Changes in the Fluorescence Emission Spectrum of *Chlorella emersonii* Induced by Cold Treatment; a Possible Regulative Feature of Energy Uptake

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Z. Naturforsch. **37 c**, 448–451 (1982); received January 19, 1982

Fluorescence Emission Spectrum, *Chlorella*, Energy Uptake

Algae, when slowly cooled down to around −5 °C, undergo a change in the fluorescence emission spectrum subsequently taken at liqu. nitrogen temperature. This change resembles the magnesium effect described by Murata [BBA **189,** 171–181, (1969)] for isolated chloroplasts.

Evidence is shown, that both effects are indeed analogous. Cooling the organisms seems to increase the permeability of the thylakoids for cations and, thus, a depletion with concomitant changes in membrane structure. The system serves as a model for the probable *in vivo* control of pigment interaction through alteration of membrane properties.

Introduction

The fluorescence emission spectrum of higher plants and algae provides valuable information about the interaction of photosynthetic systems and is, therefore, extensively used in their qualitative and quantitative assessment (reviewed in [1, 2]). Depending on the problem investigated, measurements are mainly performed at either room or liquid nitrogen temperature.

The interaction of the photosystems expresses itself, amoung others, in the ratio between the emission bands of the respective pigment complexes. A variety of agents are known, which influence this parameter, *e.g.* cations [3–5], DMSO [6], pH [5, 7] etc. The underlying, common mechanism of their action might well be a change in the physical properties of the thylakoid-membrane matrix resulting in an alteration of the distances between pigments and pigment units with either a concomitant increase or an inhibition of resonance transfer of absorbed energy. The membrane, thus, can be viewed as a pivot for the distribution of quanta. It follows, that an active influence on its physical properties through any of the afore mentioned means should constitute a regulatory feature at an early level of photosynthesis.

The reasonable assumption, that the physical properties of the membrane are underlying the control of pigment interaction, is amenable to experimental verification. The approach presented in this paper consists in an alteration of the thylakoid fluidity through reduction of the ambient temperature. A change in the emission spectrum of the photosynthetic organism used should be observed which in magnitude and scope should closely resemble that induced by Mg^{2+} [3, 5], light [8, 9] etc. proposed as a regulatory feature.

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0341-0382/82/0500-0448 $ 01.30/0

Materials and Methods

Chlorella emersonii, strain 211-8b of the Göttingen algae collection, was grown synchronally according to Lorenzen [10] using white light and a 14/10 h light-dark regime. The algae were harvested in the autospore phase immediately after beginning of the light phase: The algae mass was condensed by centrifugation about 10 fold compared to the initial concentration.

All steps of the following procedures were conducted in the dark to avoid the light induced pigment rearrangement described before [8]. After an hour's adaptation of the algae to dark under gentle stirring samples were adsorbed on cheesecloth as described previously [1] and placed, interspaced with wet filterpaper, into a copper vessel within a cryostat. Cooling the vessel to the desired pretreatment temperature, which was monitored with a build-in thermometer, usually took place within 30 minutes.

The cold pretreated or untreated control algae were fixed for fluorescence measurements by imme-

diate cooling to liquid nitrogen temperature using the quick freeze method [1]. The spectra were taken using 450 – 490 nm broad band exciting light and the same instrumental setup as described before [9]. The data are corrected for filter tailing and photomultiplier sensitivity.

Results

The temperature induced alteration of the fluorescence emission spectrum is shown in Fig. 1. As can be seen, the emission band at F_{720} is unaffected, while those of LHC and PSII are greatly reduced. The reduction is dependent on the exposure time to cold (Fig. 2), *i.e.*, the algae have to reach a certain "transition" point before the effect starts to express itself. The dependence on duration of cold incubation seems to be caused by at least two variables, namely the insulation effect of the cell body which protects the thylakoid membrane for a short period from reaching the temperature of the environment and, secondly, the time it takes within the membrane to possibly rearrange itself once a certain "transition temperature" is reached. The figure supports this view through its discontinuity of the increase of the emission ratio.

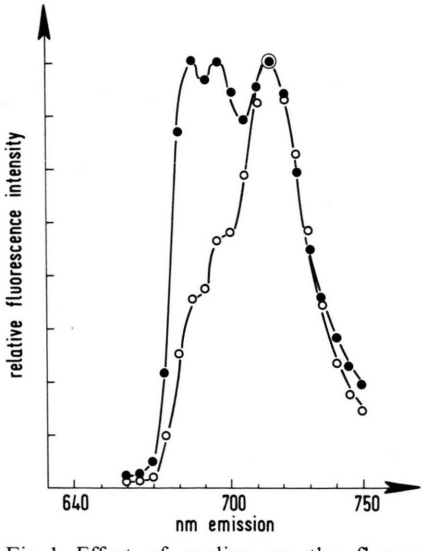

Fig. 1. Effect of cooling on the fluorescence emission spectrum of *Chlorella emersonii*. The closed circles represent the untreated control data (20 °C), while the open circles show the signal intensity after exposure to – 10 °C for 30 minutes. The data are normalized to F_{720}, the ratio between the signals of control and treated algae was 0.99.

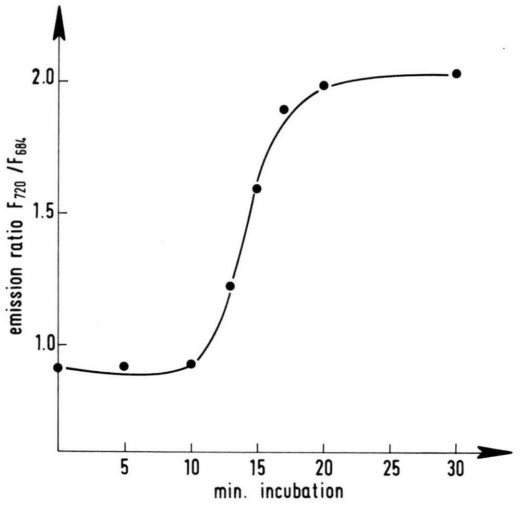

Fig. 2. Dependence of the cooling effect on the duration of incubation at – 10 °C.

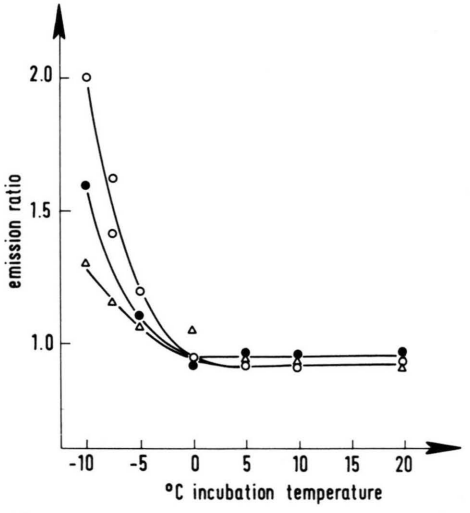

Fig. 3. Dependence of the cooling effect on the temperature of incubation. The open circles depict the ratio F_{720}/F_{684}, the closed circles that of F_{720}/F_{695}, the open triangles show the ratio F_{695}/F_{684}.

An approximation of the postulated "transition" temperature was attempted by varying the incubation temperature. Fig. 3 shows, that the effect expresses itself once 0 °C, the freezing point of water, is attained.

The effect of cooling is thoroughly *reversible*, both in a physical and a functional way. The fluorescence spectrum and the oxygen production of the control is undistinguishable at all light intensi-

ties from that of samples which have undergone a cooling/warm up cycle (Fig. 4). The reduction in LHC and PSII emission is not influenced by addition of 10^{-4} M DCMU, indicating an absence of any important influence from the electron transport chain (data not shown).

However, specifically affecting the physical parameters of the surrounding thylakoid membrane matrix results in a fluorescence spectrum similar to that of cold treated algae. Fig. 5 shows this for the treatment with 10% DMSO. On the other hand, fixation of the membranes with glutaraldehyd results in complete insensitivity to cold treatment (data not shown).

The expression of the cold induced spectral change resembles the Magnesium effect of Murata [3]. The underlying cause for the latter has been explained as a change in the thylakoid membrane environment caused by addition or depletion of magnesium [5]. If cold treatment leads to an increased permeability of the membrane barrier for magnesium at low temperature, the combined treatment of the algae with cold and EDTA should diminish the magnesium content by removing it from the thylakoid membrane and complexing it *irreversibly* within and outside the cell. An effect analogous to that of Murata [3] should be observed, which in this case cannot be reserved in a cooling/warm up cycle.

The algae were suspended in either 0.4 M sucrose-phosphate buffer pH 6.2 (Control) or the same medium containing 50 mM EDTA (sample). After incubation at -1 °C for an hour in the dark, the algae were washed in the respective media, resuspended in 0.4 M sucrose-phosphate buffer pH 6.2 and allowed to rewarm to room temperature before further processing. Fig. 6 shows the resulting alteration of the fluorescence spectrum which is precisely

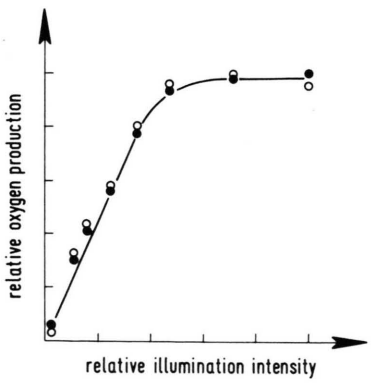

Fig. 4. Photosynthetic activity of control algae (solid circles) and algae which have undergone a cooling/warm up cycle (open circles).

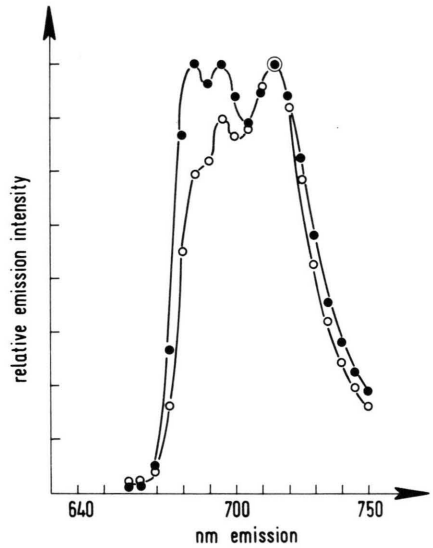

Fig. 5. Effect of treating *Chlorella emersonii* with 10% DMSO. The closed circles represent the signal of untreated controls, the open circles those of the DMSO treated algae. The data are normalized at F_{720}. The ratio of F_{720} (control) to F_{720} (DMSO treated) was 1.05.

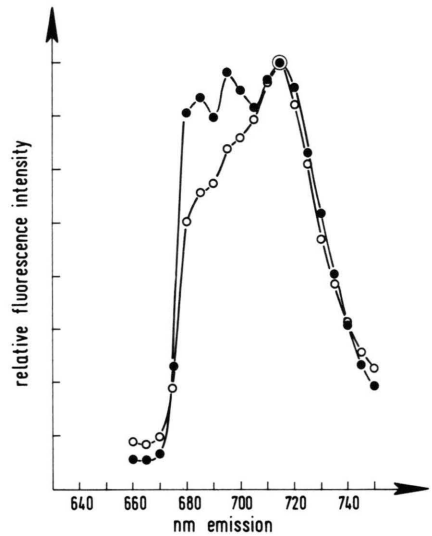

Fig. 6. Effect of combined cold and EDTA treatment on the fluorescence spectrum of algae. The treated algae (○) were, contrary to the data shown in Fig. 1, allowed to warm up to room temperature before quick freezing them for the subsequent assay.

as predicted and similar to that of the aforementioned Murata effect. The ratios F_{715}/F_{685}, – found in 8 different experiments, were 1.06 ± 0.03 SD for the control and 1.68 ± 0.05 SD for the sample.

Discussion

The data so far allow two conclusions, first of all, the change in the spectrum is related to the physical properties of the thylakoid membrane, and secondly, the "transition" temperature suggests the involvement of intraorganelle water. Murata [11, 12] has recently shown, that the temperature of cultivating the algae affects the permeability of their thylakoid membrane for ions and leads to a differential level of Magnesium ions. Thus, it can be argued, that in the above experiments the reduction in ambient temperature leads to a rearrangement of membrane structure with a consequent change in surface charges. This in turn inflicts a loss of cations, especially Mg^{2+}, which permeate out of the organelle and probably the cell as well. The similarity of the cooling effect described here and the Magnesium effect reported by Murata [3] for isolated chloroplasts supports this suggestion, which is also in accordance with the notions put forward by Wong *et al.* [5]. However, the experiments do not rule out, that the excitation transfer from accessory carotenoids is shifted between the pigment systems. In view of the current knowledge about the distribution of these compounds within the photosystems itself this notion deemes us highly unlikely.

Acknowledgement

The research was supported by a grant from the Deutsche Forschungsgemeinschaft.

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