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Novel, rapid purification of the membrane protein photosystem I by high-performance liquid chromatography on porous materials

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Abstract

New porous materials have been tested for their potential to speed up purification of membrane proteins. As an example the purification of photosystem I, a light-driven electron pump from the cyanobacterium *Synechocystis* PCC6803, was optimized. The combination of two HPLC steps (an anion-exchange chromatography followed by a hydrophobic interaction chromatography) yields homogeneous monomeric or trimeric photosystem I as determined by gel filtration and gel electrophoresis. In comparison to traditional purification schemes our method is at least three-times faster and allows for easy scale-up.

Keywords: *Synechocystis*; Purification; Photosystem I; Proteins; Membrane proteins

1. Introduction

During the past years the field of genomics has seen tremendous progress culminating in the determination of complete genomic sequences for a number of model organisms [1]. This vast sequence information also provides a lot of new information on the protein level and started the era of proteomics. For example, the genome of the unicellular cyanobacterium *Synechocystis* PCC 6803 codes for 3186 proteins from which 1422 (45%) were new to science with unknown functions. Moreover, 1032 (32%) genes code for proteins which are thought to be membrane proteins [2]. In the case of eukaryotic cells similar estimations suggest that 30 to 40% of all cellular proteins are integral membrane proteins [3]. Given the high number of membrane proteins and their importance to sustain cellular life, rapid and efficient methods to purify them are in high demand.

Traditional methods of membrane protein purification include membrane solubilization by detergents followed by various, often time consuming methods such as sucrose density gradients and chromatography on conventional supports. In 1991 a new type of support material for chromatography was developed and introduced by Regnier [4]. Conventional materials are characterized by small pores (<1000 Å) through which the analyte must pass to reach most of the active surface area. This is a diffusion process and depends critically on the flow-rate yielding decreasing resolution with increasing flow-rates. The new materials contain large pores (>1000 Å) which makes mass transport far more important

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than diffusion. This results in a resolution characteristic that is almost independent of the flow-rate. Thus chromatographic separations can be speed up several times if porous materials are used for the separation. In this report we show that the advantage of porous materials can also be used to purify membrane proteins.

Thylakoid membranes of cyanobacteria and higher plants contain an electron transport chain which is composed of three major transmembrane–protein complexes (PS II, Cyt b_6f, PS I) interconnected by soluble electron carriers. The overall reaction catalyzed by this light-driven electron transport chain leads to the oxidation of water and the reduction of $NADP^+$. To understand the molecular details of this machinery each component has to be purified and must be available in large quantities to allow a detailed analysis by biophysical methods (i.e., X-ray diffraction and electron microscopy). In this report we will focus on the purification of photosystem I (PS I), a membrane protein complex consisting of 11 subunits in cyanobacteria. Published purification protocols for PS I usually involve a detergent solubilization step followed by a sucrose gradient centrifugation for about 16 h [5]. The purity of these preparations is not high enough for crystallization trials. Rögner and co-workers were the first to use high-performance liquid chromatography (HPLC) as final purification steps [6,7]. By a combination of anion-exchange and hydroxyapaptite chromatography (HA) they achieved a purity sufficient for crystallization. Unfortunately, this procedure takes about 5 days including all steps.

Here we present a novel, extremely fast purification protocol for PS I which can be adapted – with slight modifications – for the purification of photosystem II (PS II) and the cytochrome b_6f complex (b_6f). By the use of new porous chromatographic supports it was possible to omit the sucrose density gradient step. A combination of anion-exchange chromatography followed by a hydrophobic interaction chromatography (HIC) yields pure and homogeneous PS I. Several mg protein could be purified within two days eliminating the bottleneck of purification in crystallization experiments. The described optimization procedures should allow the fast purification of any other membrane protein.

2. Experimental

2.1. Cell culture and membrane preparation

Cells of a glucose-tolerant strain from *Synechocystis* PCC 6803 were grown in liquid BG-11 using a 25-l photobioreactor (Bioengineering, Wald, Switzerland) at 30°C and 25 μE illumination. Cells were harvested and thylakoid membranes were prepared as described in Ref. [7].

2.2. PS I purification by HPLC

Membranes (at 1 mg chlorophyll/ml) were solubilized by addition of solid dodecyl-β-D-maltoside (DM, final concentration 1%, w/v). Remaining insoluble material was removed by centrifugation (200 000 *g*, 1 h, 4°C) and the supernatant was filtered (0.22 μm filter, Diagonal, Münster, Germany) before application to HPLC.

HPLC was done either on a Waters system (two pumps, Model 510, fitted with preparative pump heads, PCM module, Rheodyne injector, Model 9125) coupled to a diode-array detection (DAD) system (DAD 996, Waters, Eschborn, Germany) or on a Biocad workstation (700E, PerSeptive Biosystems, Wiesbaden, Germany). Columns were kept at a constant temperature (10°C) in an oven compartment (Jasco, Gross-Umstadt, Germany). Columns and run conditions used during the optimization process are described in Results.

For routine preparation of monomeric and trimeric PS I the following protocol was developed: after solubilization, the supernatant (in 20 m*M* MES, pH 6.5, 10 m*M* $MgCl_2$, 10 m*M* $CaCl_2$, 10 m*M* $MgSO_4$) was loaded on a self packed anion-exchange column (86×16 mm, Poros 50 HQ, PerSeptive, Wiesbaden, Germany) using a flow-rate of 6.75 ml/min. Elution was achieved within 7 min by a linear gradient of $MgSO_4$ up to 200 m*M*. Fractions containing monomeric or trimeric PS I were pooled and concentrated by ultrafiltration (Ultrafiltration cell fitted with a $100 \cdot 10^3$ rel. mol. mass cut-off membrane, Millipore, Eschborn, Germany). For HIC, ammonium sulfate was added to the prepurified monomeric and trimeric samples to yield a final concentration of 1.5 *M* $(NH_4)_2SO_4$. The samples were applied to a self

packed hydrophobic interaction column (93×16 mm, Poros 20 Butyl, PerSeptive). Elution was done at a flow-rate of 6.75 ml/min with a linear gradient of ammonium sulfate [to 0 *M* in 20 m*M* MES (pH 6.5), 10 m*M* $MgCl_2$, 10 m*M* $CaCl_2$] and monitored at 280 nm (proteins) and at 435 nm (pigments). Pure fractions of monomeric and trimeric PS I were pooled, concentrated and stored at −70°C until further use.

2.3. Biochemical standard techniques

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis was done according to Ref. [8]. Gel images were taken with a CCD camera (GelPrint 2000i, MWG, Munich, Germany).

Buffer exchange was done on small gel permeation columns (Econo-Pac 10 DG, Bio-Rad, Munich, Germany).

Spectra were measured with a DU 7400 photometer (Beckman, Munich, Germany). Chlorophyll concentrations were determined using an extinction coefficient of 74 000 M^{-1} cm^{-1} at 679 nm.

2.4. Chemicals

DM was from Biomol (Hamburg, Germany). All other chemicals were of analytical grade and mostly purchased from Fluka (Deisenhofen, Germany).

3. Results

Photosynthetic membrane protein complexes have been purified for a great number of years. However, there was always a problem because time-consuming methods like sucrose gradient centrifugation had to be used. Even the introduction of HPLC brought no decrease in preparation time because conventional materials allowed only low flow-rates (0.5–1 ml/min) [6,9]. Therefore purification was always the time-limiting process for material consuming downstream investigations, e.g., for crystallization work. With the development of new macroporous (>1000 Å, termed porous materials in this article) materials extremely fast protein purification became feasible. We already briefly described the use of a new ion-exchange chromatography (IEC) material for the purification of PS I [10]. Here we report in detail the advantages of porous materials for the improvement and speeding up of membrane protein purification.

Preliminary studies showed that strong anion-exchange material (quaternary ammonium) could resolve a membrane extract from *Synechocystis* into peaks which could be attributed to a monomeric form of PS I, to PS II and to a trimeric form of PS I. In order to find the best material for this separation, materials from different companies were tested. Fig. 1 shows that the overall separation pattern is quite similar in all three cases. The Poros material (Fig. 1A) shows, besides the flow-through, three peaks: the first, small one represents monomeric PS I, the second peak PS II and the last peak trimeric PS I. The UNO material (Fig. 1B), a novel continuous bed support, gives the same separation pattern but with a better resolved PS II peak. The Resource material (Fig. 1C) shows besides the above mentioned peaks an additional one which was not assigned.

The Poros HQ support was chosen for further optimization and scale up (see Discussion). In order to optimize and take advantage of the theoretical properties of porous materials (e.g., resolution independent from flow-rate), separation was done at different flow-rates and the elution profiles were compared (Fig. 2). An increase of the flow-rate by a factor of 6 showed almost no changes in peak resolution. The only difference is that a small peak of PS II (between the peaks containing monomeric and trimeric PS I) can be seen at a flow-rate of 2 ml/min (Fig. 2A). This peak appears only as a shoulder at 6 ml/min (Fig. 2B) and is barely visible at 12 ml/min (Fig. 2C). In summary, the elution profile of membrane proteins is almost independent of the flow-rates and hydrophobic proteins can be separated at high flow-rates with excellent efficiency.

Another parameter which we have optimized was pH. For this, the pH map feature of the Biocad 700E workstation was used. Fig. 3 gives representative elution profiles for three different pH values: whereas at pH 6.5 (Fig. 3A) monomeric and trimeric PS I are clearly separated from each other, both peaks are closer together at pH 7.5 (Fig. 3B) and elute as overlapping peaks at pH 8.5 (Fig. 3C). At more basic pH a new peak appears which can be attributed to

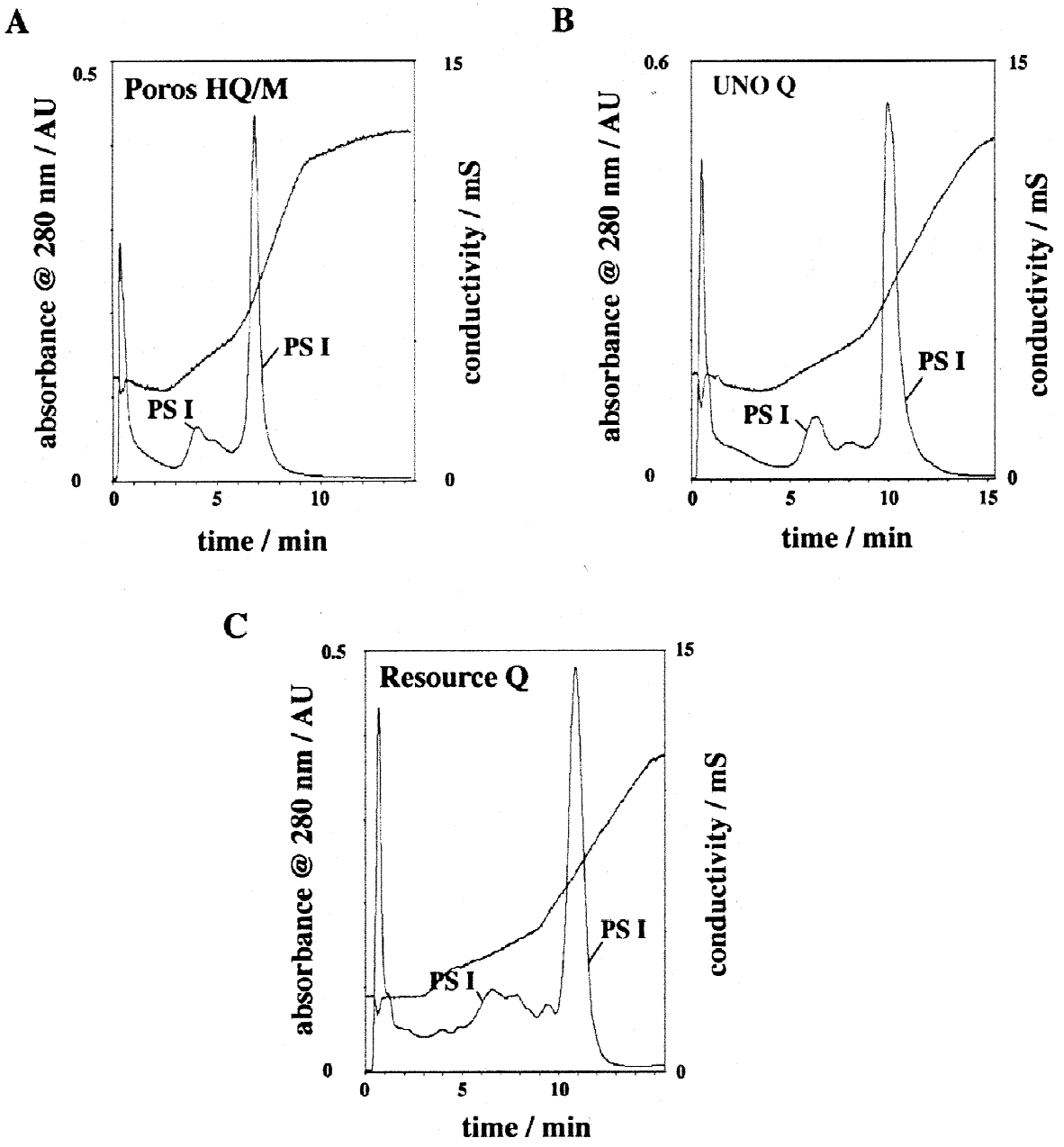

Fig. 1. Comparison of different IEC columns. Elution profiles of a thylakoid membrane extract from *Synechocystis* cells (see Experimental for details) on different IEC materials: (A) Poros HQ/M column (column dimensions: 100×4.6 mm, particle size: 20 μm, pore size: 50–100 nm, functional group: quaternary amine), (B) UNO Q column (column dimensions: 39×7 mm, particle size: continuous bed, pore size: channels, functional group: quaternary amine) and (C) Resource Q column (column dimensions: 30×16 mm, particle size: 15 μm, pore size: 20–2000 nm, functional group: quaternary amine). Each column was run at a flow-rate of 5 ml/min and elution was achieved by a linear gradient of $MgSO_4$ (200 m*M* final concentration) over 20 column volumes (CVs). Detection was at 280 nm with simultaneous measurement of the conductivity (upper trace).

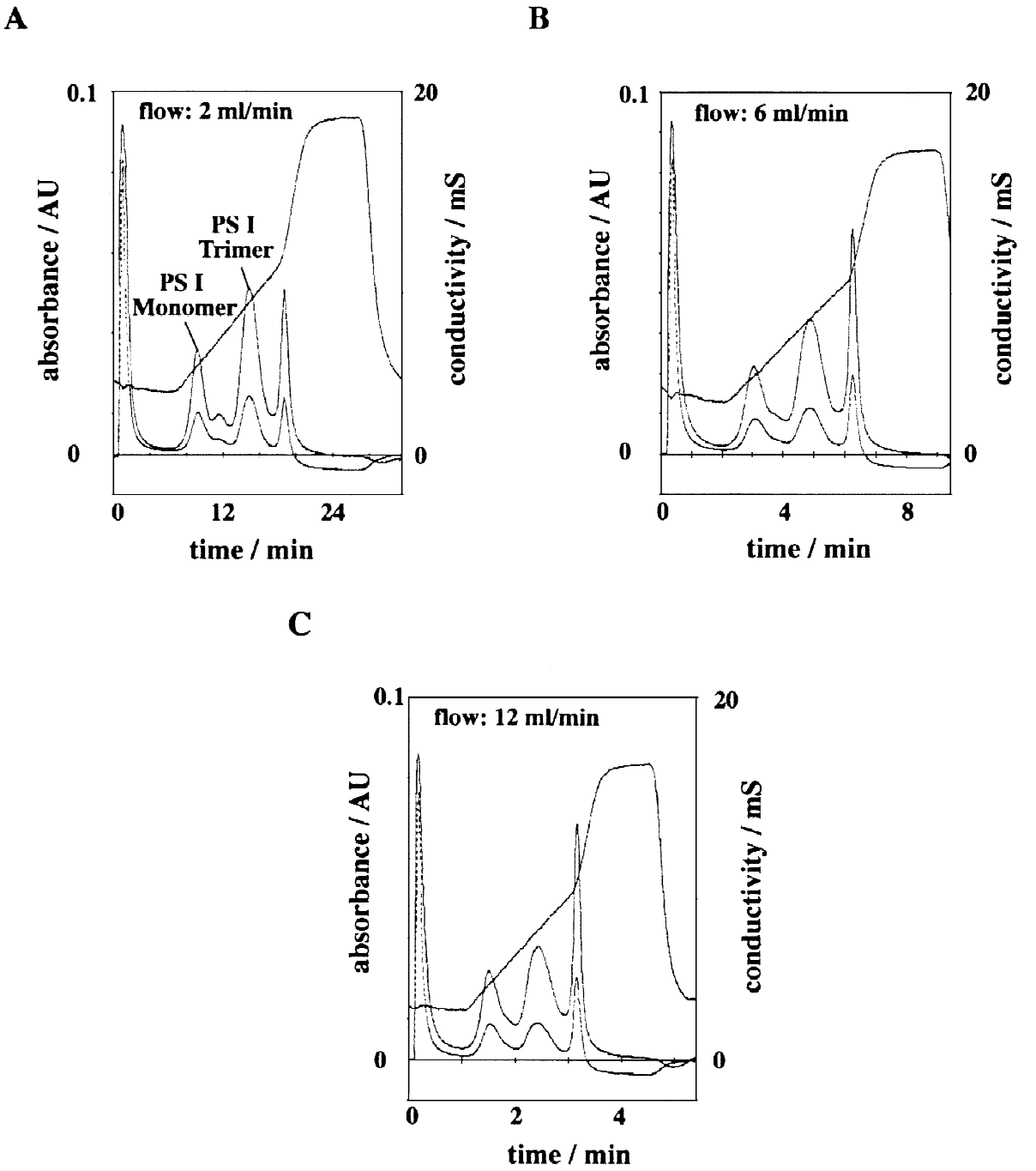

Fig. 2. Effect of increased flow-rates on resolution. Elution profiles of a thylakoid membrane extract on a Poros 50 HQ column (dimensions: 100×4.6 mm, particle size: 50 μm, pore size: 50–100 nm, functional group: quaternary amine) at different flow-rates: The flow-rate was increased from 2 ml/min (A) to 6 ml/min (B) and finally to 12 ml/min (C). Elution was done with a linear $MgSO_4$ gradient (up to 200 m*M* within 18 column volumes) and monitored simultaneously at 280 nm (proteins, lower trace) and 430 nm (pigments, middle trace). The upper trace shows the conductivity.

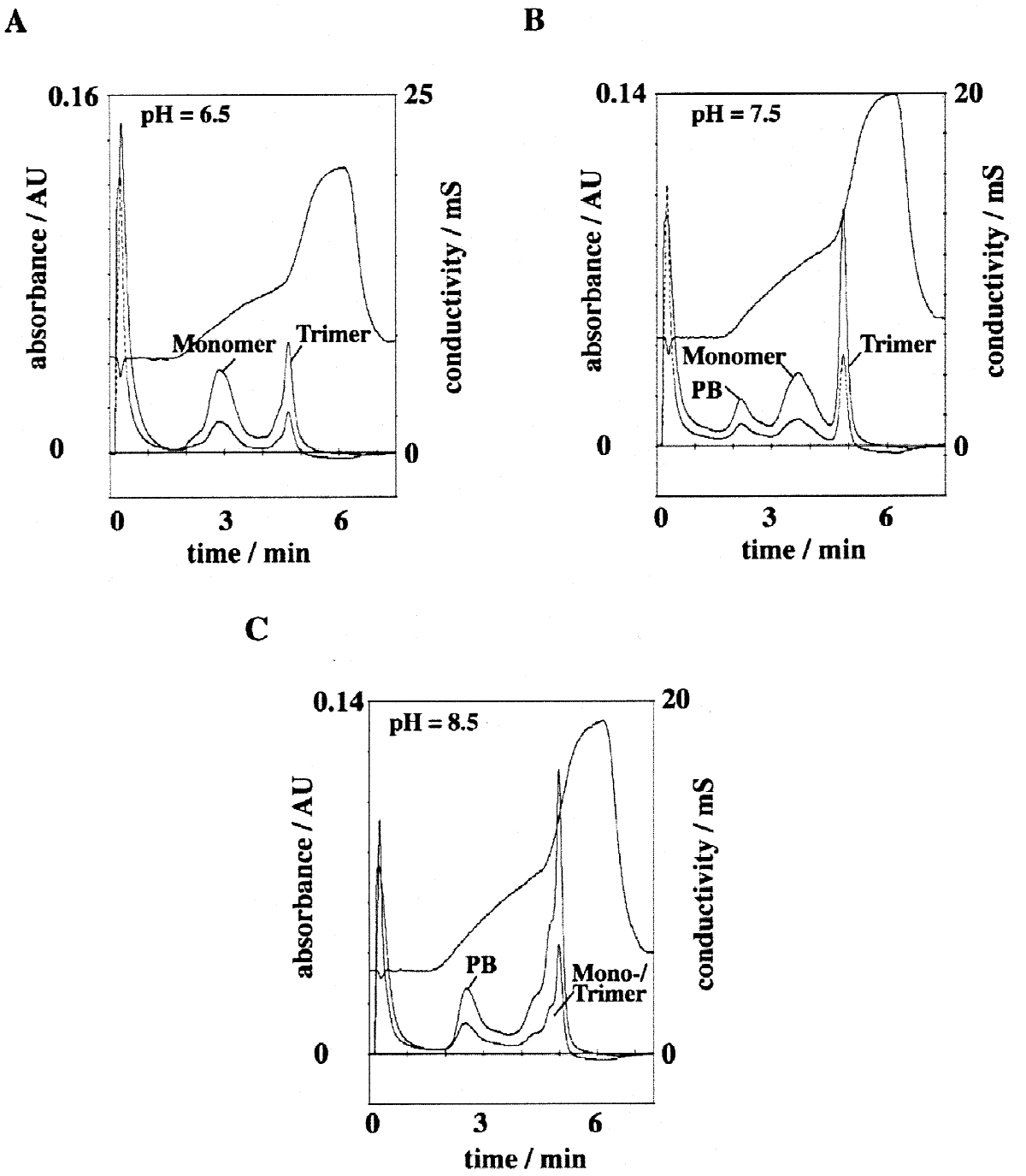

Fig. 3. pH optimization of the ion-exchange chromatography step. Elution profiles of a thylakoid membrane extract on a Poros 50 HQ column (dimensions: 100×4.6 mm, particle size: 50 μm, pore size: 50–100 nm, functional group: quaternary amine) at different pH: the pH was increased from (A) pH 6.5 to (B) pH 7.5 and finally to (C) pH 8.5 using a Tris–Bis-Tris–propane (20 m*M*) buffer system. In each case a flow-rate of 8 ml/min was used. Elution was by a linear $MgSO_4$ gradient (up to 200 m*M* within 18 column volumes) and was monitored at 280 nm (proteins, lower trace) and 430 nm (pigments, middle trace). The upper trace shows the conductivity.

phycobilin proteins (PBs) according to their unique absorption spectrum (data not shown, at pH 6.5 PBs are found in the flow-through fraction). Therefore a pH of 6.5 gives the best purification results.

After these optimization steps the purification was scaled up: instead of the analytical Poros 50 HQ column (1.67 ml bed volume) a self packed column filled with Poros 50 HQ support (17.3 ml bed volume) was used. Fig. 4 shows the elution profile after scaling up by a factor of 10. It resembles the profile of the analytical column (Fig. 2), but the peaks are not as well resolved due to high sample load. The flow-through fractions show a spectrum typical for carotenoids and free chlorophyll (data not shown). Monomeric PS I elutes next, followed by trimeric PS I and a smaller peak which must, according to gel filtration (data not shown), be attributed to aggregated PS I and PS II. A total chlorophyll amount of 1 mg, which corresponds to about 3 mg of total protein, was applied. This amount was also the maximal possible load at which sufficient resolution between monomeric and trimeric PS I could be achieved with this set-up.

After IEC the monomeric and the trimeric PS I fractions still contain contaminants (see below) which make an additional purification step necessary. HIC was not applied to the purification of photosynthetic membrane proteins before. In order to examine possible advantages of HIC, materials differing in the degree of hydrophobicity were studied (Fig. 5): An extract of membrane proteins from *Synechocystis* was applied to a Poros ET column (functional group: ethyl ether, weak hydrophobicity), a Poros PE column (functional group: phenyl ether, strong hydrophobicity) and to a Poros HP2 column (functional group: phenyl ether in high density, very strong hydrophobicity). Only the ethyl ether material proved to be useful for separation: monomeric and trimeric PS I are separated from each other and from other membrane protein complexes including phycobilin proteins and pigments (Fig. 5A). In the case of both phenyl ether supports either only phycobilin proteins (Fig. 5B) or nothing (Fig. 5C) was eluted by decreasing the ammonium sulfate concentrations. PS I was irreversibly bound to these support materials. The porous HIC materials from Pharmacia (Resource ISO, ETH, PHE) bound PS I either too strongly (PHE) or gave only low resolution (ETH, ISO) and were not employed further (data not shown). The effect of different pH for the separation was also tested, but the separation was only slightly affected by shifting pH from 6.5 to 8.0 (data not shown).

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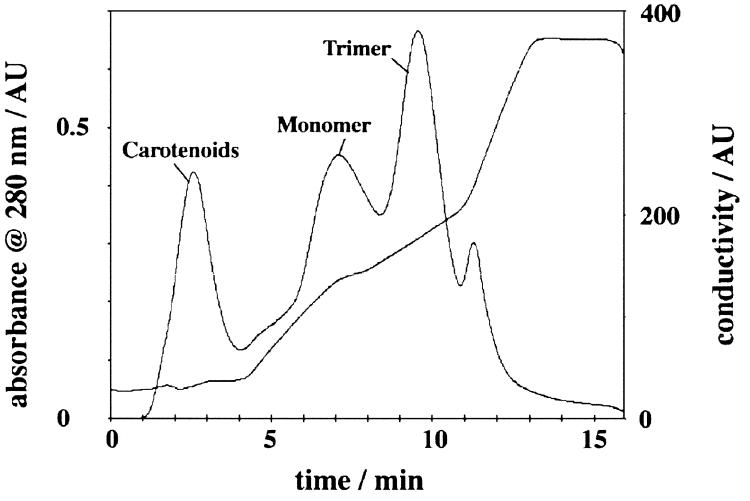

Fig. 4. Preparative separation of *Synechocystis* membrane proteins by IEC. Elution profile of a membrane extract (1 mg chlorophyll) on an IEC perfusion chromatography column (Poros 50 HQ, column dimensions: 86×16 mm, particle size: 50 μm, pore size: 50–100 nm, functional group: quaternary amine). Elution was done with a linear $MgSO_4$ gradient (10–200 m*M*, within 9 min) at a flow-rate of 6.75 ml/min. Absorbance was recorded at 280 nm (lower trace) and conductivity was measured as well (upper trace).

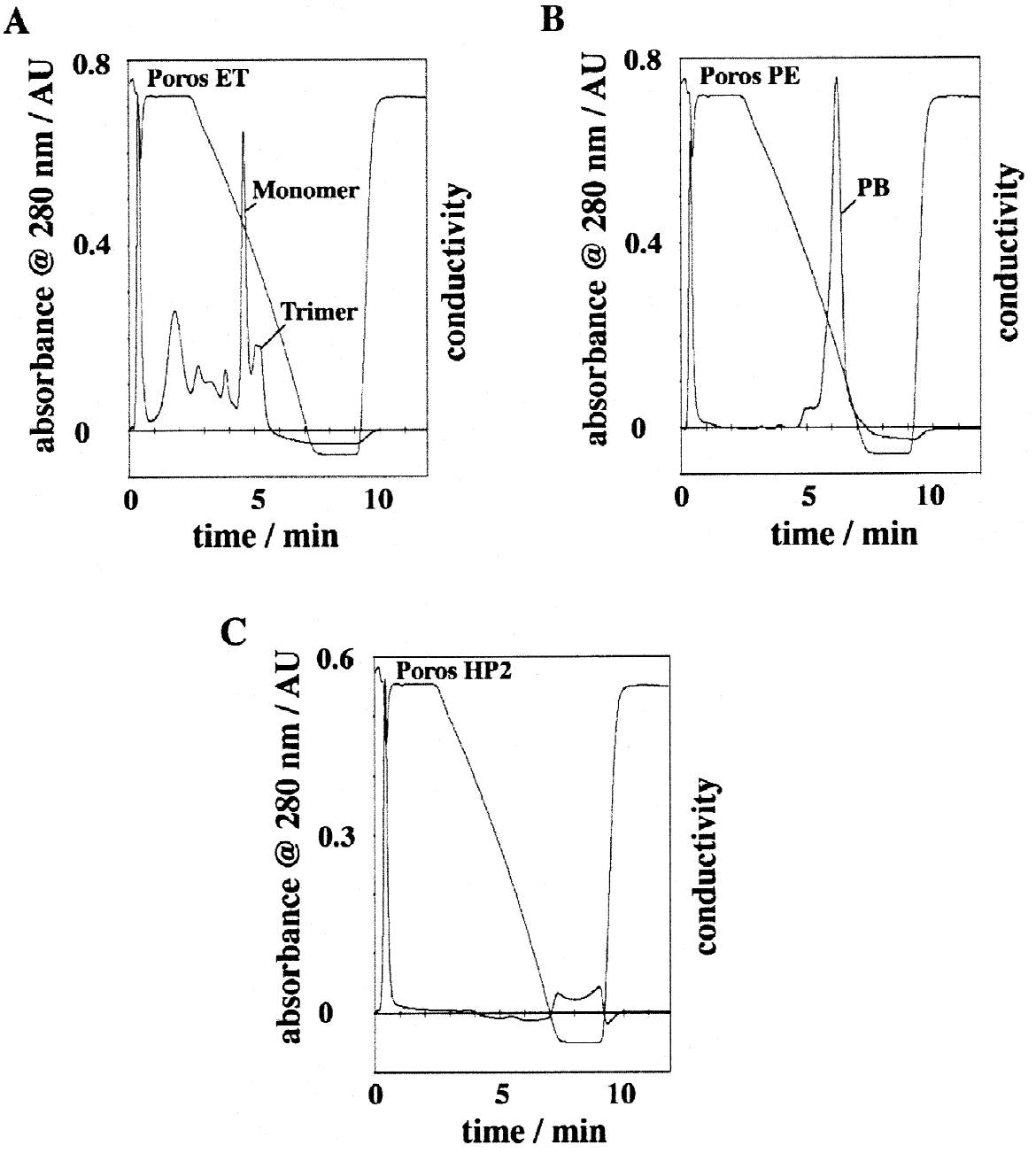

Fig. 5. Comparison of different HIC columns. Elution profiles of a thylakoid membrane extract from *Synechocystis* cells (see Experimental for details) on different HIC materials: (A) Poros ET (column dimensions: 100×4.6 mm, particle size: 20 μm, pore size: 50–100 nm, functional group: ether, PerSeptive, Wiesbaden, Germany), (B) Poros PE (column dimensions: 100×4.6 mm, particle size: 20 μm, pore size: 50–100 nm, functional group: phenyl ether, PerSeptive) and (C) Poros HP2 (column dimensions: 100×4.6 mm, particle size: 20 μm, pore size: 50–100 nm, functional group: phenyl ether but at a higher density, PerSeptive). All columns were used with a flow-rate of 4.5 ml/min and elution was by a linear gradient of $(NH_4)_2SO_4$ (1.5 *M* to 0 *M*) within 5 min. The elution profile was recorded at 280 nm (lower trace) and at 430 nm (middle trace). The gradient itself was also monitored by measuring the conductivity (upper trace).

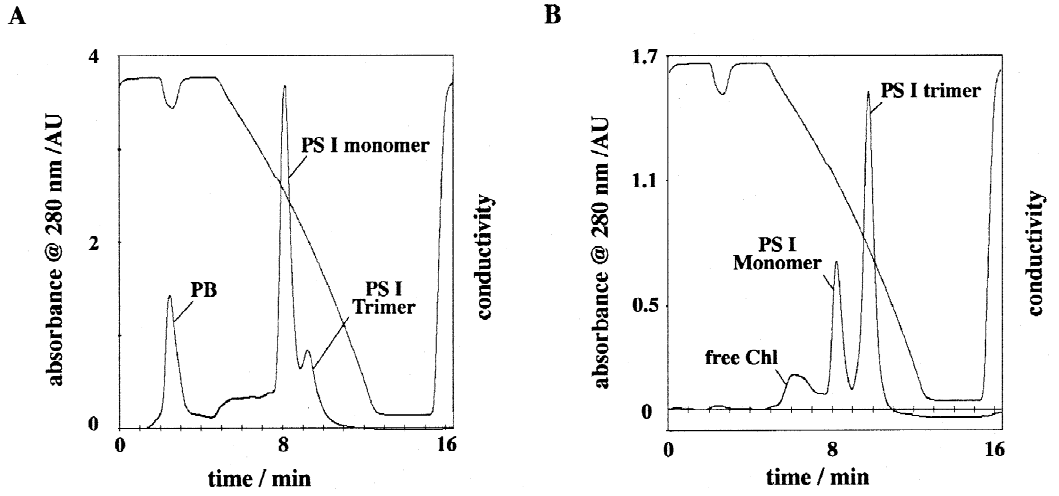

Fig. 6. Final, preparative purification of *Synechocystis* PS I by HIC. After IEC, either monomeric or trimeric PS I containing fractions were pooled, concentrated and adjusted to 1.5 *M* $(NH_4)_2SO_4$ (see Experimental). These samples (0.8 mg chlorophyll) were loaded on a preparative HIC perfusion chromatography column (Poros Butyl, column dimensions: 86×16 mm, particle size: 20 μm, pore size: 50–100 nm, functional group: butyl, PerSeptive, Wiesbaden, Germany). (A) The elution profile of monomeric PS I after IEC, (B) the elution profile of trimeric PS I after IEC. In both cases elution was achieved by a linear $(NH_4)_2SO_4$ gradient (1.5–0 *M*, within 8 min) at a flow-rate of 6.75 ml/min. Absorbance was recorded at 280 nm (lower trace) together with the conductivity (upper trace).

shows identical separation characteristics as the ethyl ether material) was chosen as second and final purification step (Fig. 6). Monomeric and trimeric fractions from the IEC column (Fig. 4) were concentrated, adjusted to 1.5 *M* ammonium sulfate and applied to a self-packed Poros Butyl column (bed volume 18.7 ml). Chromatographic conditions for the analytical scale were only corrected for the larger column volume and used immediately for the scale-up by a factor of 10. The pre-purified monomeric PS I sample elutes from HIC with a large peak of monomeric PS I besides some trimeric PS I and some phycobilin proteins both of which could be separated from the monomer (Fig. 6A). A pre-purified trimeric PS I sample shows, besides the main peak containing trimeric PS I, peaks resulting from monomeric PS I and from free chlorophylls all showing almost baseline separation (Fig. 4B). Monomeric and trimeric peak fractions were pooled, concentrated and stored at −70°C. Both oligomeric forms were stable in detergent solution (0.03%, w/v, DM) after purification (data not shown).

To check the purification, samples were taken at all steps and analyzed by SDS–PAGE on Schaegger–Jagow gels (Fig. 7). The Coomassie stained gel shows the efficiency of the separation strategy. While the membrane shows many proteins (Fig. 7, lanes 1 and 2), the extract is already enriched in PS I (Fig. 7, lanes 3 and 4). After IEC, both the pooled monomeric PS I and the pooled trimeric PS I still contain other proteins as contaminants (Fig. 7, lanes 5 and 6). Therefore a second purification step is absolutely necessary. After the final purification by HIC, both monomeric and trimeric PS I show all known PS I subunits while no other contaminants can be seen (Fig. 7, lanes 7 and 8). In summary, extremely pure and homogeneous PS I either in a monomeric or in a trimeric form can be prepared by our purification scheme. Within two days, 10 to 20 mg of pure PS I were prepared which provides enough material for material consuming experiments such as crystallization trials.

In order to demonstrate the general versatility of our purification protocol we decided to apply our method to the purification of PS I from the filamentous cyanobacterium *Spirulina platensis*. This bacterium lives in alkaline lakes at a pH of 10. Fig. 8 shows the first step in purification, a chromatography on Poros 50 HQ. Although we could use the same strategy, we had to replace $MgSO_4$ as eluent with

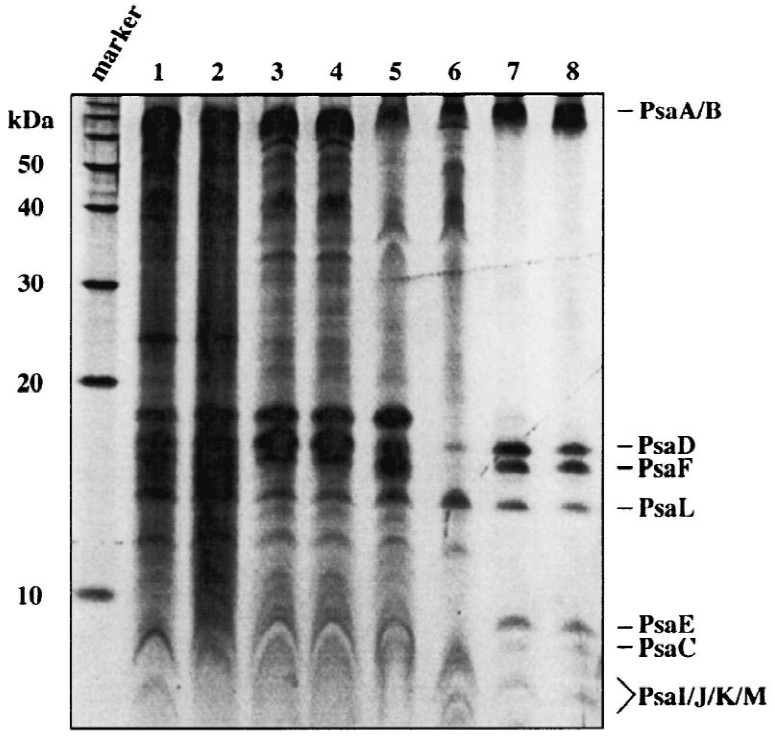

Fig. 7. SDS–PAGE analysis of the purification process. Samples taken at different points during the purification were applied to a SDS–PAGE system. The Coomassie Blue stained gel was digitized by a CCD camera. Applied samples were as follows: lanes 1 and 2, thylakoid membranes which were the starting material for PS I purification. Lanes 3 and 4, solubilized membrane proteins before IEC. Lane 5 shows the monomeric and lane 6 the trimeric PS I fraction after IEC. Monomeric PS I (lane 7) and trimeric PS I (lane 8) after final purification using HIC.

NaCl, in order to separate monomeric from trimeric PS I. After a second chromatography on Poros Butyl, which was done essentially as for *Synechocystis* proteins, we could obtain highly purified and homogeneous monomeric and trimeric PS I (data not shown). This material could further be used in reconstitution assays to investigate the trimerization process in vitro [11].

4. Discussion

Purification of hydrophobic membrane proteins is still a challenge for the biochemist. In order to solubilize them and to keep them in a soluble state, detergents must be present in every purification step. To preserve function and/or oligomeric complexes, additives such as sugars have been used which often lead to high viscosity and high column backpressures. This in turn makes preparations time consuming and presents often the bottleneck for a detailed biophysical characterization of the membrane protein of interest.

The aim of this report was to investigate the potential of porous support materials in the course of membrane protein purification. Porous materials allow extremely high flow-rates which enables fast and thorough optimization of purification protocols and efficient as well as fast purification on a preparative scale. These advantages of porous materials, which were already demonstrated for the purification of soluble proteins [12], hold also in the presence of detergents, e.g., during purification of highly hydrophobic proteins, as shown in this report.

The three major protein complexes involved in photosynthetic energy conversion (PS I, PS II, Cyt b_6f) are localized in thylakoid membranes. Thylakoid membrane preparations are the starting material

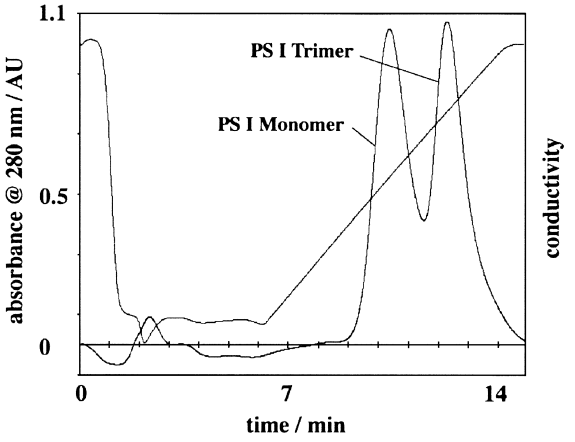

Fig. 8. PS I purification from *Spirulina platensis*. Elution profile of a membrane extract (1 mg chlorophyll) from *Spirulina platensis* on an IEC perfusion chromatography column (Poros 50 HQ, column dimensions: 86×16 mm, particle size: 50 μm, pore size: 50–100 nm, functional group: quaternary amine). Elution was done with a linear NaCl gradient (10–300 m*M*, within 9 min) using a flow-rate of 6.75 ml/min. Absorbance was recorded at 280 nm (lower trace). The conductivity is shown on the upper trace.

for further purification of these complexes. In recent years the focus has been on deciphering the molecular structure of these complexes. However, only time-consuming preparation methods for these complexes were available. Therefore we redesigned a purification method published originally by Rögner et al. [7] to allow rapid purification of photosynthetic membrane protein complexes in high purity and high yield. Instead of a sucrose density gradient followed by two HPLC steps (IEC and hydroxyapatite) we used IEC followed by HIC both based on new porous materials.

All porous anion-exchange materials tested could be applied successfully. We have chosen the Poros 50 HQ material for further use because this material is available in bulk quantities and can be used with medium pressure systems (particle size 50 μm). The same is true for the Source Q material (e.g., the Resource Q column) which could be used alternatively without any disadvantages. The Uno Q column features a continuous bed and therefore it is not possible to pour your own columns. In addition, though the UNO Q column is excellent in terms of resolution, the bed is very sensitive and more difficult to clean. On the other hand the Poros material is chemically stable against organic solvents, which is important for the removal of lipids binding readily to the column. The sucrose gradient separates lipids which were solubilized by the detergent treatment from photosynthetic membrane protein complexes. This step became redundant when using Poros material. If after each use a thorough cleaning with 100% methanol was done, then the column could be reused at least 100 times without loss of resolution. In addition it was found that sucrose as additive is not necessary to preserve function or oligomeric nature. The high flow-rates (>5 ml/min) possible with porous materials (in comparison with a maximum 1.5 ml/min for conventional supports) makes very fast and economic method development possible. Within one day as diverse parameters as pH, eluent and gradient can be optimized, which would not be feasible with conventional materials and their very long separation times.

After IEC, both the monomeric and the trimeric PS I still contained other proteins so that a second purification step was necessary to achieve sufficient purity for crystallization. In the old protocol [7] a chromatography step based on HA was used. This column had a maximum flow of 1 ml/min leading to extremely long separation times (in the range of hours). Another problem associated with HA is the high probability of calcium phosphate crystal formation (if using Ca^{2+} containing buffers) on the column which frequently leads to high-pressure shutdowns. To avoid these problems we decided to apply HIC as second column and we tested various HIC materials. Only the very weak ethyl or butyl ether Poros supports were able to separate monomeric and trimeric PS I from each other and from other proteins. All other hydrophobic supports tested were too hydrophobic so that the photosynthetic complexes bound irreversibly. HIC has, to our knowledge, not been used before in the purification of photosynthetic membrane proteins and it is somehow surprising that HIC can be used at all for very hydrophobic membrane proteins. The combination with IEC as a first column proved to be ideal as the samples after elution from IEC in high salt buffer could be used directly (after addition of ammonium sulfate) for HIC. A time-consuming buffer exchange is not necessary. In contrast to the HA column, HIC columns could be used at much higher flow-rates

(>5 ml/min). Recently, porous HA materials became available which remain to be tested but the problem of calcium phosphate precipitation on the column will remain even with porous materials.

The traditional method allowed for the purification of several mg pure PS I within one week whereas by the new procedure the same amount could be purified within two days. Both, the purity (subunit composition) and photochemical activity are identical with PS I complexes purified according to the old protocol.

The use of porous materials in purification of photosynthetic membrane proteins have also been reported by Tjus et al. [13]. They used an IEC column (Poros Q) to isolate subcomplexes of PS I and LHC I but the first step of their purification was still a sucrose gradient centrifugation. Also they did not exploit the full potential of porous materials: at a flow-rate of 1 ml/min, their separations still took nearly one h. We have shown in this report that the flow-rate can be increased without loss in resolution when purifying membrane proteins as it is the case with soluble proteins [12].

We have shown the versatility of our method which was developed for *Synechocystis* by applying it to PS I purification of *Spirulina*, an organism which lives in a completely different habitat. Such a universal method should be very useful for crystallization projects: Often a protein from only one organism out of many gives crystals of high quality. If the purification method is very fast, a larger number of organisms can be screened to find the best organisms for crystallization work. Similarly, mutant proteins can be purified fast and tested for improved crystallization properties.

Work in our institute has shown that by this combination of IEC and HIC on porous materials the purification of the Cyt b_6f complex [14] and the PS II complex [15] is also possible.

In summary, our results demonstrate the advantages of porous support materials for the purification of membrane proteins. With the field of proteomics becoming more important, extremely fast and efficient purification methods are needed to allow for high-throughput purification of several hundred proteins, both soluble and hydrophobic. This should be possible with the new porous materials and further automation and miniaturization in chromatographic hardware.

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