemistry-inducing flash. The value of F_0 ('dead fluorescence') was determined by firing the detecting flash immediately prior to discharging the actinic flash. The detecting flash was judiciously attenuated to insure that it could not induce any noticeable fluorescence increase, even after repetitive (~10) applications. ϕ_{max} (the maximum fluorescence yield) was obtained by subjecting the sample to 30 sec of saturating illumination from a tungsten filament lamp ($\lambda \sim 450$ nm), and determining the fluorescence intensity immediately after illumination by means of the detecting flash.

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3. Results and discussion

Previous reports from this lab have documented the intriguing observation that the fluorescence yield increases to only 1.5 × F_0 following a saturating flash (either from a flashlamp or laser ($E \sim 1$ J)) given to dark-adapted chloroplasts or *Chlorella* frozen to 77°K [3, 4]. However, it has now been shown that the magnitude of this single-flash fluorescence yield is temperature dependent, showing only minimal increase from 77°–150°K and a rather pronounced change from 150°–240°K. Fig. 1 illustrates this behavior and presents corresponding data for F_0 and ϕ_{max}. As can be seen, ϕ_{max} also remains relatively constant to 150°K, then rapidly decreases as higher temperatures are selected. A similar behavior for fluorescence intensity monitored at 687 nm (F_{687}) has been reported by Cho and Govindjee [7]. F_0, however, slowly decreases over the entire temperature range examined and does not exhibit the break at 150°K. This data may be presented in an alternative form if the fluorescence yield is assumed to be a manifestation of the rate of fluorescence divided by the sum of the rate constants of all processes (e.g. nonradiative, radiative or chemical) which lead to the deactivation of the chlorophyll excited singlet. Utilizing this premise the logs of F_0', ϕ 300 μsec and ϕ_{max} are plotted against the reciprocal temperatures yielding an analog of the well-known Arrhenius plot (fig. 2). Although F_0 exhibits a monotonic increase in magnitude as T decreases, ϕ_{300sec} and ϕ_{max} show a more complex behavior.

Similar behavior has recently been noted in cytochrome oxidation half-times in photosynthetic bacteria [8], in the rate of photo-reduction of C550 in Photosystem 2 [3], in rates of charge recombination between the photooxidized donor and reduced primary acceptor in photosynthetic bacteria [9], and in Photosystem 1 of green plants and algae [10]. The temperature insensitive portion of these Arrhenius plots indicates a negligible activation energy and characterizes electron or proton tunneling processes [9, 11].

The molecular mechanism of electron or proton transfer in photosynthetic systems has not been established, although water has been implicated as a structural participant in the Photosystem 1 reaction center chlorophyll, P700 [12]. It is therefore note-

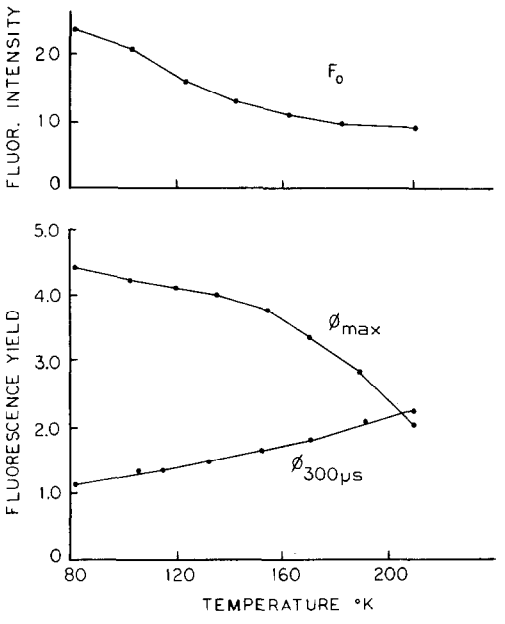

Fig. 1. The variation of F_0, ϕ_{max} and $\phi_{300\,\mu sec}$ as a function of temperature (80° – 220°K) in *Chlorella pyrenoidosa*.

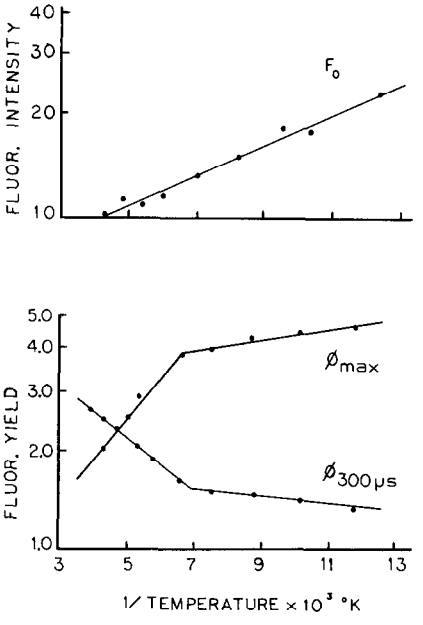

Fig. 2. The variation of F_0, ϕ_{max} and $\phi_{300\,\mu sec}$ as a function of temperature shown in the manner of an Arrhenius plot.

worthy that water-ice undergoes a phase transition from an amorphous to a cubic structure at 150°K [13]. This transition in the crystal structure of ice was first invoked by Cho and Govindjee to explain the temperature dependence of fluorescence in *Chlorella* [7]. Kihara and McCray have recently invoked the hypothesis that electron transfer between membrane bound carrier molecules may be effected by hydrogen atom transfer via water bridges [8]. If this supposition is correct, then the phase transformations of ice (e.g. water associated with biological membranes) may influence biological oxidation–reduction reactions. It is suggested here that the phase transition of ice at 150°K induces a conformational change or redistribution of electron carriers in the proximity of the Photosystem 2 reaction center chlorophyll, which results in the partial decoupling of the primary donor from its acceptor complex. The strong temperature dependence of $\phi_{300\mu s}$ and ϕ_{max} at temperatures above 150°K would then arise from increased diffusion of electron or proton carrier molecules (e.g. water) or from a population of higher vibrational energy levels of the donor–acceptor complex in the reaction center [11] leading to thermally-aided electron transfer. This alteration of the environment of the Photosystem 2 reaction center at low temperatures is reflected in the reduced quantum efficiency for reduction of the primary acceptor [14, 15] during steady-state illumination or during single flashes [4].

Alternatively, the fluorescence behavior at low temperatures may mirror the reduction of the oxidized primary donor, $P680^+$, by cytochrome b_{559} [16]. This photo-oxidation of cytochrome b_{559} has been shown to be temperature independent [16, 17] at low temperatures and also to follow closely the fluorescence yield increase during irradiation of chloroplasts at 77°K [16]. If the extent of reduction of $P680^+$ by cytochrome b_{559} is indeed a determinant of the fluorescence yield, then the limited single-flash-induced fluorescence yield increase noted at low temperatures may arise from hindered electron transport (e.g. resulting from the ice phase transition) from cytochrome b_{559} to $P680^+$. Further transient optical and fluorescence kinetic studies are required to determine whether inefficient photo-reduction of the primary acceptor or inefficient photo-oxidation of cytochrome b_{559} at 77°K is the principal factor leading to a low fluorescence yield increase following a single flash.

The findings in this communication suggest that water in photosynthesis may participate in electron transfer events as well as serving as the substrate for oxygen production. In this regard, future studies should reinvestigate the role of isotopic substitution on the primary processes of Photosystem 2. Additionally, a search for evidence in Photosystem 2 of enthalpy–entropy compensation [18], a diagnostic test for the participation of water in macro-molecular processes, may provide further clues into the role of water in biological energy transduction systems.

Acknowledgments

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