A NEW NON-PHOTOREDUCIBLE PROTOCHLOROPHYLL(IDE)–PROTEIN: *P*-649–642 FROM CUCUMBER COTYLEDONS

NADPH mediation of its transformation to photoreducible *P*-657–650

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1. Introduction

In higher plants, the photoreduction of protochlorophyll(ide) is an essential light-dependent step in chlorophyll(ide) biosynthesis. Some workers have studied this step in vitro using isolated etioplasts and prolamellar body membranes [1–6]. Two classes of protochlorophyll(ide)–proteins have been described: convertible and non-convertible, respectively, into chlorophyll(ide)–proteins by illumination [7–14]. Of the photoreducible protochlorophyll(ide)s *P*-657–650 has been found to be the main component in etiolated leaves or suspensions of etioplasts, *P*-647–638 being less important. *P*-630–629 has been described as the only non-photoreducible protochlorophyll(ide) so far; it has been shown to be transformed to *P*-657–650 by the addition of NADPH [3–5].

A new non-photoreducible protochlorophyll(ide)–protein complex, *P*-649–642, is described here; it is transformed into *P*-657–650 when NADPH is supplied.

2. Material and methods

Cucumber (*Cucumus sativus*, cv. 'long vert de Chine') seedlings were grown in darkness at 20 ± 1°C on a moist mixture of vermiculite (50%)–perlite (50%). Etiolated cotyledons (5 g) from day 7 seedlings were ground at 4°C in 10 ml ice cold medium (0.2 M Tris–HCl, 0.5 M sucrose, 10% or 5% glycerol, pH 8.0). The homogenate was filtered through 8 layers of cheese-cloth and 1 layer of 30 μm mesh nylon tissue then centrifuged at 200 × *g* for 3 min at 4°C. The supernatant was centrifuged at 1500 × *g* for 7 min at 4°C. The final pellet was resuspended in 5 ml isolation medium (fresh suspension). All manipulations were carried out in dim green light.

The fresh suspensions were incubated in the dark at 3°C, 10°C or 24°C. The incubated suspensions were illuminated, when desired, using a photographic flash-light (light energy 125 J; Multiblitz Report Porba 50 E). NADPH was purchased from Boehringer.

Absorption spectra were recorded at −196°C using a Cary 17 spectrophotometer. Emission spectra were recorded at −196°C using the apparatus described [8].

3. Results

During dark incubation, the absorbance of the etioplast suspension increased at 629 nm and decreased at 650 nm, while the emission at 632 nm increased relative to that at 657 nm. This is seen in fig.1 which compares spectra recorded before (curves a, a′) with spectra recorded after dark incubation for 6 h (curves b, b′). Such a spectral change is always observed when *P*-657–650 is inactivated [9,13,14].

After incubation, a 1 ms flash illumination caused

Abbreviation: P_{x-y}, forms of protochlorophyll(ide)–proteins with low temperature emission at *x* and absorption at *y* nm

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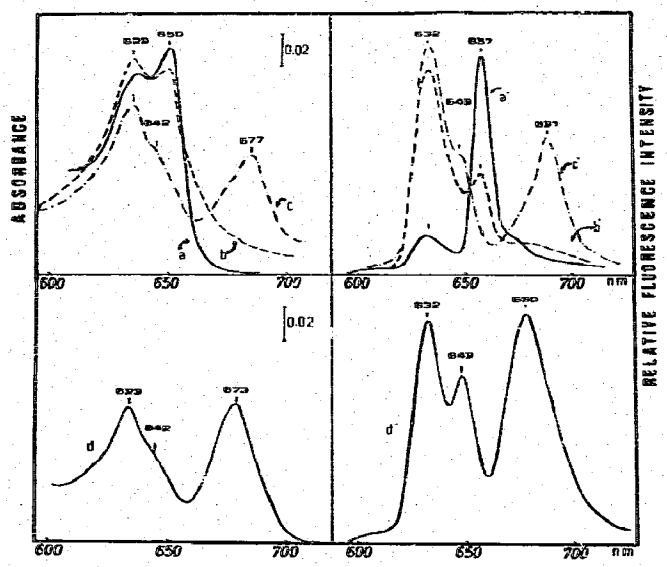


Fig.1. Low-temperature (−196°C) absorption (on the left) and fluorescence emission (on the right) of a fresh Cucumber etioplast suspension (a, a'); (b, b') the same after a 6 h dark incubation at 10°C (10% glycerol in the medium); (c, c') the same after 2 successive 1 ms, light flashes. (d, d') are low-temperature (−196°C) absorption and fluorescence spectra of Cucumber etioplasts incubated for 3 h in the dark at 10°C in a medium containing 5% glycerol and then further incubated under dim, fluorescent white light (3.5 W.m^{-2}) for 30 min at 10°C.

the disappearance of the A_{650} nm absorption band and its replacement by another band with an absorption maximum at 677 nm. Similarly, the fluorescence band at 657 nm was replaced by one at 691 nm. This is seen in curves c and c' (fig.1) which also show that, after illumination, the absorption spectrum had a shoulder at 642 nm, while the corresponding emission spectrum had a shoulder at 649 nm.

These two shoulders were never seen when a fresh suspension was illuminated without previous dark incubation. They both were seen after a 10 h dark incubation at 3°C, or after a 6 h dark incubation at 10°C. Both disappeared completely after further incubation for 2 h at 24°C. Once formed at 3–10°C both remained visible after several flashes of light. Hence they are supposed to belong to a particular protochlorophyll(ide)–protein complex appearing when an etioplast suspension is incubated in the dark then illuminated; in this complex, protochlorophyll(ide) is not photoreducible. We call it *P*-649–642. Absorption and emission bands of *P*-649–642 are particularly well seen in fig.1, curves d, d'.

When NADPH was added to a suspension containing *P*-649–642, the shoulders at 642 nm (absorption) and at 649 nm (emission) disappeared, being replaced by bands at 650 nm (absorption) and 657 nm (emission) during further dark incubation. This dark change did not occur in the control sample without the addition of NADPH. The bands at 650 nm (absorption) and at 657 nm (emission) of the NADPH-treated sample disappeared after flash illumination for 1 ms (fig.2).

4. Discussion

To our knowledge the *P*-649–642 non-photoreducible protochlorophyll(ide)–protein has not been noticed previously. It appears to be formed during etioplast incubation in the dark when the level of NADPH is low and remains stable after illumination of the incubation mixture. When NADPH is added, the spectral characteristics of *P*-649–642 disappear and the spectral bands of the photoreducible *P*-657–650 form appear (fig.2):

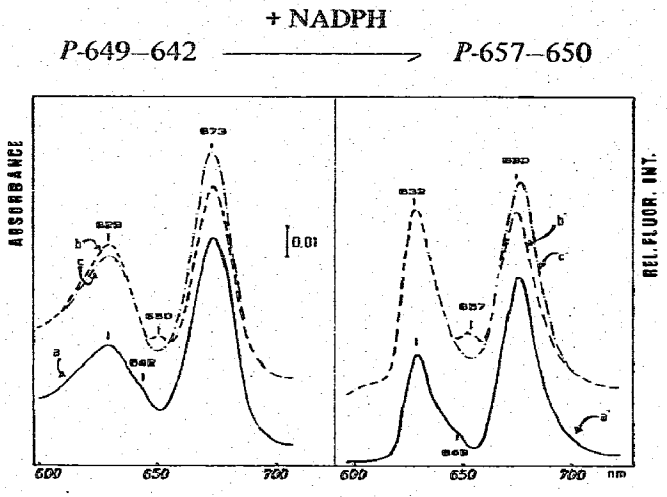
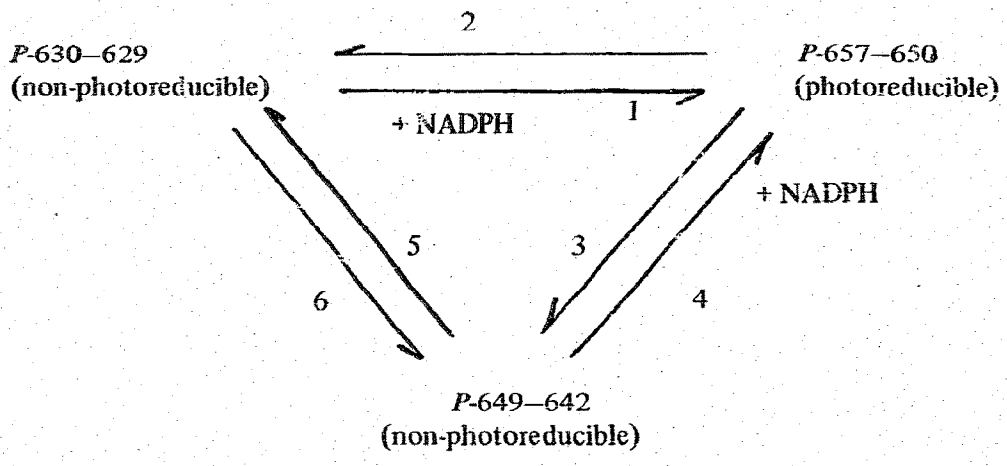


Fig.2. Low-temperature (−196°C) absorption (on the left) and fluorescence emission (on the right) spectra of Cucumber etioplasts incubated for 3 h in the dark at 10°C, then illuminated by 2 successive 1 ms flashes (curves a, a'). NADPH was supplied after the flashes at final conc. 0.15 mM, and the etioplasts were kept again for 30 min in the dark at 10°C (curves b, b'). Finally the suspension received a 1 ms flash (curves c, c').

Griffiths [3,4] and Brodersen [5] have already noted the NADPH dependence of the transformation of the non-photoreducible *P*-630–629 protochlorophyll(ide)–protein into the photoreducible *P*-657–650 protochlorophyll(ide)–protein. The following diagram includes the new form, *P*-649–642 as well:



Pathway 1 is described in [3–5]. The present paper deals with pathways 2–4. Pathway 2 is also mentioned in [9,11,13,14]. Pathway 5 is demonstrated by incubating, at 24°C, a suspension in which *P*-649–642 had been formed.

There is as yet no evidence for pathway 6.

Acknowledgements

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