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SCIENCE DIRECT®
Bioelectrochemistry 63 (2004) 103–106
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Enrichment of cardiolipin content throughout the purification procedure of photosystem II

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Received 23 June 2003; received in revised form 18 September 2003; accepted 22 September 2003

Dedicated to Prof. Mario Della Monica (January 9, 1932–September 9, 2003)

Abstract

Photosystem II is a multisubunit membrane complex which performs the water oxidation process in the higher plants.

Core dimers and monomers of photosystem II have been isolated from thylakoid membranes by sucrose density gradient centrifugation.

Lipids extracted from different photosystem II-enriched fractions obtained from spinach thylakoids have been analysed by thin layer chromatography.

Cardiolipin is enriched throughout the purification of photosystem II complexes; in particular dimers contained two times more cardiolipin than their monomeric counterparts.

Keywords: Photosystem II; Cardiolipin; Thin layer chromatography

1. Introduction

Biological membranes are not just inert physical barriers, but are metabolically active and support a wide range of key biochemical processes, including photosynthesis, respiration, transport, motility, cellular recognition and signal transduction. The physical and chemical properties of integral membrane proteins are critically determined by the lipid environment in which they are embedded. It is widely accepted, in fact, that specific protein–lipid interactions are relevant for the structural and functional integrity of many prokaryotic and eukaryotic membrane proteins [1].

A variety of phospholipids, glycolipids and other amphipatic molecules concur to the lipid composition of cell membranes. The relative amounts of the lipids can significantly vary, depending on the considered organisms and the specific growth conditions. Amongst lipids, anionic phospholipids such as phosphatidyl glycerol (PG) and cardiolipin (CL) play an important role in several cellular processes, contributing both to the physical properties of the membrane and to the interaction with proteins [2]. In particular CL has been shown to co-isolate with mithocondrial cytochrome *c* oxidase during the protein purification [3], and to play a role in optimising its functioning [4]. Recently CL together with two other lipids, a phosphatidylcholine (PC) and a glucosylgalactosyl diacylglycerol, have been found in the crystals of Bacterial Reaction Center [5], a light-driven electron transfer protein complex of the cytoplasmic membrane of photosynthetic bacteria, and its interaction with the protein has been examined by X-ray diffraction [6].

As well documented in literature, the thylakoid membrane of higher plants contains essentially monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG), PG and PC [7]; an involvement of PG molecules in Photosystem II (PSII) dimer-monomer interconversion has been previously reported [8].

The PSII complex is composed by several protein subunits, the main being the reaction center proteins (D1 and D2), the inner antenna proteins (CP47, CP43), the extrinsic protein of the oxygen evolving complex (33, 23 and 17 KDa

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doi:10.1016/j.bioelechem.2003.09.031

protein), the outer chlorophyll *a*/*b* binding antenna composed of LHCII and CP29, CP26 and CP24.

In this short communication we report the results obtained from a study on lipid composition of spinach photosynthetic membrane, starting from chloroplast and proceeding to fractions progressively enriched in PSII, by means of TLC. Here, evidence of an enrichment of CL in the purified PSII complexes is for the first time documented.

2. Materials and methods

2.1. Isolation of chloroplasts, thylakoids, membrane fractions enriched in PSII (BBYs), OG-core complexes, PSII core monomers and dimers

Chloroplasts, thylakoids, BBYs, OG-core complexes, as well as purified monomeric and dimeric PSII core complexes, were isolated from market spinach according to Hankamer et al. [9]. Briefly, chloroplasts were obtained from spinach leafs by differential centrifugation and thylakoids from chloroplasts by their rupture in a hypotonic solution. Thylakoids were solubilized with Triton X-100 (Sigma) in order to extract PSII-enriched membranes (BBYs). These last membranes were solubilized with *n*-dodecyl-β-D-glucopyranoside (Sigma) to detach the LHCII proteins from the PSII core (OG-core complexes). Sucrose gradients, supplemented with *n*-dodecyl-β-D-maltoside (Sigma), were employed to obtain oxygen evolving PSII core monomers and dimers which lack of the 23 and 17 KDa extrinsic proteins and the CP29, CP26, CP24 chorophyll binding proteins.

2.2. Extraction and purification of lipids from PSII preparations

Polar lipids and non polar pigments (chlorophylls and carotenoids) were extracted from chloroplasts, thylakoids, BBYs, OG-core complexes, PSII core monomers and dimers according to Bligh and Dyer [10].

Before the extraction, chloroplasts, thylakoids, BBYs and OG-core complexes were concentrated by centrifugation and resuspended in the appropriate buffer. PSII core monomers and dimers were concentrated using an Amicon Centricon 100 concentrator and subsequently resuspended in the buffer solution, in order to reduce the detergent amount.

According to Bligh and Dyer, the aqueous suspensions containing 1 mg of chlorophyll/ml of water were sequentially stirred with the vortex in methanol and chloroform (Aldrich), in order to obtain a monophasic system, keeping the ratio between solvents ($H_2O/CH_3OH/CHCl_3$) to 0.8:2:1. The protein fractions were removed by centrifugation.

The surnatants were mixed with additional water and chloroform, in order to obtain a byphasic system, in which the ratio between solvents ($H_2O/CH_3OH/CHCl_3$) was 1:1:0.9.

The organic phase was dried in a rotatory evaporator and the lipids and pigments obtained were redissolved in chloroform before being stored at -20 °C.

2.3. Analysis of PSII lipid components by TLC

The lipid mixtures extracted from chloroplasts, thylakoids, BBYs, OG-core complexes, PSII core monomers and dimers were resolved into their components by TLC for qualitative analysis. The selected amounts of each sample were dispensed onto Silica Gel 60 TLC plates (10 × 20 cm, Merck).

The lipid components were then resolved by using a 75% chloroform, 13% methanol, 9% acetic acid and 3% water mobile phase. The separated phospholipids were stained with molybdemun blue. PE was also detected using a solution containing acetone (18 ml), lutydine (2 ml) and ninydrine (50 mg) in order to specifically evidence the amino group of this lipid.

MGDG, DGDG and SQDG were detected spraying the TLC plates with an acidic aqueous solution (5% sulphuric acid) and incubating them at 120°C for 5 min.

Lipid standards were purchased from Sigma.

3. Results and discussion

The PSII is a multisubunit complex embedded in the thylakoid membranes of higher plants, algae and cyanobacteria. It uses light energy to catalyze a series of electron transfer reactions resulting in the splitting of water into molecular oxygen, protons and electrons. The purification procedure of this integral membrane complex involves the fragmentation of chloroplast membranes in several fractions progressively enriched in PSII (thylakoids, BBYs, OG-cores, and PSII dimer and monomer complexes), replacing the membrane lipids with the detergent molecules. Notwithstanding, several lipid molecules remain bound to these different complexes.

In order to analyse the lipid composition of the different fractions separated during the PSII purification we have performed a TLC of the lipid extract obtained from each sample.

In Fig. 1 the lipid profile of chloroplasts, thylakoids and different PSII membrane complexes is shown. Besides the pigments at the solvent front, the main lipid components of the extracts were identified by their Rf values relative to those of standard markers (not shown) and by their staining behaviour with specific reagents. Some minor lipid bands could not be identified at this stage.

Among the glycolipids, MGDG and DGDG constitute a large portion of all samples analyzed; the SQDG band, well distinct in chloroplasts and OG-cores (Fig. 1a), is masked in PSII monomer and dimer lanes by the presence of the

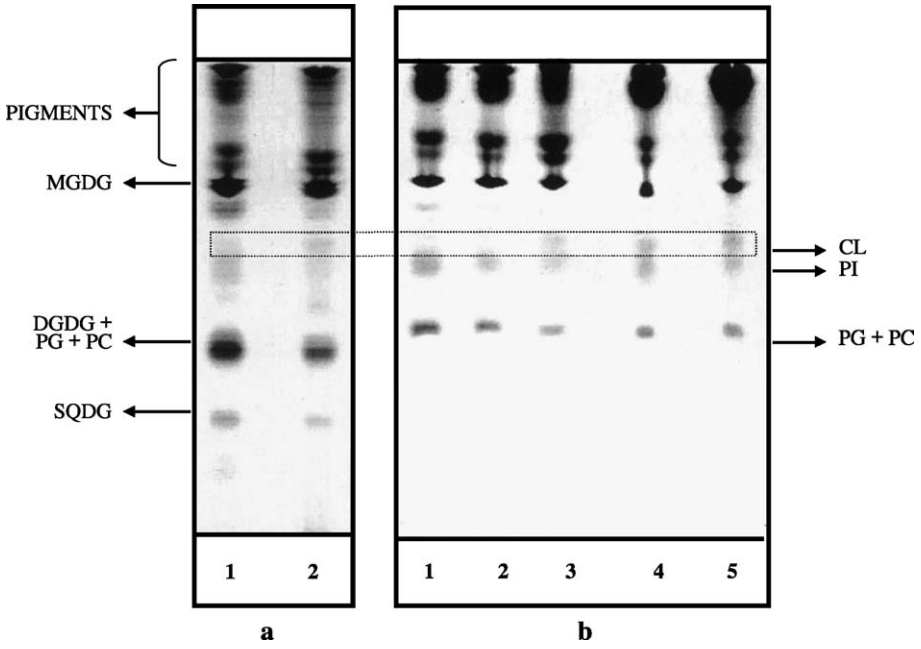

Fig. 1. TLC separation of polar lipids isolated from chloroplasts, thylakoids and different PSII membrane complexes. (a) Lane 1, chloroplasts; lane 2, OG-core complexes; 100 μg of lipids of each sample were loaded and the plate stained by 5% H_2SO_4 solution. (b) Lane 1, chloroplasts; lane 2, thylakoids; lane 3, OG-core complexes; lane 4, PSII core monomers; lane 5, PSII core dimers. Lanes 1–3: 100 μg of lipids loaded; lanes 4 and 5: 30 μg of chlorophyll of each preparation loaded. This plate was stained by molybdenum blue.

detergent *n*-dodecylmalthoside (not shown). PG almost comigrates with PC and both of them overlaps with DGDG.

Blue molybdenum staining allowed to better distinguish the phospholipids present in the different fractions. The plate illustrated in Fig. 1b evidences a clear CL enrichment in the OG-core, monomer and dimer samples respect to chloroplasts and thylakoids, indicating as this lipid is specifically bound to the reaction center of PSII complexes.

Although the band behind CL can be either assigned to phosphatydilinositol or phosphatydiletanolamine, which have almost the same Rf, the absence of a ninhydrin positive band in the samples allows its attribution to phosphatydilinositol.

The amount of CL in PSII monomer and dimer samples has been estimated by means of a TLC calibration. A value of 2.8 μg and of 4.5 μg has been obtained for monomer and dimer, respectively. Using the Chl/reaction center molar ratio of (36:1) and (40:1) for the PSII core monomers and dimers [9], it has been possible to calculate a CL/reaction center ratio of 2:1 and of 4:1 for the PSII monomer and dimer, respectively.

Although the presence of CL in the chloroplast has been previously reported [11], its presence in PSII complexes is here documented for the first time. The observed enrichment of this lipid in the monomer and dimer suggests, in addition, its possible involvement in the maintenance of the structural and functional properties of the PSII complexes.

Further qualitative and quantitative analyses of the lipids associated to these photosynthetic proteins are in progress.

Acknowledgements

This work was made possible thanks to the financial support of the ‘‘Organizzazione sopramolecolare di porfirine naturali e sintetiche’’ Cofin-MIUR 2002 Grant.

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