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Improved purification of the membrane-bound hydrogenase–sulfur-reductase complex from thermophilic archaea using ε-aminocaproic acid-containing chromatography buffers

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Abstract

A hydrogenase–sulfur reductase (SR) complex was purified from membrane preparations of the extremely thermophilic, acidophilic archaeon *Acidianus ambivalens* using a combination of sucrose density gradient centrifugation and column chromatography (FPLC). All chromatographic steps were performed in the presence of 0.5% ε-aminocaproic acid resulting in the elution of the SR complex as a sharp peak. In contrast, chromatography using buffers without ε-aminocaproic acid, or in the presence of detergents, were not successful. The purified *A. ambivalens* SR complex consisted of at least four subunits with relative molecular masses of 110 000, 66 000, 39 000 and 29 000, respectively. A similar procedure was applied to purify the membrane-bound hydrogenase from *Thermoproteus neutrophilus,* a non-related extremely thermophilic but neutrophilic archaeon, which consisted of only two subunits with relative molecular masses of 66 000 and 39 000, respectively.

Keywords: Purification; Enzymes; Hydrogenase–sulfur reductase complex; ε-Aminocaproic acid

1. Introduction

The extremely thermophilic, acidophilic and chemolithoautotrophic archaeon *Acidianus ambivalens* grows optimally at 80°C and pH 2.5 either aerobically by oxidation of sulfur to sulfuric acid, or anaerobically by reduction of sulfur to hydrogen sulfide [1,2]. In contrast, the related archaeon *Thermoproteus neutrophilus* grows at 85°C and pH 6.8 only in the absence of oxygen [3]. Both organisms gain energy by sulfur respiration with hydrogen as electron donor [4–6]. Two membrane-bound enzymes are involved in this pathway: a hydrogenase which oxidizes hydrogen and transfers the electrons to a primary acceptor, and a sulfur reductase (SR) which reduces elemental sulfur to H_2S [5,7]. The electron acceptors of the hydrogenases and the electron donors of the SR are not known. However, there is evidence that quinones and/or cytochromes are involved (Laska and Kletzin, unpublished, [7]).

One of the crucial points in membrane protein purification is the choice of the optimal detergent for solubilization of the proteins from their lipid environment and for keeping them in solution during all purification steps. Archaea differ from other organisms by their unusual lipid composition. Archaeal membranes consist mainly of phytanyl ether lipids in contrast to bacteria and eukaryotes whose membranes consist mainly of phosphoester lipids (reviewed in Refs. [8,9]). In addition, the membranes

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of most of the extremely thermophilic archaea consist of a monolayer of membrane-spanning C_{40} tetraether lipids [10,11]. This feature is thought to be a special form of adaptation in order to proliferate in high-temperature environments [12]. Therefore, new methods have to be developed for the solubilization and purification of membrane proteins from these organisms.

In this contribution, we describe the purification of the membrane-bound SR complex from *A. ambivalens* and of the hydrogenase from *T. neutrophilus*. We used ε-aminocaproic acid (6-aminohexanoic acid) in buffers to improve the performance of column chromatography. ε-Aminocaproic acid is a lysine analog used for hemostasis enhancement in the treatment of blood coagulation disorders. The substance acts as an indirect inhibitor of fibrinolysis by inhibition of plasminogen activating proteases [13]. ε-Aminocaproic acid has been used biochemically as inhibitor of serine proteases during protein purification [14]. The agent is not a detergent itself. However, it has been used to improve the separation of membrane proteins during gel electrophoresis under non-denaturing conditions [15]. The application of ε-aminocaproic acid for purification of hydrophobic membrane proteins in column chromatography has not been described so far. We show in this contribution that the use of ε-aminocaproic acid improves the resolution of chromatographic procedures.

2. Experimental

2.1. Organism and growth conditions

Acidianus ambivalens (DSM 3772) and *Thermoproteus neutrophilus* (DSM 2338) were grown anaerobically without addition of organic substrates according to published procedures [2–4,16].

2.2. Chemicals

NADP, NADPH and DNase I were from Roche Diagnostics (Mannheim, Germany). ε-Aminocaproic acid (6-aminohexanoic acid), colloidal Coomassie blue (Roti-Blue), 3-[(cholamido-propyl)-dimethylamino]-1-propanesulfonate (CHAPS), and Tris were purchased from Roth (Karlsruhe, Germany). *N,N*-Dimethyl-*p*-phenylendiamine, methyl viologen and the molecular mass standard for sodium dodecyl sulfate (SDS) gels (SDS-7) were from Sigma (Munich, Germany). The molecular mass standard (HMW calibration kit) for gel permeation chromatography was obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Triton X-100 and Coomassie Brilliant Blue G (Serva Blue) were from Serva (Heidelberg, Germany). β-Octyl-D-glucopyranoside was purchased from Riedel de Haen (Seelze, Germany). 2,3-Dimethyl-1,4-naphthoquione (DMN) was a kind gift from A. Kröger, Frankfurt, Germany. Powdered sulfur was obtained from Merck (Darmstadt, Germany; No. 7987; average diameter 20 μm) as were all other chemicals (reagent grade).

2.3. Apparatus

All chromatographic protein purification steps were performed using a fast protein liquid chromatography (FPLC) system LCC 501-Plus equipped with a dual-wavelength UV monitor WM 2141 (Amersham Pharmacia Biotech). The Superose-6 HR 10/30 and Mono P HR 5/20 chromatography columns and the DEAE Sepharose Fast Flow material were from Pharmacia. The DEAE fast flow material and the cellulose were packed into a 10 cm×10 mm column from Pharmacia (7.8 ml). The cellulose packing material was from Roth. Nanosep 30 microconcentrators were used for ultrafiltration of small volumes (≤500 μl) and Jumbosep 30 centrifugal concentrators were used for volumes of 10–65 ml (Pall Gelman, Northborough, UK).

2.4. Enzyme purification

All steps were performed at room temperature under anaerobic conditions in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI, USA) under an N_2/H_2 atmosphere.

The cell pellet (8 g) was suspended in 10 ml of double deionized (dd) water. The pH was adjusted to pH 7.5 with 1 *M* potassium phosphate buffer (KP_i), pH 8. For lysis, the cells were frozen three times in liquid nitrogen and thawed at 37°C in a water bath. After DNase treatment for 30 min at 37°C (50 μg/ml), the crude extract was centrifuged for 20 min

at 3000 *g* for removal of sulfur particles and intact cells. The supernatant was centrifuged at 120 000 *g* at 4°C in an ultracentrifuge for 1 h (Rotor SW 41, Beckman Instruments, Palo Alto, CA, USA) for separation of membranes and the cytoplasmic fraction. The pellet (membrane fraction) was washed once with 100 m*M* KP_i buffer, pH 8.0 and centrifuged as described above. For solubilization, the pellet was resuspended in the same buffer containing containing 1% (w/v) sodium deoxycholate. The resuspended membrane fraction was transferred anaerobically to a stoppered 50-ml serum bottle (Zinsser Analytic, Frankfurt, Germany). The bottle was placed in a sonication bath and was sonified for 30 min at room temperature (Model RK 103 H; Bandelin Electronic, Berlin, Germany). The solubilized proteins were separated from insoluble particles by ultracentrifugation as described above (''membrane solubilizate''). The fractions of the different protein purification steps were stored anaerobically in serum bottles under a nitrogen atmosphere at −20°C.

The membrane solubilizate was loaded on a linear 20–35% (w/v) sucrose density gradient in Tris–acetate buffer, pH 7.7 containing 0.1% (w/v) sodium deoxycholate (3 ml of solubilizate/42 ml tube) and centrifuged for 70 h at 140 000 *g* at 15°C using an SW 28 rotor. Fractions (3 ml) of the gradient were collected and analyzed for enzymatic activities. The fractions showing highest hydrogenase and sulfur reductase activities were pooled and concentrated using Jumbosep ultrafiltration concentrators. During this procedure, a buffer exchange was performed to the loading buffer of the following chromatography step.

The concentrated protein solution was further purified by gel permeation or alternatively by anion-exchange chromatography. The chromatography buffers (Table 1) were degassed before each run. The FPLC runs were performed outside of the anaerobic chamber. However, all fractions were collected anaerobically under constant gasing with CO_2. The gel permeation chromatography using a Superose-6 column was performed at a constant flow-rate of 0.5 ml min^{-1}. The concentrated active fractions were applied onto the column using a 0.5-ml sample loop. The flow-rate for both anion-exchange chromatography columns (Mono P and DEAE) and the cellulose was 1 ml min^{-1}. The columns were equilibrated with loading buffer. In each case, the protein samples were applied using a 2-ml sample loop. A gradient from 0–25% elution buffer (Table 1) was used to elute the *A. ambivalens* SR complex from the DEAE Sepharose column. For the elution of the *T. neutrophilus* SR complex from the Mono P column, a gradient from 0–100% elution buffer was applied (Table 1). The proteins were applied to the cellulose column in 20 m*M* Tris–HAc buffer, pH 7.7. They were eluted with 0.1 *M* glucose or 0.5% (w/v) Triton X-100 in the same buffer.

2.5. Enzyme assays

Hydrogenase activity was routinely measured at 75°C by following the reduction of methyl viologen with H_2 in a DU 640 photometer (Beckman Instruments) at 600 nm in anaerobic cuvettes [17]. The reaction mixture contained 5 m*M* methyl viologen in 100 m*M* KP_i buffer, pH 8.0. The reduction of DMN by H_2 was recorded photometrically at 75°C by following the change in the absorption at 270 nm. The background absorption at 290 nm was subtracted. The reaction mixture contained 200 μ*M* DMN in 100 m*M* KP_i buffer, pH 8.0 under hydrogen

Table 1
Buffers used for column chromatography

Column		Tris–HAc	NaCl	ε-Aminocaproic acid
Superose-6 (gel permeation chromatography)		50 m*M*, pH 7.7	1.5 M	0.5% (w/v)
DEAE Sepharose,	Loading buffer	50 m*M*, pH 7.0	–	0.5% (w/v)
	Elution buffer	50 m*M*, pH 7.0	1.5 M	0.5% (w/v)
Mono P Sepharose	Loading buffer	50 m*M*, pH 7.7	–	0.5% (w/v)
	Elution buffer	50 m*M*, pH 7.7	1.5 M	0.5% (w/v)

atmosphere [18–20]. The reduction of horse heart cytochrome *c* under a hydrogen atmosphere was recorded photometrically at 550 nm and 75°C. The reaction mixture contained 0.5 m*M* cytochrome *c* and 2 m*M* NADP in 100 m*M* KP_i buffer, pH 8.

Sulfur reductase activity was determined by incubating samples in 100 m*M* *N*-(2-hydroxyethyl)-piperazine-*N′*-3-propane-sulfonic acid (HEPPS from Serva) buffer (pH 7.0) containing 0.1% (w/v) Triton X-100, 5% (w/v) sulfur powder, 100 μ*M* cytochrome *c* or 200 μ*M* DMN and 1 m*M* NADPH under a hydrogen atmosphere for 45 min at 75°C. The product hydrogen sulfide was determined colorimetrically by the methylene blue method [21,22]. Polysulfide reductase was determined at 75°C with DMN as artificial electron acceptor according to published procedures [20,23].

2.6. Analytical procedures

Protein concentrations were determined with the bicinchoninic acid reagent (Pierce, Rockford, IL, USA) or photometrically by measuring the absorption of aromatic amino acids at 280 nm in a Beckman DU 640 photometer. Cytochrome spectra (dithionate-reduced minus air-oxidized) were recorded on a Beckman DU 640 photometer at room temperature. Proteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE) [24]. After electrophoresis, the proteins were stained with colloidal coomassie blue. A two-dimensional electrophoresis with a blue native (BN) PAGE in the first dimension and an SDS–PAGE in the second dimension was performed according to published procedures [15]. Proteins were separated with BN-PAGE under non-denaturing conditions in a Bis-Tris/Tricine system containing 0.002% Coomassie Blue G in the cathode and sample buffers. In addition, the gel buffers contained 0.5 *M* ϵ-aminocaproic acid. After detection of the hydrogenase with activity staining as described [6] the enzymatically active band was excised and placed on top of a standard denaturing SDS gel [15].

3. Results

3.1. Purification of membrane bound hydrogenase and sulfur reductase from Acidianus ambivalens

A SR complex with hydrogenase and sulfur reductase activity was purified from *Acidianus ambivalens* cells grown under anaerobic conditions. For the preparation of membrane fractions, cells were disrupted, and the cytoplasmic and membrane fractions were separated by ultracentrifugation. The pellet (membrane fraction) contained 92% of the total hydrogenase and 90% of the total sulfur reductase activity (Table 2). From the washed membrane fraction the proteins were solubilized by sonication with sodium-deoxycholate in KP_i buffer. After ultracentrifugation 90% of the hydrogenase and 74% of the sulfur reductase activities were found in the supernatant (‘‘solubilizate’’, Table 2).

Table 2
Distribution of hydrogenase and sulfur reductase activities after different steps of purification of the SR complex from *A. ambivalens*

	Hydrogenase (methyl viologen)					Sulfur reductase			
	Amount of protein	Specific activity	Total activity	Yield (%)	Enrichment	Specific activity	Total activity	Yield (%)	Enrichment
	(mg)	(U/mg)	(U)			(U/mg)	(U)		
Crude extract	904.5	22.1	266	100	1	6.3	66	100	1
Cytoplasmic fraction	325.2	2.6	9.8	–	–	0.38	6.1	–	–
Membrane fraction	310.8	27.1	245	92	1.2	16.2	59.1	90	1.9
Membrane solubilizate	296.4	32.4	240	90	1.4	17.5	48.7	74	2.1
Density gradient[a]	79.6	138.7	180	67	6.2	22.3	38.3	58	2.7
Superose-6[b]	0.485	170.0	136	51	7.9	45.0	36.6	56	5.4
DEAE Sepharose[b]	0.252	114	129	48	5.2	47.5	25.8	39	5.7

[a] Activity of the pooled fractions 8–10 (see Fig. 1).
[b] Superose-6 and DEAE chromatography was run alternatively with active fractions from the density gradient centrifugation.

The SR complex was further enriched by sucrose density gradient centrifugation (20–35%, w/v, sucrose). Five out of 14 fractions collected after centrifugation contained hydrogenase and sulfur reductase activity (Fig. 1 and Table 2). The fractions with the highest enzymatic activity were pooled. The specific hydrogenase activity in the pooled fractions was 346 U (mg protein)$^{-1}$, determined with DMN as electron acceptor and 138 U (mg protein)$^{-1}$, determined with methyl viologen. Cytochrome *c* was not reduced. The specific sulfur reductase activity was 22.3 U (mg protein)$^{-1}$, determined with powdered sulfur as substrate. Polysulfide was not reduced. A summary of the enrichment procedure is given in Table 2.

3.2. Gel permeation chromatography on Superose-6

After density gradient centrifugation the fractions containing the desired enzyme activities were concentrated. After a buffer exchange they were applied to the Superose-6 column. The running buffer contained 0.5% (w/v) ϵ-aminocaproic acid and 1.5 *M* sodium chloride to improve the separation of solubilized proteins. The SR complex eluted as a sharp peak at an elution volume of 16.2 ml. This corresponded to an apparent relative molecular mass of 254 000 (Fig. 2). Four strong protein bands were visible in Coomassie stained SDS–PAGE results of the active fraction (relative molecular masses: 110 000, 66 000, 39 000 and 29 000, Fig. 3). The specific activity of the hydrogenase was 883 U (mg protein)$^{-1}$ with DMN at 80°C, and 170 U (mg protein)$^{-1}$ with methyl viologen at 75°C. The sulfur reductase activity was 45 U (mg protein)$^{-1}$. After gel permeation chromatography in presence of 0.1% (w/v) Triton X-100, however, the enzyme activities were found in four fractions with different elution volumes. The specific hydrogenase activity for the most active fraction was only approximately 10% of the value obtained in presence of ϵ-aminocaproic acid. In the absence of ϵ-aminocaproic acid or

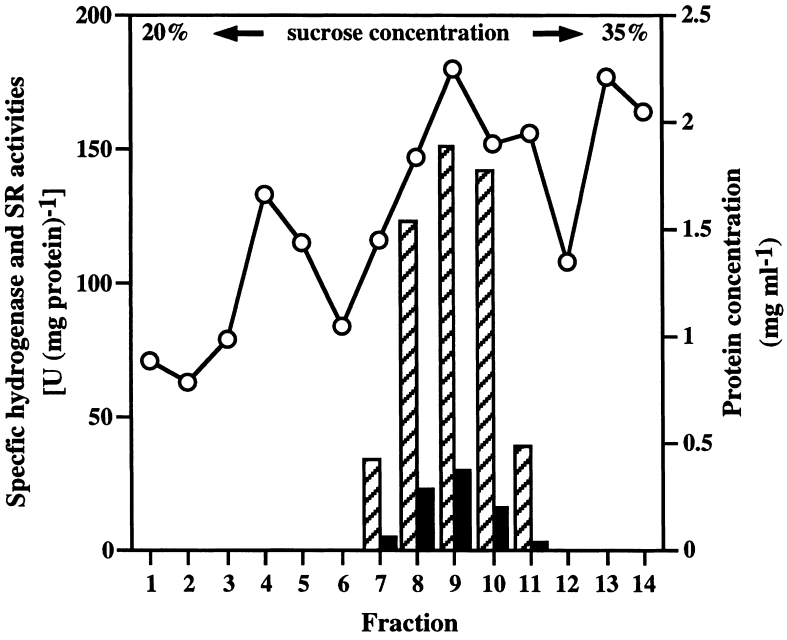

Fig. 1. Elution profile of the solubilized *A. ambivalens* membrane proteins after sucrose density gradient centrifugation. Fractions (3 ml) were collected anaerobically, the protein concentration (right axis) and the specific hydrogenase and SR activities (left axis) were determined separately for the individual fractions.

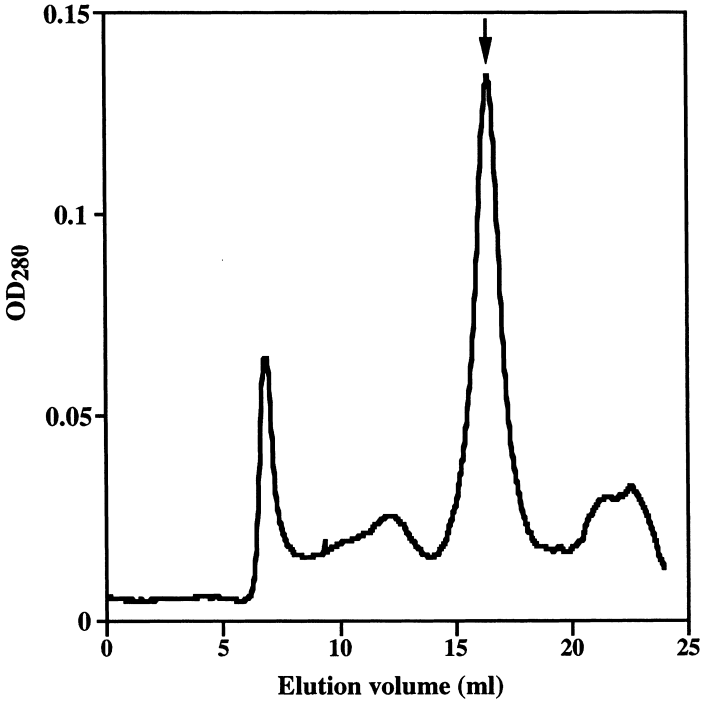

Fig. 2. Separation of the *A. ambivalens* SR complex by gel permeation chromatography using a Superose-6 HR 10/30 column. The bed volume was 24 ml and the flow-rate of the separation was 0.5 ml min^{-1}. The arrow marks the enzymatically active fraction. Left axis: O.D. at 280 nm.

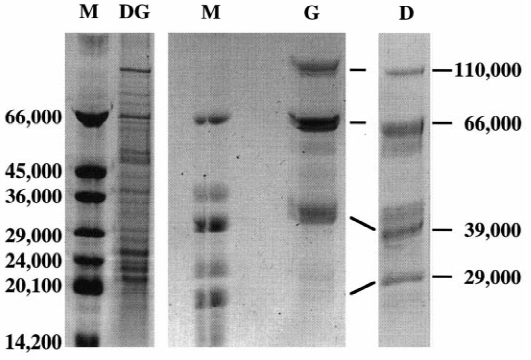

Fig. 3. SDS–PAGE of the enzymatically active fractions of the SR complex from *A. ambivalens*. M, molecular mass markers; DG, pooled active fractions 8–10 (see Fig. 1) after the sucrose density gradient centrifugation; G, active fraction after gel permeation chromatography (see Fig. 2); D, active fraction after DEAE chromatography (see Fig. 4). Bands with a relative molecular mass of 110 000, 66 000, 39 000 and 29 000 are visible in both enrichments and belong most probably to the SR complex.

detergents, hydrogenase and SR activities were not recovered from the column.

3.3. Anion-exchange chromatography on DEAE Sepharose

Alternatively, the SR complex was purified by anion-exchange chromatography on DEAE Sepharose in ϵ-aminocaproic acid-containing Tris buffers, pH 7.0. Under these conditions, the SR complex eluted as a sharp peak at an NaCl concentration of 0.36 *M* (Fig. 4). No other fraction contained hydrogenase and sulfur reductase activity. The specific activity of the hydrogenase was 624 U (mg protein)$^{-1}$ with DMN at 80°C, and 114 U (mg protein)$^{-1}$ with methyl viologen at 75°C. The sulfur reductase activity was 48 U (mg protein)$^{-1}$. The enrichment of the hydrogenase was 5.5-fold with a yield of 48% compared to the crude extract. The enrichment of the sulfur reductase was 5.7 and the yield was 40% (Table 2). In SDS–PAGE at least

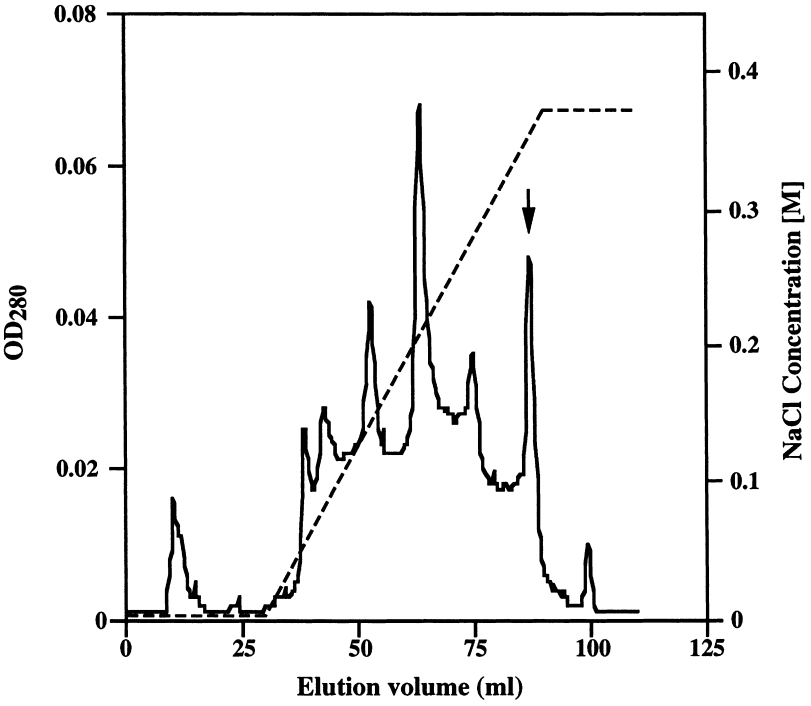

Fig. 4. Separation of the *A. ambivalens* SR complex by DEAE anion-exchange chromatography. The bed volume was 7.8 ml and the flow-rate of the separation was 1 ml min^{-1}. The arrow marks the enzymatically active fraction. Straight line, left axis: O.D. at 280 nm; dashed line, right axis: sodium chloride concentration of the elution buffer.

four protein bands appeared with similar molecular masses as those purified using a gel permeation column (110 000, 66 000, 39 000 and 29 000; Fig. 3).

In contrast, DEAE chromatography in the absence of detergent or with 0.1% (w/v) Triton X-100, 0.1% (w/v) β-octyl glucopyranoside, and 0.5% (w/v) CHAPS, respectively, was not successful. The enzymatic activities were distributed over a wide range of different fractions and variable sodium chloride concentrations. It was concluded that the SR complex bound unspecifically to the agarose-based column matrix. This interpretation was supported by the observation that hydrogenase bound non-specifically to materials such as dialysis tubing, cellulose-based ultrafiltration membranes (e.g., Centricon microconcentrators; Amicon, Danvers, MA, USA), and pure cellulose. We tried to purify the SR complex over a cellulose column. However, no purification was achieved, since other membrane proteins showed a similar behavior. The highest specific hydrogenase activity after anion-exchange chromatography with ϵ-aminocaproic acid-containing buffers was approximately 20-fold higher compared to Triton X-100-containing buffers. The specific sulfur reductase activity was approximately 25-fold higher.

3.4. Enrichment of hydrogenase and sulfur reductase from Thermoproteus neutrophilus

Membrane fractions of autotrophically grown *T. neutrophilus* cells were prepared to enrich an SR complex which also consists of a hydrogenase and a sulfur reductase. The disruption of the cells, the separation of membrane and cytoplasmic fractions and the solubilization of the membrane proteins were performed by the same procedure as already described. The membrane fraction contained 99% of the total hydrogenase and 98% of the total sulfur reductase activity. After solubilization and centrifugation, only 38% of the total hydrogenase and 74% of the sulfur reductase activity were found in the

supernatant. The SR complex was enriched from the supernatant by sucrose density gradient centrifugation as described. Six out of 14 fractions contained hydrogenase and sulfur reductase activity. However, the yields were low (19% for the hydrogenase; 9% for the sulfur reductase). In contrast to the *A. ambivalens* hydrogenase, the *T. neutrophilus* enzyme did not reduce DMN, but cytochrome *c*. The specific hydrogenase activity for the membrane solubilizate was 32.7 U (mg protein)$^{-1}$ with cytochrome *c*.

3.5. Anion-exchange chromatography

The SR complex consisting of hydrogenase and sulfur reductase activities was enriched from the pooled active fractions by anion-exchange chromatography on Mono P columns using loading and elution buffers containing 0.5% (w/v) ϵ-aminocaproic acid in Tris–HAc, pH 7.7. The complex bound to the column and was eluted in a narrow fraction at a sodium chloride concentration of approximately 0.7 *M* (Fig. 5). The specific methyl viologen-dependent hydrogenase activity was 212 U (mg protein)$^{-1}$. The specific sulfur reductase activity was 12 U (mg protein)$^{-1}$. A 5.7-fold enrichment of the hydrogenase and a 2.2-fold enrichment of the of sulfur reductase was achieved. However, the purification of the *T. neutrophilus* SR complex over a Mono P column resulted in the specific enrichment of the hydrogenase, which consisted of two subunits with relative molecular masses of 39 000 and 66 000 (Fig. 6). Two-dimensional gel electrophoresis combining in the first dimension a blue native PAGE and in the second dimension an SDS–PAGE revealed that these two subunits constitute the hydrogenase (data not shown). The apparent molecular mass of the native hydrogenase was 90 000, determined by gel permeation chromatography as described above. After the gel permeation step, the sulfur reductase activity was lost. Apparently, the two activities were

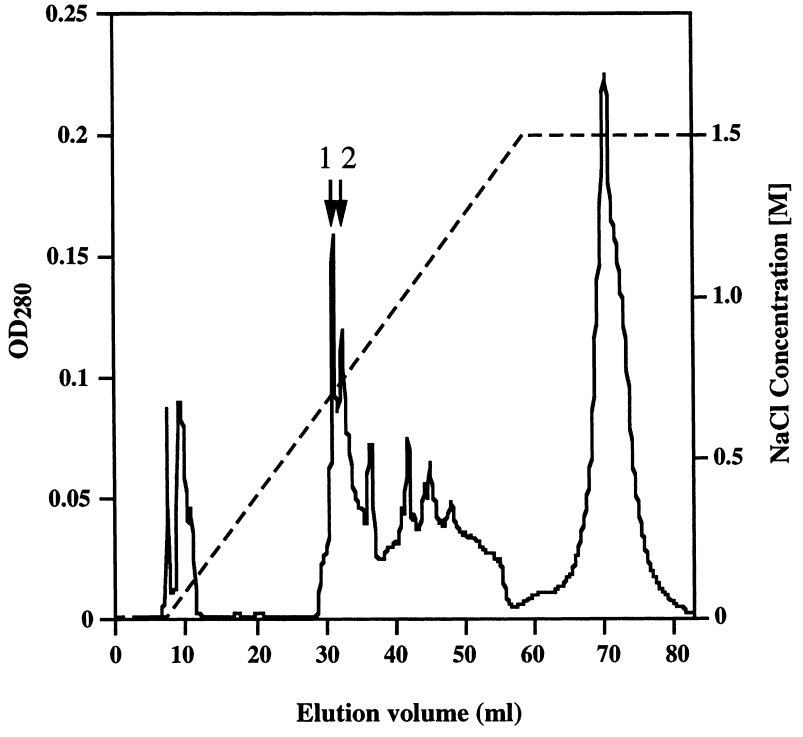

Fig. 5. Results of the separation of the *T. neutrophilus* hydrogenase and SR reductase by anion-exchange chromatography over a Mono P HR 5/20 column (4 ml bed volume, flow-rate 1 ml min^{-1}). The arrows mark the enzymatically active fractions (see Fig. 6). Straight line, left axis: O.D. at 280 nm; dashed line, right axis: sodium chloride concentration of the elution buffer.

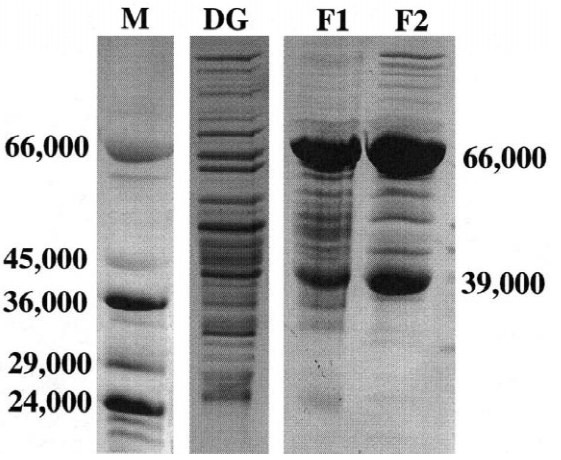

Fig. 6. SDS–PAGE of the active fraction from density gradient centrifugation (DG) and from two neighboring Mono P-enriched enzymatically active fractions of the SR complex and *T. neutrophilus* (F1 and F2; see Fig. 5), the two major bands constitute the hydrogenase.

separated. For the sulfur reductase, the subunit composition could not yet be determined.

3.6. Determination of cytochrome contents

Difference spectra (dithionate-reduced minus air oxidized) were recorded from membrane solubilizates of anaerobically grown cells of *A. ambivalens* and *T. neutrophilus*, respectively. Spectra from *A. ambivalens* solubilizates showed peaks at 418, 438 and 585 nm, typical for cytochrome *a* (data not shown). In addition, a faint peak appeared at 556 nm, giving evidence for the presence of cytochrome *b*. The difference spectrum of *T. neutrophilus* membranes showed α, β and γ peaks at 558, 528, 467 and 428 nm indicating the presence of cytochrome *b*.

4. Discussion

In this report, we describe the purification of two membrane proteins from two phylogenetically unrelated species of extremely thermophilic archaea. The solubilization of membrane proteins from *A. ambivalens* with sodium deoxycholate was very effective, more than 90% of the hydrogenase and SR activities were found in the supernatant (solubilizate) after ultracentrifugation. The solubilization of *T. neutrophilus* membrane proteins was less effective, only 53% of the hydrogenase and 58% of the total SR activity were found in the supernatant. With sucrose density gradient centrifugation, the methyl viologen dependent hydrogenase of *A. ambivalens* was enriched 6.2-fold, the SR 2.7-fold. The enrichment factors of the *T. neutrophilus* hydrogenase and SR compared to the crude extract were only 1.4- and two-fold, respectively. Most probably, this was due to the poor solubilization.

With an optimized chromatographic purification using either a gel permeation or a DEAE Sepharose column in the presence of ϵ-aminocaproic acid the hydrogenase and SR from *A. ambivalens* was almost enriched to electrophoretic homogeneity (Fig. 3). However, there was little increase in the specific activity. Some subunits might have been lost or inactivated despite the anaerobic purification procedure. Obviously, the SR subunits dissociated partially from the complex during chromatography. The intensities of the four protein bands constituting the complex vary on SDS–PAGE (Fig. 3). However, 51% of the total hydrogenase and 56% of the total SR activity were recovered after gel permeation chromatography, whereas 48% of the hydrogenase and 40% for SR were recovered after the DEAE chromatography (Table 2). Due to the low growth yields of the chemolithotrophic archaea (0.16 g wet mass l^{-1}), the purification procedure was not yet optimized by combination of different column chromatographic techniques.

In each case, the purification was only successful when ϵ-aminocaproic acid was added to the chromatography buffers. Without this agent, the proteins bound non-specifically to the matrix of the columns and/or eluted in several fraction. The application of detergents like Triton X-100, β-Octyl glucopyranoside and CHAPS in the chromatographic steps did not improve the performance. The hydrogenase bound non-specifically to materials such as cellulose-based dialysis tubing, ultrafiltration membranes and pure cellulose. From these observations, we concluded that the hydrogenase bound non-specifically to most carbohydrate-based materials including Sepharose. Similar results were obtained with the *T. neutrophilus* SR complex.

At present, we can only speculate about the

mechanism how the lysin analog ϵ-aminocaproic acid influences the separation. In a different approach to solve this problem, a 24-amino-acid peptide with a defined sequence had been designed and applied for the solubilization of membrane proteins [25]. In this case, hydrophobic domains are masked. In contrast, ϵ-aminocaproic acid is no detergent, it cannot solubilize proteins from their lipid environment. But the mechanisms might be the same or similar. We concluded that ϵ-aminocaproic acid improves the chromatographic separation of membrane proteins by keeping already solubilized proteins in solution without much interference with the chromatographic separation. We could show that the agent can be applied to several different chromatographic techniques.

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