

Purification of the MglC/E membrane proteins of the binding protein-dependent galactose transport system of *Salmonella typhimurium*

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The high affinity galactose transport system of *Salmonella typhimurium* consists of four proteins, a periplasmic galactose binding protein (the MglB protein), and three inner membrane-associated proteins, the MglA, MglC and MglE proteins. We purified the MglC/E proteins from an MglC/E hyperproducing strain after solubilisation of inclusion bodies in guanidine hydrochloride followed by renaturation in a detergent-containing buffer and affinity chromatography on a MglB-Sepharose column. The MglC/E proteins are devoid of ATPase activity and they complement an extract from a strain carrying a plasmid with the *mglA* gene for reconstitution of the MglB-dependent galactose transport in proteoliposomes.

Binding protein-dependent transport; Membrane protein; Proteoliposome

1. INTRODUCTION

The binding protein-dependent transport systems of Gram-negative bacteria form a class of at least thirty permeases implicated in the active transport of ions, sugars, amino acids and oligopeptides (reviewed in [1,2] and belong to a superfamily of membrane proteins which extends to eukaryotic cells and includes the multidrug-resistance P-glycoprotein [3] and the cystic fibrosis transmembrane conductance regulator [4]. Each of these binding protein-dependent transport systems consists of a periplasmic substrate-binding protein, which serves as the primary recognition site for transport, and three or four additional inner membrane-associated proteins implicated in substrate translocation and energy coupling. The binding protein-dependent galactose transport system of *Salmonella typhimurium* (formerly called the methyl-galactose permease) consists of a periplasmic galactose-binding protein (the *mglB* gene product) and three inner-membrane proteins (the *mglA*, *mglC*, *mglE* gene products) [5]. Several results suggest an implication of ATP in the energisation of binding protein-dependent transport systems [6–10]. In a recent study, we reported the reconstitution of the binding protein-dependent galactose transport in proteoliposomes [11]. In the present study we report the purification of two proteins whose molecular weights (29,000 Da and 21,000 Da) correspond to those of the

MglC and MglE proteins, as determined by molecular cloning experiments [5]. The purified proteins are devoid of ATPase activity and they complement an extract prepared from an MglA-hyperproducing strain for reconstitution of the MglB-dependent galactose transport in proteoliposomes.

2. MATERIALS AND METHODS

2.1. Bacteria

The strain, *Escherichia coli* K12 LA 5709 (F⁻ *mglS12 lacY galE ptsF arg recA srl*), is defective for the *mgl* operon and carries plasmids pHG16 or pHG15, two pBR322 derivatives containing, respectively, the *mglC* and *mglE* genes, or the *mglA* and *mglB* genes of *S. typhimurium*. It was obtained from the laboratory of Prof. W. Boos (Fachbereich Biologie, Konstanz University, Germany) [5]. The strains were grown in LB-rich medium supplemented with 0.4% glucose (in order to repress the *mgl* genes) to an absorbance of 0.5 at 600 nm, and then transferred for 2 h, in the same medium but without glucose, to obtain expression of the *mgl* genes.

2.2. Purification of the MglC/E proteins

The purification procedure was done at 4°C (except when indicated) and started with 4 g of bacteria (LA 5709 pHG16). The bacteria were washed with 30 ml of 100 mM Tris-HCl (pH 7.4) and lysed by suspension at 20°C in 3 ml of 50 mM potassium phosphate (pH 6.8), 2 mM dithiothreitol, 5 mM ATP, 10 mM galactose, 20% sucrose, followed by addition of 1 ml of 1 mg/ml lysozyme (freshly prepared), 0.1 g of sonicated phospholipids (asolectin) in 1 ml of water and EDTA (added slowly (2 min)) to a concentration of 5 mM, 2% Triton X-100, 0.1 mg of pancreatic DNase and 5 mM MgCl₂ were then added. The whole was incubated for 1 h at 0°C, centrifuged for 10 min at 5,000 × g to eliminate the cellular debris, and the supernatant was centrifuged for 10 min at 4,000 × g. The white pellet, containing protein aggregates [12], was washed three times with buffer containing 50 mM potassium phosphate, 0.5 mM EDTA, 5 mM MgCl₂, 2 mM dithiothreitol, 0.1 mg/ml asolectin, 2 mM ATP. It was solubilised in 10 ml of 6 M guanidine-HCl, 1% CHAPS, 1 mM dithiothreitol, 50 mM Tris-HCl, pH 7.4 (1 h at 37°C), and renatured by dilution in 100 ml of 20 mM

Abbreviation: CHAPS:(3-(3-Cholamidopropyl)-dimethylammonio)-1-propanesulfonate).

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Tris-HCl, pH 8, 0.5 mM EDTA, 5 mM MgCl₂, 2 mM dithiothreitol, 15% glycerol, 0.7% CHAPS, 0.2 mg/ml aroclorin at 20°C (buffer A). The protein solution was allowed to stand for 2 h at 20°C, clarified by centrifugation (10 min at 20,000 × g), concentrated to 5 ml by ultrafiltration, and dialyzed overnight against 50 ml of the same buffer; the concentrated solution was used for the purification. The protein solution was loaded at 20°C on an MglB-Sepharose column (made as described by the manufacturer by coupling 10 mg of the purified MglB protein to 0.5 g of CNBr-activated Sepharose (Pharmacia Fine Chemicals)) equilibrated in buffer A; the column was washed with 20 ml of a similar buffer with a CHAPS concentration reduced to 0.5% (buffer B), and eluted with 20 ml of a linear gradient of 0–1 M NaCl in buffer B. The eluted fractions were dialysed against buffer B, and concentrated by ultrafiltration.

2.3. Preparation of the protein extract from the MglA-producing strain

This was made by following the same procedure as the preparation of the MglC/E proteins. The strain, LA 5709 pHG15, was used instead of strain LA 5709 pHG16. The preparation was the same as above until the overnight dialysis step against buffer B.

2.4. Purification of the MglB protein

This purification was made from the *E. coli* strain LA 5709, containing plasmid pHG15 by an osmotic shock procedure, followed by two column chromatographies on hydroxylapatite and DEAE cellulose as described in [13]. The MglB protein was deprived of bound ligand as described previously [13].

2.5. Gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed by the method of Laemmli [14] by using 12.5% polyacrylamide gels. Coloration of protein bands was made by silver staining [15]. Protein fractions were incubated in sample buffer for 1 h at 37°C (instead of 3 min at 100°C) before electrophoresis.

2.6. ATPase

ATPase activity was measured by adding protein in 2 µl of buffer B to 3 µl of 100 µM [³H]ATP (1.5 Ci/mmol), 1 mM MgCl₂ and 10 mM galactose. ATP and ADP were separated by chromatography on polyethyleneimine cellulose, as described in [16].

2.7. Proteoliposomes

Proteoliposomes were formed by a dilution procedure as follows: 0.1 ml of MglC/E protein in buffer B (50 µg), 0.1 ml of MglA extract in buffer B (400 µg), 0.1 ml of soybean phospholipids (50 mg/ml) (arolectin from Associated Concentrates) previously resuspended by sonication and solubilized with 10 µl of 10% octylglucoside, were incubated for 1 h at 0°C, freeze-thawed once, and the whole was diluted 40 times in 12 ml of 20 mM Tris-HCl pH 8.0, 0.5 mM EDTA, 5 mM MgCl₂, 0.3 mM dithiothreitol, 10 mM ATP at 18°C. Proteoliposomes were collected by centrifugation for 1 h at 200,000 × g and resuspended in 50 µl of the preceding buffer without ATP. Proteoliposomes, devoid of the MglC/E proteins or of the MglA protein, were prepared in the same manner by replacing the missing protein by the same volume of buffer B.

2.8. Transport assay

15 µl of proteoliposomes were added to 15 µl of 200 µM galactose and 15 µl of 100 µM MglB protein at 20°C; at the indicated times, 8 µl aliquots were poured into 1 ml of a buffer containing 20 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 5 mM MgCl₂, 15% glycerol at 20°C; the whole was immediately filtered through 0.22 µm cellulose esters filters (Millipore GSWP) and washed with 2 × 1 ml of the preceding buffer at 0°C.

2.9. Materials

ATP (disodium salt) was obtained from Sigma. [³H]ATP and [³H]galactose were obtained from Amersham and were used at 1.5 Ci/mmol. Triton X-100 was from Boehringer and arolectin (purified

soybean phospholipids) was from Associated Concentrates, Woodside, Long Island, NY 11377, USA. All other chemicals were obtained from Sigma and were of reagent grade.

3. RESULTS

3.1. Purification of the MglC/E proteins

The insoluble protein aggregates from the *E. coli* strain LA 5709 carrying plasmid pHG16 (containing the *mglC* and *mglE* genes of *S. typhimurium*) were solubilized in guanidine hydrochloride and CHAPS, renatured by dilution in a CHAPS-containing buffer and purified on an MglB-Sepharose affinity column (as described in section 2). The protein fractions which elute from the column around 0.5 M NaCl represent 7% of the loaded proteins and show two bands at 29,000 Da and 21,000 Da when analyzed on a sodium dodecyl sulfate polyacrylamide gel (Fig. 1). Their molecular weights correspond to those of the *mglC* and *mglE* gene products, according to molecular cloning experiments [5]. The faster migrating band around 15,000 Da was not consistently seen in these preparations. The purified fractions do not display any ATPase activity (not shown).

3.2. Reconstitution of galactose transport in proteoliposomes

The components used for the reconstitution of galactose transport in proteoliposomes comprise the two membrane proteins eluted from the MglB protein affinity

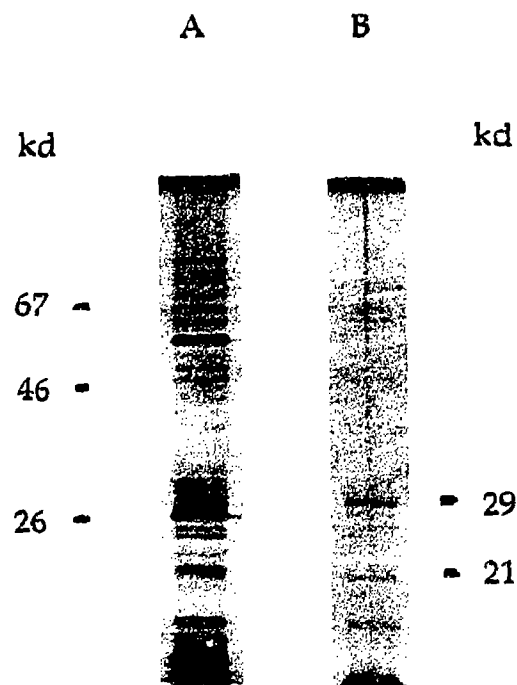


Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the MglC/E proteins. Migration was from top to bottom. The concentration of acrylamide was 12.5%. Kd, kilodaltons. (A) Proteins solubilized from inclusion bodies from the MglC/E-hyperproducing strain. (B) Protein retained on the MglB-Sepharose affinity column.

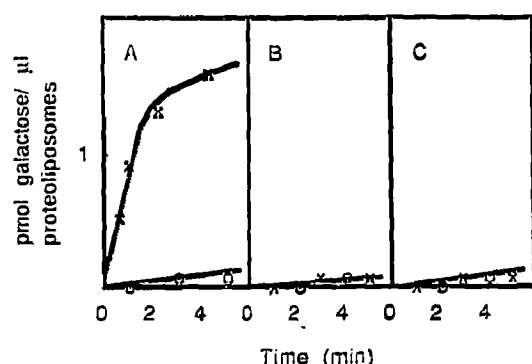


Fig. 2. Reconstitution of galactose transport in proteoliposomes. Uptake was assayed at 20°C in the presence of 10 mM trapped ATP, 66 μ M [3 H]galactose and in the presence (x), or in the absence (o) of the MglB protein at a concentration of 66 μ M. (A) Proteoliposomes made with the MglA extract and the MglC/E proteins. (B) Proteoliposomes made with the MglA extract. (C) Proteoliposomes made with the MglC/E proteins.

ity column (MglC and MglE proteins), the protein extract prepared from the MglA-hyperproducing strain and the MglB protein (the periplasmic galactose-binding protein). Proteoliposomes made with the MglA extract and the purified MglC/E proteins, and containing trapped ATP, accumulate galactose when the galactose-binding protein (MglB protein) is added to the transport assay (Fig. 2A). Omission of the galactose-binding protein in the transport assay (Fig. 2A), or omission of ATP during the formation of the proteoliposomes (not shown) results in a considerable reduction in galactose accumulation. Proteoliposomes prepared with the MglC/E proteins alone (Fig. 2B), or with the MglA extract alone (Fig. 2C) do not show significant galactose accumulation.

4. DISCUSSION

The present results suggest that the purified proteins described in this study are the MglC and the MglE proteins: (i) the purified proteins possess a molecular weight similar to that of the *mglC* (29,000 Da) and *mglE* (21,000 Da) gene products, as determined by molecular cloning experiments; (ii) the purified proteins complement an extract from a MglA-hyperproducing strain for reconstitution of the MglB-dependent galactose transport in proteoliposomes; (iii) the proteins have been purified on the basis of their affinity for an MglB-Sepharose column, a result in accordance with their postulated affinity for the MglB protein [5]. The MglC/

E proteins are devoid of ATPase activity. This is in accordance with the absence of a nucleotide binding site in the sequence of similar proteins such as the HisQ or HisM proteins [1]. The purification of the MglC/E proteins described in the present study represents the first purification of the hydrophobic membrane proteins of a periplasmic binding protein-dependent transport system (the maltose transport system has been purified as a complex containing also the peripheral MalK protein [17], and it should help in the characterisation of the MglA protein. The purification of the four proteins implicated in the binding protein-dependent galactose transport should allow a determination of the function of each of them [1], and it should also help to unravel the mechanism of other related transport systems, such as the multidrug-resistance efflux pump [3] or the cystic fibrosis transmembrane conductance regulator [4].

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