Volume 224, number 2, 343–347 November 1987

Isolation of two different subpopulations of the light-harvesting chlorophyll *a/b* complex of photosystem II (LHCII)

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Received 1 October 1987

Isolated LHCII from spinach has been solubilized and fractionated by non-denaturing isoelectric focusing to yield two subpopulations with different polypeptide but equal chlorophyll composition. One LHCII subpopulation contains only a 27 kDa polypeptide while the other contains the 27 and 25 kDa polypeptides in about equal amounts. The polypeptide patterns of the two subpopulations closely correspond to those suggested previously for the inner LHCII and peripheral LHCII, respectively.

Light-harvesting; Photosystem II; Photoregulation; Isoelectric focusing; Chlorophyll-protein

1. INTRODUCTION

The light-harvesting chlorophyll *a/b*-protein complex of photosystem II (LHCII) is the dominant constituent of the thylakoid membrane of plants and green algae [1]. The LHCII is composed of several subunits, all in the 25–30 kDa region [2,3], which are coded for by small multigene families in the nuclear DNA [4]. When photosystem II becomes overexcited, LHCII is phosphorylated resulting in certain structural and functional rearrangements of the thylakoid pigment bed [5,6]. Recent studies on spinach thylakoids have revealed that LHCII has a heterogeneous arrangement around the photosystem II core and two subpopulations can be defined [7–10]. One LHCII population (inner) is tightly bound to the photosystem II core: it contains predominantly a 27 kDa polypeptide and shows quite slow phosphorylation kinetics [9,10]. The other is more loosely or peripherally attached to photosystem II from which it can be released due to phosphorylation or increased temperatures [10,11]. This latter LHCII pool, which is very rapidly phosphorylated, contains a relatively high proportion of a 25 kDa subunit supplementing the 27 kDa subunit [10]. The commonly employed Triton X-100-based isolation procedure for LHCII [12] gives a preparation which contains a mixture of both subpopulations [10].

Here, such an LHCII preparation [12] has been further subfractionated by preparative column isoelectric focusing. Two distinct LHCII subpopulations were isolated with polypeptide com-

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

position typical of the peripheral and inner LHCII pools.

2. MATERIALS AND METHODS

Thylakoid membranes were isolated from spinach leaves in 0.4 M sorbitol, 0.1 M Tricine-NaOH (pH 7.8) and washed twice in the same medium [12]. Isolation of the LHCII was performed using Triton X-100 solubilization of thylakoids followed by sucrose gradient centrifugation mainly according to Burke et al. [12] as modified by Ryrie et al. [13]. The final LHCII preparation was suspended in 2 mM Tricine-NaOH (pH 7.5). For isoelectric focusing, the isolated LHCII was washed once in 5 mM EDTA (pH 7.5) and pelleted at 42000 × *g* for 30 min. The pellet containing 5 mg chlorophyll was solubilized by addition of 0.5 ml of 10% (w/v) Triton X-100 and 1.0 ml of 2.5% (w/v) *n*-octyl-*β*-glucopyranoside giving a final ratio of Triton X-100:*n*-octyl-*β*-glucopyranoside:chlorophyll of 10:5:1. After stirring for 15 min on ice, 188 μl of 40% (w/v) ampholine (pH 3.5–5.0), 85 μl of 30% (w/v) ampholine (pH 2.5–4.5), 500 μl of 10% (w/v) glycine, 2.7 g sucrose and water were added to a final volume of 5 ml. The final concentrations in the sample cocktail were: 1 mg chlorophyll/ml, 1% Triton X-100, 0.5% *n*-octyl-*β*-glucopyranoside, 1.5% ampholine (pH 3.5–5.0), 0.5% ampholine (pH 2.5–4.5), 0.5% glycine and 54% sucrose.

Isoelectric focusing was conducted using an LKB 8100 ampholine electrofocusing column. The cathode solution was 0.25 M NaOH, 60% sucrose. The solubilized sample was placed on a 55% sucrose cushion which overlaid the cathode solution. Ampholine containing a 5–50% sucrose gradient was then carefully cast on top of the sample. The gradient was prepared by mixing equal amounts of 5 and 50% sucrose solutions each containing 0.5% Triton X-100, 0.2% *n*-octyl-*β*-glucopyranoside, 1.5% ampholine (pH 3.5–5.0), 0.5% ampholine (pH 2.5–4.5) and 0.5% glycine. The anode solution was 0.25 M H_3PO_4. The electrofocusing was run for 72 h at 10 W constant power in the dark at 4°C. The fractions were collected from the top of the gradient. Chlorophyll concentration was determined in 80% acetone according to [14]. SDS-PAGE analyses were performed as in [15] using 12–22.5 or 13–30% polyacrylamide gels. The gels were stained with Coomassie brilliant blue and scanned using a laser densitometer. The polypeptides were quantified from their relative peak areas.

3. RESULTS

In an attempt to isolate the two subpopulations of LHCII suggested from previous thylakoid studies [10], we used a two-step fractionation procedure. The first step consists of isolating total LHCII from destacked thylakoid membranes with Triton X-100 followed by sucrose centrifugation [12,13]. In the second step, this 'bulk' LHCII is further solubilized by Triton X-100 and *n*-octyl-*β*-glucopyranoside and then subfractionated by preparative isoelectric focusing. The bulk LHCII preparation had a chlorophyll *a*/*b* ratio of 1.1–1.2. The main absorbance maxima in the red region of the spectrum were at 675.0 nm, predominantly due to chlorophyll *a*, and 652.0 nm, predominantly due to chlorophyll *b*. The ratio between the two main polypeptides (27 and 25 kDa) was determined to be 3.5–4.0. Apart from the dominant 27 and 25 kDa polypeptides, this preparation also contained small amounts of other polypeptides, especially a 29 kDa polypeptide band. The latter polypeptide band, which occasionally carries some chlorophyll, probably corresponds to the photosystem II chlorophyll-protein CP29 [16].

Isoelectric focusing of the isolated LHCII, after further solubilisation in Triton X-100 and *n*-octyl-*β*-glucopyranoside, gave rise to three major green bands (fig.1). These focused at pH 4.30, 4.38 and 4.45, respectively. A splitting of the band focusing at pH 4.45 into two narrow bands was occasionally seen but was always collected as one fraction. Typically fractions 1, 2 and 3 contained 30, 40 and 30% of the starting chlorophyll, respectively. Depending on the purity of the bulk LHCII preparation, a faint green band could be seen focusing at approx. pH 4.55. This band probably corresponds to the contaminating CP29 in accordance with the work of Mullet [17].

The polypeptide composition of each fraction was analyzed by SDS-PAGE (fig.2). In all three fractions mainly the 27 and 25 kDa polypeptides

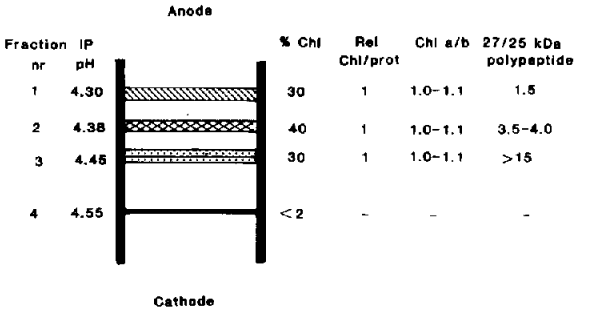

Fig.1. Polypeptide pattern and chlorophyll content of subfractions obtained by isoelectric focusing of purified spinach LHCII.

could be resolved. After quantification, it was found that the stoichiometric amounts of the two polypeptides were quite different in the three fractions. Fraction 3, focusing at pH 4.45, was heavily enriched in the 27 kDa polypeptide having a 27/25 kDa polypeptide ratio as high as 15, compared to 3.5–4.0 in the initial LHCII preparation. In contrast, fraction 1, which focused at pH 4.30, had a 27/25 kDa polypeptide ratio as low as 1.5. The middle fraction contained the two polypeptides in a ratio close to that of unfractionated LHCII (3.5–4.0). The amount of chlorophyll associated with fraction 2 decreased as the detergent/chlorophyll ratio increased. It is therefore likely that this fraction contains undissociated LHCII. Strikingly, the polypeptide compositions of fractions 1 and 3 are in good agreement with the values suggested for the peripheral and inner pools of LHCII, respectively [10].

As indicated in fig.1 the chlorophyll distribution between the three fractions was rather even. When loaded on SDS-PAGE on an equal chlorophyll basis the three fractions showed the same degree of total Coomassie binding to the 27 and 25 kDa polypeptides. This indicates that all LHCII subfractions have the same protein/chlorophyll ratio. The fractions contained chlorophyll *a* and *b* in essentially the same amount giving a chlorophyll *a/b* ratio of between 1.00 and 1.10 (fig.1). The absorption spectra of the different fractions were almost identical (not shown), with absorbance maxima at 673 .0 and 651.0 nm in the red region and 437.0 and 470.0 nm in the blue region of the spectrum.

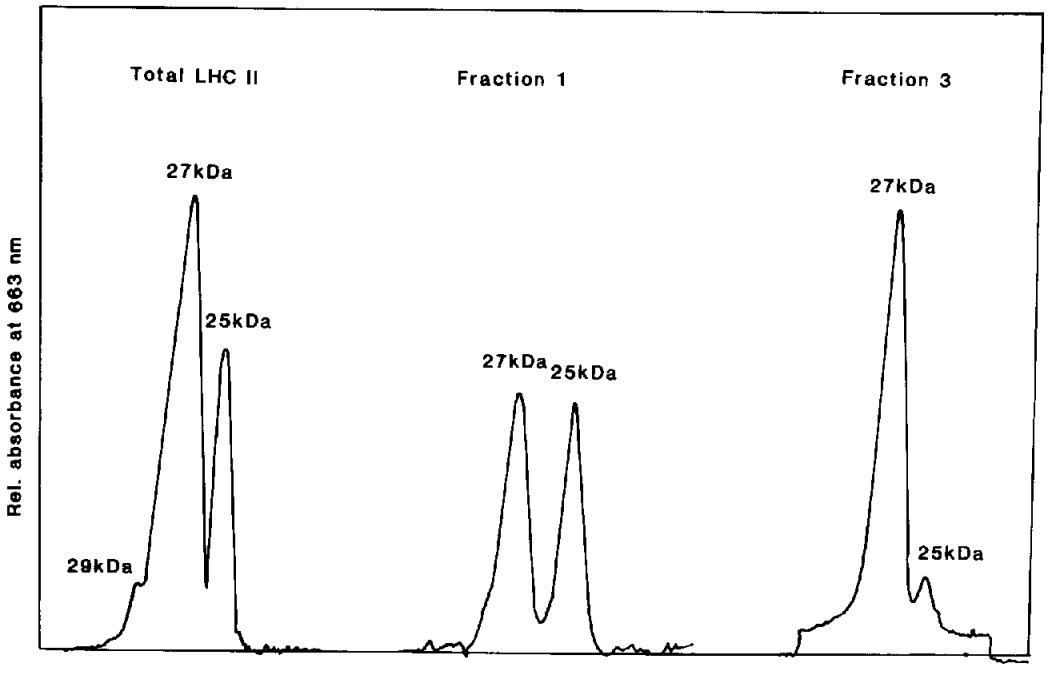

Fig.2. Laser densitometer scans of SDS-PAGE analyses of total LHCII and fractions 1 and 3 from the isoelectric focusing experiment.

4. DISCUSSION

Here, we have shown that isolated LHCII from spinach thylakoids can be further solubilized and fractionated into two distinct populations by preparative isoelectric focusing. The most basic LHCII band contains only the 27 kDa polypeptide which corresponds to the peptide composition suggested for the inner subpopulation of LHCII [10]. The peripheral LHCII has been predicted to contain a 27 and a 25 kDa polypeptide in more equal amounts [10] which agrees very well with the 27/25 kDa polypeptide ratio of 1.5 for our most acidic LHCII fraction.

The peripheral pool of LHCII is thought to play a major role in the short-term regulation of the balance of excitation energy between the two photosystems [9,10]. Moreover, recent evidence [18] has shown that this peripheral pool of LHCII is also responsible for the long-term adaptation of the photosystem II antenna to variations in the light environment. The present isolation of the two different LHCII subpopulations should therefore provide new possibilities for elucidating the molecular mechanism of photoregulation of the photosynthetic apparatus. Of particular interest is the determination of how gene expression for polypeptides of the peripheral and tightly bound subpopulations of LHCII is regulated under different light conditions.

An unsolved question has been whether both the major apopolypeptides of LHCII carry the same amount of chlorophyll and whether they both contain chlorophyll *a* and *b* [1]. As shown in fig.1, both fractions 1 and 3, despite their very different polypeptide compositions, have the same relative protein/chlorophyll content and the same chlorophyll *a/b* ratio. These results provide strong evidence that both the 27 and 25 kDa apopolypeptides of LHCII have identical chlorophyll content and composition. Compared to the initial LHCII preparation there was a small shift in the absorbance maximum from 675 .0 to 673.0 nm and 652.0 to 651.0 nm after isoelectric focusing. The reason for this could be either that the LHCII subpopulations are further purified from the contaminating chlorophyll carrying CP 29, which has an absorption maximum of 675.0 nm [16], or that the pigment-protein interactions within the LHCII are disturbed during isolation. The somewhat lower chlorophyll *a/b* ratios of the three LHCII subfractions compared to the initial LHCII preparation are probably due to removal of CP 29 which has a chlorophyll *a/b* ratio around 3.5 [16].

In a study by Mullet [17], isolated pea LHCII was fractionated into five different fractions by isoelectric focusing. A direct comparison between our isoelectric focusing system and that used by Mullet is not straightforward, since we included both more detergent and more carrier ampholytes and glycine in our system. It appears that the two major bands of Mullet correspond to our fractions 3 and 2, containing the 27 kDa polypeptide and undissociated LHCII, respectively. Any correlation to the inner and peripheral pools of LHCII could not be made from the work of Mullet [17] and the stoichiometric relationship between the polypeptides in the fractions was not reported.

Attempts to obtain clearly defined three-dimensional crystals of LHCII have so far been unsuccessful [19]. One reason for this may lie in the heterogeneous nature of the bulk LHCII. A more homogeneous preparation of LHCII, like our present subfractions, may therefore be better suited for successful crystallization studies.

ACKNOWLEDGEMENTS

This study was supported by the Swedish Natural Science Research Council (NFR). J.M.A. was a visiting Professor under the auspices of the NFR.

REFERENCES

[1] Thornber, J.P. (1986) in: Encyclopaedia of Plant Physiology (Arntzen, C.J. and Staehelin, L.P. eds) vol.19, pp.98–142.
[2] Delepelaire, P. and Chua, N.-H. (1981) J. Biol. Chem. 256, 9300–9309.
[3] Hoober, J.K., Millington, R.H. and D'Angelo, L.P. (1980) Arch. Biochem. Biophys. 202, 221–234.
[4] Dunsmuir, P. (1985) Nucleic Acids Res. 13, 2503–2518.
[5] Larsson, U.K., Jergil, B. and Andersson, B. (1983) Eur. J. Biochem. 136, 25–29.
[6] Kyle, D.J., Kuang, T.-Y., Watson, J.L. and Arntzen, C.J. (1984) Biochim. Biophys. Acta 765, 89–96.

[7] Staehelin, L.A. and Arntzen, C.J. (1983) J. Cell Biol. 97, 1327–1337.
[8] Larsson, U.K. and Andersson, B. (1985) Biochim. Biophys. Acta 809, 396–402.
[9] Jennings, R.C., Islam, K. and Zucchelli, G. (1986) Biochim. Biophys. Acta 850, 483–489.
[10] Larsson, U.K., Sundby, C. and Andersson, B. (1987) Biochim. Biophys. Acta, in press.
[11] Sundby, C. and Andersson, B. (1985) FEBS Lett. 191, 24–28.
[12] Burke, J.J., Ditto, C.L. and Arntzen, C.J. (1978) Arch. Biochem. Biophys. 187, 252–263.
[13] Ryrie, I., Anderson, J.M. and Goodchild, D.J. (1980) Eur. J. Biochem. 107, 345–354.
[14] Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
[15] Laemmli, U.K. (1970) Nature 227, 680–685.
[16] Camm, E.L. and Green, B.R. (1980) Plant Physiol. 66, 428–432.
[17] Mullet, J.E. (1983) J. Biol. Chem. 258, 9941–9948.
[18] Larsson, U.K., Anderson, J.M. and Andersson, B. (1987) Biochim. Biophys. Acta, in press.
[19] Kühlbrandt, W. (1984) Nature 307, 478–480.