Stability of etiochloroplasts isolated from pine cotyledons as studied by means of low temperature absorption and fluorescence spectroscopy

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Abstract: 77 K absorption and fluorescence spectra are measured during incubation of etiochloroplast isolated from pine cotyledons and suspended in buffers with or without various co-factors (GSH, ATP, CoA*) and NADPH. It is shown that, in the absence of co-factors, rapid spectral changes due to denaturation of the protein–pigment complexes occur. The spectral changes differ according to whether denaturation occurs in the light or in the dark. The rate of denaturation of the Chl–protein complexes is significantly lowered when co-factors are added and a protective effect of NADPH on the PChlide–protein complexes was observed.

Introduction

Dark-grown gymnosperm seedlings form Chl in complete darkness. The green plastids developed in the dark contain crystalline prolamellar bodies as well as lamellae and grana [5]; they are called etiochloroplasts.

In the same conditions, angiosperm seedlings synthesize PChl(id) which can be converted enzymatically by light into Chl(id); the etioplasts developed in the dark contain prolamellar bodies bearing a few perforated double membrane sheets. Until now, very little was known about dark biosynthesis of Chl in gymnosperms. The fact that small amounts of PChl can be found in dark-grown pine cotyledons and that occasional accumulation of this pigment in pine has been reported [6, 13] may indicate that PChlide is probably used in the pathway of Chl biosynthesis in darkness.

Etiochloroplast suspensions may be more useful than entire cotyledons for studying the biochemical mechanism of Chl formation in gymnosperms in the dark. Such preparations avoid the problem of the variability of biological material and allow the addition of substances such as co-factors, reducing agents, inhibitors or Chl precursors.

In recent years, considerable efforts have been made to improve procedures

*Abbreviations: PChl(id) = protochlorophyll(id); PChlidesF657 = protochlorophyllide–lipoprotein complex with fluorescence maximum at 657nm; Chl(id) = chlorophyll(id); PEG = polyethylene glycol; DCIP = 2–6–dichlorophenol indophenol; ALA = 5–aminolevulinic acid; ATP = adenosine 5′ triphosphate; CoA = coenzyme A; NADPH = nicotinamide–adenine dinucleotide phosphate, reduced.

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of preparations of chloroplastic and sub-chloroplastic particles from gymnosperms. Both PEG (25%) and high sucrose concentrations in the extraction buffer were reported to be necessary to protect the isolated chloroplasts against phenolic compounds and resins which are liberated upon homogenization of leaf tissues and against ageing [7, 8, 9, 11]. Chloroplasts from coniferous leaves isolated in such media exhibit Hill activity [7, 8], DCIP photoreduction [9] and photophosphorylation [11].

In such preparations, the state of the lipoprotein-pigment complexes can change rapidly on account of progressive denaturation during incubation in the isolation medium.

Until now, no study of the evolution of the spectral properties of isolated pine etiochloroplasts had been published.

This paper deals with the measurement and the analysis of the 77 K fluorescence and absorption spectra of pine etiochloroplasts pre-treated or not with ALA during incubation in buffers known to allow preparation of active particles. A method is proposed for estimating the level of denaturation of the pigment–protein complexes from the fluorescence spectra. It is shown that a decrease in the denaturation rate occurs when co-factors (GSH, ATP, CoA, NADPH) are added in the re-suspension medium. It is also shown that the spectral forms appearing during denaturation differ according to whether denaturation occurs in the light or in the dark.

Material and methods

Plant material

Seedlings of *Pinus jeffreyi* (obtained from Vilmorin, Paris, France) were grown on vermiculite soaked with a nutrient solution A4 (according to Arnon [1]) at 23°C in complete darkness for 15–17 days. The handling of the plants was carried out in the dark or under a dim green light.

Etiochloroplast preparations

Two to three grams of 15–17 day old cotyledons were harvested, cut into small pieces and then ground in a mixer (Janke and Kukel K9 type A10) for 15 s at +2°C with 30 ml of the extraction buffer, which consisted of 0.5 M sucrose, 0.01 M NaCl, 0.05 M Tris–HCl pH 7.8, 0.2% (w/v) bovine serum albumin and 25% (w/v) PEG 4000 (Carbowax 4000 – Fluka AG Buchs S4), according to Oku and Tomita [7]. The homogenate was filtered through a layer of cheesecloth (Blutex–50 from Tripette et Renaud, Paris, France) and the filtrate was centrifuged at 200g for 3 min. Etiochloroplasts in the supernatant were collected by centrifuging at 1500g for 7 min. The pellet was re-suspended and homogenized in 10 ml of 0.5 M sucrose, 0.2 M Tris–HCl pH 7.7, 4 mM MgCl2, 0.2% (w/v) bovine serum albumin (unfortified re-suspension buffer).

Fortified etiochloroplasts were obtained by re-suspending the pellet of