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Rapid Report

The use of excitation fluorescence spectroscopy in the study of short-term chloroplast membrane reorganisation

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The advantages of the use of excitation fluorescence spectroscopy in the investigation of PS I absorption cross-section dynamics during phosphorylation of membrane proteins or heat treatment have been shown. After phosphorylation in the light, there occurred only 15–20% enhancement in absorption cross-section of F735 – PS I peripheral antenna – at the expense of chlorophyll *b* and short wavelength chlorophyll *a* species in the region 674–676 mm, which is attributed to the light-harvesting pigment complex of Photosystem II. Phosphorylation in the dark with dithionite caused increases of 30–35% in PS I absorption cross-section at the expense of Chl *b*, Chl *a* absorbing at 674–676 mm and long-wavelength PS II internal antenna chlorophyll at 685–687 nm. The same changes in the region of 685–687 mm were found after heat treatment of chloroplasts. The application of excitation fluorescence spectroscopy made it possible to estimate directly for the first time not only the extent of changes in absorption cross-section of PS I but also to describe which complex of PS II is responsible.

There is much evidence that the reorganisation of chloroplast membranes during phosphorylation of chlorophyll-proteins or heat treatment seems to be a molecular basis for photosynthetic state transitions [1]. In both cases there takes place a lateral migration of PS II complexes from the grana towards the stroma lamellae where PS I is situated [1]. It is still unclear whether this migration may lead to enhancement in excitation energy redistribution between PS I and PS II or not. It was shown by Allen and Melis [2] that there is no change in absorption cross-section of P700 after phosphorylation of membrane proteins. However, there is evidence that PS I-enriched vesicles, isolated after phosphorylation, reveal about a 20% increase in rate of P700 oxidation [3,4]. Similar results were achieved when P700 measurements had been done after heat treatment of chloroplasts [5]. Measurements of PS I electron transport after phosphorylation or heat treatment have shown pronounced increase in rate [5–8]. Application of Butler's method of low-temperature chloroplast fluorescence induction at 685, 692–696 and 735 nm regions also prove significant changes in energy redistribution between PS I and PS II [9–11]. There is some evidence that phosphorylation may increase the absorption cross-section of PS I, based on excitation fluorescence measurements in the Soret band region of the long-wavelength antenna, F735 [11,12], which used to be described as the 'peripheral antenna' of PS I [13,14]. Unfortunately, analysis of these spectra seems to be very difficult due to the complex and undetermined structure of the 'blue' chloroplast absorption band. In this paper I have applied 'red region' excitation fluorescence spectroscopy of F735 because the structure and origin of absorption bands here have been well investigated [15] (see also reviews, Refs. 16, 17). In all cases of chloroplast membrane reorganisation (light and dark phosphorylation, heat treatment) I have found pronounced changes in excitation spectra showing enhancement in absorption cross-section of the PS I antenna. Comparative analysis of spectral changes induced by light phosphorylation, dark phosphorylation and heat treatment revealed differences in the composition of the PS II chlorophyll complexes which provide PS I with additional excitons.

Abbreviations: PS I, PS II, Photosystems I & II; Chl, chlorophyll; F735 and F695, low-temperature fluorescence at 735 and 695 nm; P700, PS I reaction centre; LHC-II, light-harvesting complex; *a*/*b*, ratio of Chl *a* to Chl *b* concentration; CP43 and CP47, PS II proximal antenna pigment-proteins.

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Chloroplasts were isolated from pea plants grown under supplemented light in a greenhouse for 2 weeks in soil culture as described in Ref. 18. Reaction medium contained 15 mM Tricine buffer (pH 7.6), 10 mM sodium chloride, 5 mM magnesium chloride and 100 mM sucrose. The phosphorylation of membrane proteins was carried out for 15 min, either in white light (300 μE m^{-2} s^{-1}) or in the dark with dithionite, both in the presence of 200 μM ATP and 10 mM sodium fluoride. Heat treatment was carried out in a water bath at a temperature of 40 ° C for 5 min. Low-temperature fluorescence and excitation fluorescence spectra of chloroplasts were measured immediately after treatment using the fluorescence spectrometer described in Ref. 19, modified to allow computer control and data analysis. The concentration of chlorophyll in the samples was 10 μg/ml. The sample thickness was 1 mm. Excitation monochromator band width was 4 nm and the measuring monochromator band width was 0.8 nm and 2.8 mm for fluorescence and excitation spectra, respectively. For the excitation spectra fluorescence has been measured at 735 nm whilst scanning the excitation light from 635 to 720 nm. The influence caused by the scattered light from the exciting beam on the amplitude of the spectra at 705 nm was not more than 4%. Moreover, this was diminished by means of correction of the excitation spectra to the corresponding wavelength redistribution of the scattered light. The level of noise was not more than 0.1% and 0.5% of full scale for fluorescence and excitation spectra, respectively.

Fig. 1 shows a comparison between the low-temperature fluorescence spectra of pea chloroplasts after different treatments. Light phosphorylation caused a less pronounced effect then did heat treatment and dark phosphorylation, when the plastoquinone pool was completely reduced. Changes of the F735/F685 ratio used to be regarded as a sign of energy redistribution between PS II and PS I [20], because of the origin of F735 and F685 from the PS I and PS II antennae, respectively [21]. Different degrees of enhancement in this ratio may reflect different increases of absorption cross-section of PS I antenna, assuming we do not have artificial quenching. However, the use of this method, alone does not allow us to estimate the absorption characteristics of chlorophyll species of PS II responsible for developing the additional cross-section of PS I.

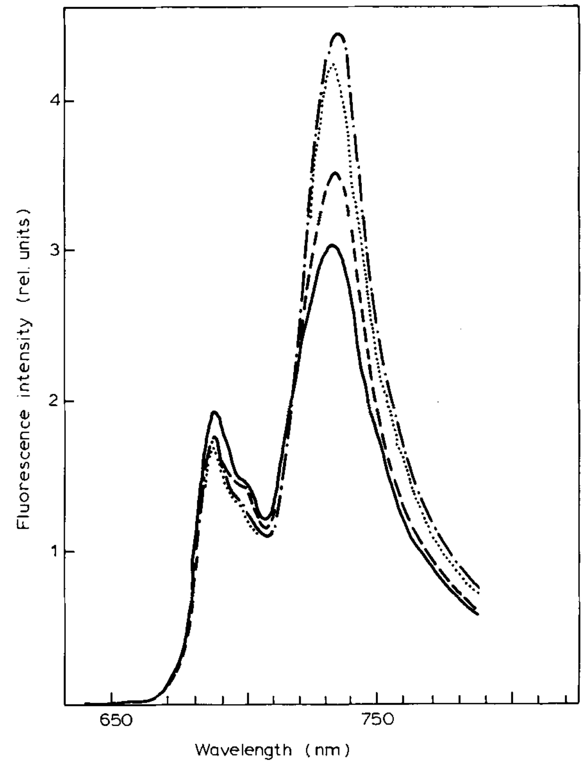

Fig. 1. Low-temperature fluorescence spectra of pea chloroplasts. (———) control, (— — —) phosphorylation in light (light intensity was 300 μE m^{-2} s^{-1}). (······) after heat treatment (5 min, 40 ° C) and (·–·–·) dark phosphorylation with dithionite. Spectra were normalised at 683 nm, the excitation beam wavelength was 435 nm, the chlorophyll concentration in the sample was 10 μg/ml.

Excitation fluorescence spectra of F735 for each case of treatment are shown in Fig. 2 compared to a spectrum of untreated chloroplasts. It is evident that each kind of treatment led to an increase in the shoulder at 650 nm, which belongs to chlorophyll *b*, and a shift of the main maximum to the shorter wavelength region. This indicates that part of the short-wavelength Chl *a* species is responsible for enhancement of the absorption cross-section of F735, the absorption maximum of which may well be recognised, in these spectra, as a shoulder near 705–715 nm. Fig. 3a demonstrates significant differences in absorption cross-section spec-

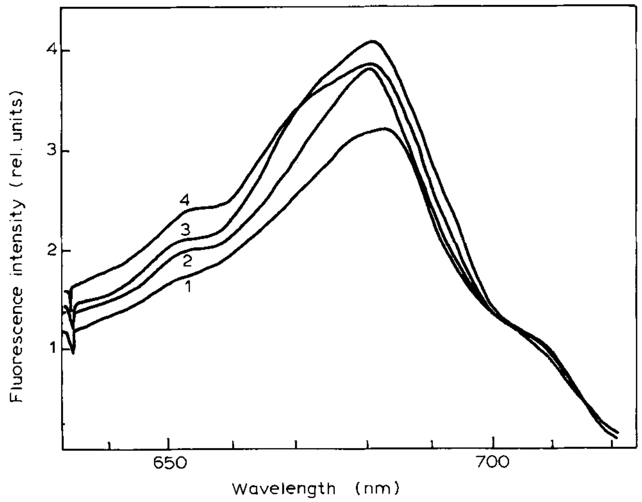

Fig. 2. Low-temperature F735 fluorescence excitation spectra of chloroplasts. (1) Control. (2) Light phosphorylation. (3) Heat treatment. (4) Dark phosphorylation. Excitation monochromator band width was 4 mm, the measuring monochromator band width was 2.8 nm and 735 nm, the chlorophyll concentration in the sample was 10 μg/ml. Spectra were normalised at 705 nm.

tra (treatment–control) for different conditions of membrane reorganisation. It is clear that, in the case of light phosphorylation, there is an increase of the PS I antenna cross-section at the expense of Chl *b* and Chl *a* at 674–676 nm. These species have been attributed to LHC-II [22]. Therefore, this proves a point of view that phosphorylation in the presence of magnesium leads to the transfer of only part of PS II – the distal antenna or LHC-II – towards the stroma and PS I [23]. Our results show that it may well interact with the F735 antenna of PS I. Calculations of variable absorption cross-section spectra ((treatment–control)/(control)) allow us to estimate the wavelength dependence of the efficiency of cross-section enhancement of PS I (Fig. 3b). Light phosphorylation caused an increase of 15–20% in PS I cross-section in the region of LHC-II. The shape of the difference spectrum (treatment–control) for dark phosphorylation was similar to the light variant in the region 650–6756 nm, but contained a shoulder at 685–687 nm (Fig. 3a). The variable spectrum has revealed more pronounced changes in absorption cross-section of PS I compared to light phosphorylation (30–35%), and new bands at 668 and 687 nm appeared (Fig. 3b). This suggests the possibility of a participation in the enhancement of PS I absorption cross-section of complexes other than mobile LHC-II.

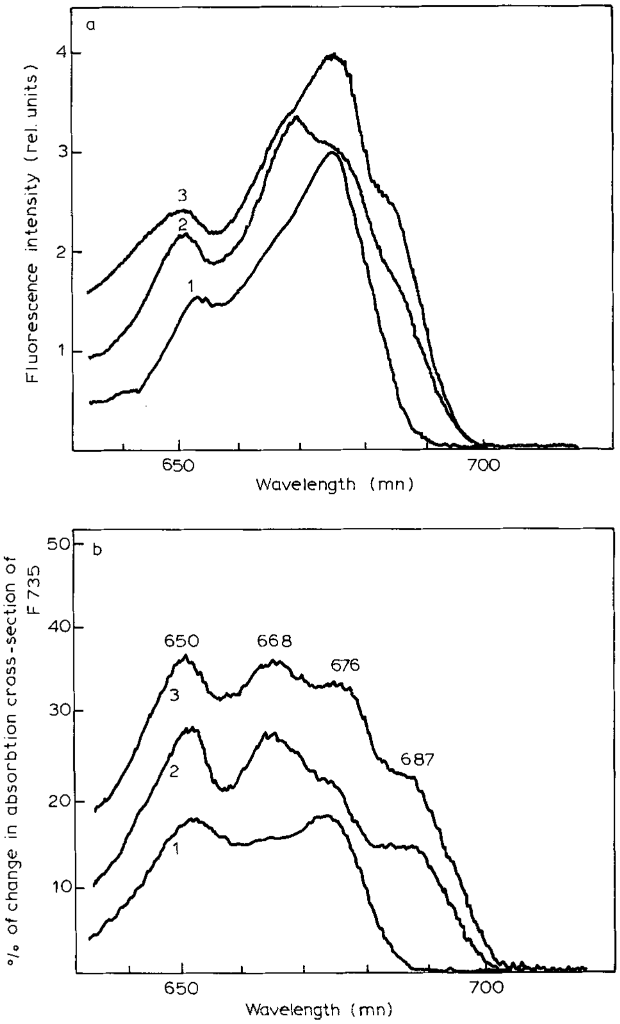

Fig. 3. (a) Difference excitation spectra (difference absorption cross-section spectra) for F735 of chloroplasts. (1) Light phosphorylation–control. (2) Heat treatment–control. (3) Dark phosphorylation–control. (b) Variable absorption cross-section spectra of chloroplasts (difference spectra were divided by the excitation spectrum of control chloroplasts and then multiplied by 100 to obtain the percentage change in the F735 excitation spectrum). (1) Light phosphorylation. (2) Heat treatment. (3) Dark phosphorylation.

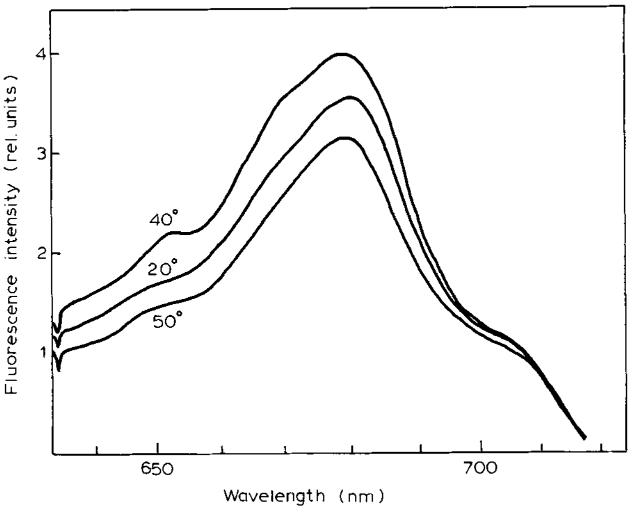

Fig. 4. Low-temperature F735 excitation spectra of pea chloroplasts after different temperature treatments. 20 ° C – control of spectrum chloroplasts incubated at 20 ° C; 40 ° – 5 min incubation at 40 ° C; 5 ° – incubation at 5 ° C for 20 min after treatment at 40 ° C.

Temperature-induced changes in PS I cross-section were more similar to those induced by dark phosphorylation except for a more pronounced maximum at 668 nm (Fig. 3a, b). To prove the reversible (undamaged) character of heat-induced reorganisation, I carried out an incubation of chloroplasts at 5 ° C for 20 min immediately following the heat treatment (Fig. 4). Heat-induced spectral changes were absolutely reversible, which may be regarded as an example of chloroplast membrane dynamics avoiding damage during heat treatment.

The data presented here show that excitation fluorescence spectroscopy in the 'red region' is a valuable method for examination of changes in PS I absorption cross-section during chloroplast membrane reorganisation. One of the advantages of these measurements is that, even if we have a quenching of F735 fluorescence yield during treatment (photoinhibition, heat destruction, etc.), we can still get correct information on changes in the absorption cross-section, because the shape of an excitation spectrum reflects only the relative efficiency of energy transfer to F735 and the absorption of chlorophyll involved, but does not depend on the original F735 yield.

Calculations of cross-section spectra and relative cross-section spectra changes (Fig. 3a, b) made it possible to identify chlorophyll species involved in providing PS I with additional excitons after membrane reorganisation, and estimate their percentage (Fig. 3b). Only phosphorylation in light caused α-transfer: the increase of absorption cross-section of PS I at the expense of LHC-II, because the cross-section spectrum has contained related chlorophyll species [22] (Fig. 3). 15–20% of the additional excitons of the PS I antenna, after phosphorylation, coincide with data achieved in P700 measurements [4,24] and calculations of low-temperature fluorescence induction curves [11,25] according to the bipartite model of W. Butler [26].

Dark phosphorylation-induced PS I cross-section changes in excitation spectra were more pronounced than in the case of light phosphorylation. The main difference took place in the region 685–687 nm – absorption of long-wavelength PS II chlorophyll species. There are a few contradictory points of view on its origin. One hypothesis which has been discussed is that long-wavelength fluorescence species of PS II at 695 nm, with correspondent absorption near 685–690 nm, may belong to pheophytin – a PS II reaction centre component [27]. However, significant progress in PS II structure investigations has shown that apparently this species belongs to CP47 – a component of the internal antenna of the PS II reaction centre which is responsible for the assembly and function of the reaction centre core [17]. Therefore, it is reasonable to say that dark phosphorylation also caused enhancement in absorption cross-section of PS I, at the expense of the internal antenna of the PS II reaction centre. This conclusion is in good agreement with the suggestion of Jennings et al. [28], that more extensive phosphorylation of PS II polypeptides may cause changes in spillover, i.e. the interaction of PS I and PS II reaction centre complexes. In addition, Fig. 3 reveals that the enhancement of cross-section of the PS I antenna is also due to the 668 nm band, which appears to be the absorption maximum of the CP43 complex – the other internal PS II antenna component [17] (the PS II reaction centre complex also possesses this maximum [29]). This shows that extensive phosphorylation may cause the interaction of both internal PS II antenna polypeptides with PS I, and, because they are tightly bound to the reaction centre of PS II, PS II core transfer should also take place.

Heat treatment has a similar effect on energy redistribution towards PS I as does dark phosphorylation: the presence of bands at 687 and 668 nm (Fig. 3) is in good agreement with the point of view of Anderson et al. that during heat treatment a transfer of PS II core complex into stroma lamellae takes place [1]. My results have shown that this led to reversible changes (Fig. 4) in energy redistribution between PS I and PS II in which spillover seems to be involved.

Finally, the intensively discussed point on the reversible energy interaction of PS II components with PS I, which is supposed to be a basis for the short-term photosynthetic membrane adaptation, seems to be possible to clarify using absorption cross-section spectroscopy in different cases of membrane rearrangement. Probably, this has to vary depending on conditions of plant growth, lipid arrangement in the chloroplast membrane, temperature, redox state of the plastoquinone, the transmembrane proton gradient, etc. This will be a subject of future study.

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