ATP-induced quenching of chlorophyll fluorescence in chloroplasts of higher plants. Dependence on structural properties of the membranes

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Abstract. The ATP-induced quenching of chlorophyll fluorescence in chloroplasts of higher plants is shown to be inhibited when the mobility of the protein complexes into the thylakoid membranes is reduced. Its occurrence also requires the presence of LHC complexes and the ability of the membranes to unstack.

These observations, in addition to a slight increase of charge density of the surface — as indicated by 9-aminoacridine fluorescence and high salt-induced chlorophyll fluorescence studies — and partial unstacking of the membranes — as monitored by digitonin method and 540 nm light scattering changes — after phosphorylation, suggest that the ATP-induced quenching of chlorophyll fluorescence could reflect some lateral redistribution of membrane proteins in the lipid matrix of the thylakoids.

Introduction

A protein kinase present in isolated thylakoids catalyses the light-dependent and DCMU-sensitive phosphorylation of membrane proteins [11–13]. A consequence of phosphorylation of a particular protein, the light harvesting chlorophyll a/b chlorophyll b protein complex (LHC), is the ATP-induced quenching of chlorophyll fluorescence of isolated chloroplasts, which has been identified with a change in energy distribution between photosystem two (PS2) and photosystem one (PS1) [14, 15].

Very recently, an hypothesis has been postulated concerning the mechanism responsible for the phosphorylation associated change in distribution of energy between PS2 and PS1 [8, 10]. It was suggested that phosphorylation would increase the net negative charge on the exposed surface of the LHC protein, enough to cause coulombic repulsion between the phosphorylated surfaces within the partition gap of the membranes and bring about partial unstacking. This would allow migration of the phosphorylated protein

Abbreviations: ATP adenosine triphosphate; 9-AA 9-aminoacridine; Chl chlorophyll; EDTA ethylenediaminetetraacetate; GDA glutaraldehyde; Hepes N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid; LHC light-harvesting chlorophyll a/b complex PS photosystem.
complex, LHC-PS2 or LHC alone, from the stacked regions into the non-appressed, PSI-enriched regions [5], where spillover or redistribution of energy to PS1 would be favoured.

The phosphorylation process occurs at the threonine groups of a positively charged 2000 M.Wt. component of the 25 to 30 Kdalton polypeptide of LHC [3, 5, 14, 21, 22]. This process in some way alters the properties of its interaction with both photosystems [15].

Since reduced plastoquinone serves to activate the kinase, the regulation of distribution of light energy seems to be mediated by the redox state of the plastoquinone pool [3, 21, 22].

The above described mechanism of protein diffusion should be possible in the lipid matrix of the thylakoid, because of its relatively high fluidity at room temperature [4, 24, 26] and has already been invoked in the thylakoid stacking process, induced by electrostatic screening [7]. Therefore, ATP-induced quenching of fluorescence should be inhibited under any condition which reduces the protein mobility. That is, the model predicts an inhibition of the ATP induced quenching of fluorescence under conditions which would prevent the proposed phosphorylation associated unstacking.

In this paper, some experiments were conducted to test the above predictions.

Materials and methods

Envelope-free chloroplasts were prepared from pea leaves, as previously described [9] and stored on ice, in a medium containing 0.1 M sorbitol, 1 mM KOH and 1 mM Hepes (pH 7.5).

To investigate effects of ATP on chlorophyll fluorescence, chloroplasts (10 µg Chl/ml) were suspended in a medium consisting of 0.1 M sorbitol, 10 mM KCl, 5 mM NaF, 0.5 µM nigericin, 0.5 µM valinomycin, 10 mM Hepes (pH 7.8, KOH) and MgCl₂, whose concentration depended on the initial level of fluorescence (around 1.5 mM; see explanation in results). ATP (0.15 mM) was added with an equimolecular amount of MgCl₂, to maintain the free Mg²⁺ concentration constant.

The yield of chlorophyll fluorescence was measured using a blue-green light (about 30 W/m²) filter and the emission was detected by an EMI 9558B S20 photomultiplier shielded by a Balzer B40 693 nm interference filter and a Schott RG 695 nm cut-off filter. 90° light scattering was detected by a photomultiplier shielded by a Balzer B40 542 nm.

Changes in redistribution of energy between PS2 and PS1 were monitored by chlorophyll fluorescence spectra recorded at 77°K, as previously described [29].

Digitonin treatment of the membranes was performed as in Refs. 9 and 16. The chloroplast suspension contained 100 µg Chl/ml, 0.1 M sorbitol, 10 mM KCl, 1.5 mM MgCl₂, 5 mM NaF, 1 µM nigericin, 1 µM valinomycin,