Nuclear pea mutants deficient in chlorophyll b and the major polypeptide of the light-harvesting chlorophyll a/b protein of photosystem II

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Abstract. Eight chlorophyll b deficient nuclear mutants of pea (Pisum sativum L.) have been characterized by low temperature fluorescence emission spectra of their leaves and by the ultrastructure, photochemical activities and polypeptide compositions of the thylakoid membranes. The room temperature fluorescence induction kinetics of leaves and isolated thylakoids have also been recorded. In addition, the effects of Mg$^{2+}$ on the fluorescence kinetics of the membranes have been investigated. The mutants are all deficient in the major polypeptide of the light-harvesting chlorophyll a/b protein of photosystem II. The low temperature fluorescence emission spectra of aurea-5106, xantha-5371 and -5820 show little or no fluorescence around 730 nm (photosystem I fluorescence), but possess maxima at 685 and 695 nm (photosystem II fluorescence). These three mutants have low photosystem II activities, but significant photosystem I activities. The long-wavelength fluorescence maximum is reduced for three other mutants. The Mg$^{2+}$ effect on the variable component of the room temperature fluorescence (685 nm) induction kinetics is reduced in all mutants, and completely absent in aurea-5106 and xantha-5820. The thylakoid membranes of these 2 mutants are appressed pairwise in 2-disc grana of large diameter. Chlorotica-1206A and -130A have significant long-wavelength maxima in the fluorescence spectra and show the largest Mg$^{2+}$ enhancement of the variable part of the fluorescence kinetics. These two mutants have rather normally structured chloroplast membranes, though the stroma regions are reduced. The four remaining mutants are in several respects of an intermediate type.

Introduction

A functional or structural role has in the last decade been assigned to several of the polypeptides of chloroplast membranes. An important way of approaching or supporting some of these identifications has been through detailed biophysical and biochemical analyses of mutants with lesions in the photosynthetic apparatus. This strategy has been used effectively for the PSI-associated polypeptides, the polypeptide associated with the reaction center

Abbreviations: Chl = chlorophyll, CPI = Chl-protein complex I, $F_o$, $F_v$ and $F_m$ = parameters of room temperature chlorophyll fluorescence induction kinetics, F685, F695 and F-1 = components of low temperature chlorophyll emission with maximum at 685, 695 and ca. 735 nm, respectively, PSI = photosystem I, PSII = photosystem II, LHCI and LHCII = light-harvesting chlorophyll a/b complexes associated with PSI and PSII, respectively, SDS = sodium dodecyl sulfate.
of PSII, and for the major light-harvesting chlorophyll a/b complex (LHCII) [14].

The present study characterizes eight mutants of pea that are deficient in the major polypeptide of LHCII and which have more or less abnormally structured thylakoid membranes.

Materials and methods

Plant material

Wild type pea (Pisum sativum L.) of cultival Weibull's Fenomen was used as reference material. Stocks of the mutants xantha-5820, -5371, -862, aurea-5106, chlorotica-5766 and chlorina-5535 were obtained from Dr. S. Blixt at the Pism Gene Bank, Weibullsholm Collection, Sweden. The mutants chlorotica-130A and chlorotica-1206A were obtained from Professor W. Gottschalk, University of Bonn, West Germany. The xantha, aurea and chlorina mutants do not survive to maturity and are kept as heterozygous stocks, while the chlorotica mutants are non-lethal [2, 3, 4, 12, 24]. The plant material was grown in vermiculite in a growth chamber at 18°C and with a 14h light/10h dark cycle (light intensity 8 W·m⁻²).

Chloroplast membrane isolation

All operations were carried out at 0–5°C. Five to 15 g of the leaves were ground with 3–5 ml grinding medium per g leaf for 2 × 5 s in a Sorvall Omnimixer. The grinding medium was 50 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), pH 7.5, 330 mM mannitol, 10 mM β-mercaptoethanol and 0.1% bovine serum albumin. The homogenate was filtered through mesh nylon. The chloroplast membranes were sedimented for 10 min at 5000g and resuspended in 20 mM Hepes, pH 7.5 with 10 mM EDTA (Hepes-EDTA buffer). For electrophoresis the membranes were centrifuged and the pellet was frozen. For measurements of photochemical activities and fluorescence induction kinetics the membranes were washed once more with Hepes-EDTA buffer and finally resuspended in small volumes of Hepes-EDTA buffer.

Assays of photochemical activities

PSI activity was measured as rate of oxygen uptake with a Clark-type electrode. The reaction vessel was thermostated at 22°C. The reaction mixture contained 50 mM Hepes, pH 7.5, 25 mM EDTA, 3 mM Na isoascorbate, 0.2 mM TMPD (N-tetramethyl-p-phenylenediamine), 0.1 mM methyl viologen, 25 μM DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), and an appropriate amount of chloroplasts in a final volume of 3 ml. Oxygen uptake in white light (300 W·m⁻²) was corrected for the uptake in the dark.

The concerted PSI and PSII activity was measured as rate of oxygen