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Purification, Reconstitution, and Characterization of Na^+/Serine Symporter, SstT, of *Escherichia coli*[1]

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A gene encoding Na^+/serine symporter (SstT) of *Escherichia coli* has been cloned and sequenced in our laboratory [Ogawa *et al.* (1998) *J. Bacteriol.* 180, 6749–6752]. In an attempt to overproduce the protein and purify it, we first constructed a plasmid pTSTH in which the modified *sstT* gene (*sstT* gene with 8 successive codons for His at the 3′-terminus) is located downstream from the *trc* promoter. Upon induction by IPTG, the His-tagged SstT protein was overproduced (about 15% of total membrane proteins), and showed activity as high as the wild type SstT. The His-tagged SstT was solubilized with octylglucoside and purified to homogeneity using a nickel nitrilotriacetic acid (Ni^{2+}-NTA) affinity resin. The N-terminal sequence (20 amino acid residues) of the purified protein showed that the sequence was identical to that deduced from the DNA sequence of the *sstT* gene and that the initiation methionine was excised. The purified His-tagged SstT was reconstituted into liposomes by the detergent dilution method. Reconstituted proteoliposomes mediated the transport of serine driven by an artificially imposed electrochemical Na^+ gradient. The K_m and the V_{max} values for serine transport with the proteoliposomes were 0.82 μM and 0.37 nmol/min/mg protein, respectively. Serine transport was inhibited by L-threonine, but not by other amino acids. The purified protein was stable for at least 6 months at –80°C.

Key words: histidine-tag, purification, serine, SstT, transporter.

Four transport systems for serine have been identified in *Escherichia coli*. One is the Na^+/serine symporter, SstT, which is the principal serine transporter in *E. coli* K-12 (*1, 2*). This system mediates the uptake of serine and threonine. The second system is a serine-specific SdaC, which is induced by leucine, and is an H^+/serine symporter (*3, 4*). The third system, the TdcC system, has been reported as a threonine transport system that also mediates serine transport (*5*). This is also an H^+-coupled symporter (*6*), and is not present when *E. coli* cells are grown under aerobic conditions (*5*). The fourth system is a leucine-isoleucine-valine system (LIV-1) (*7*). A periplasmic binding protein is involved in this transporter and it is an ATP-driven system (*7*). The presence of multiple transport systems for serine in *E. coli* made it difficult to isolate a serine-transport defective mutant, which would be very useful for cloning gene(s) encoding the serine transporter(s). We have succeeded in isolating a mutant that lacks the principal serine transporter, SstT (*6*), making it easy to clone the serine transporter genes. In fact, we succeeded in cloning the *sstT* gene and *tdcC* gene using the mutant as the cloning host (*6, 8*). Thereafter, we sequenced the *sstT* gene (*8*). The SstT protein has been deduced to comprise 414 amino acid residues with about 9 hydrophobic domains (*8*).

So far, biochemical analyses of the SstT Na^+/serine symport system have been performed with either whole cells or right-side-out membrane vesicles (*1, 2*). In order to investigate structure–function relationships in the SstT protein at the molecular level, it is necessary to purify the protein. Gene cloning and gene manipulation are valuable methods for the overproduction of proteins, which is useful for purifying a protein. A very attractive and useful strategy for protein purification, the attachment of a polyhistidine peptide (His tag) to the N- or C-terminal extremity of a protein, confers a high affinity for nickel to the recombinant protein (*9*). The tagged protein is subsequently purified by affinity chromatography using Ni-NTA resin. This strategy has been successfully applied to the purification of a large number of soluble recombinant proteins, and its utilization has been extended to the purification of detergent-solubilized membrane proteins (*10–15*).

In the present paper, we report the overproduction of the His-tagged SstT protein, and the purification, N-terminal sequencing, reconstitution into liposomes, and functional properties of the SstT Na^+/serine transport protein.

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Abbreviations: NTA, nitrilotriacetic acid; OG, *n*-octyl-β-glucoside; IPTG, isopropyl-β-D-thiogalactopyranoside; PCR, polymerase chain reaction; PMSF, phenylmethylsulfonylfluoride; CCCP, carbonyl cyanide-*m*-chlorophenylhydrazone.

MATERIALS AND METHODS

Materials—[^{14}C]Serine was from Amersham Life Science. OG was from Sigma. Other detergents were from Wako Pure Chemicals (Osaka), and Ni^{2+}-NTA agarose was from Qiagen. *E. coli* phospholipid was purchased from Avanti Polar Lipids. All other reagents were of reagent grade and purchased from commercial sources.

Bacterium and Growth—The *E. coli* strain used in this study was WAT9 (*6, 8*), which lacks the SstT Na^+/serine transport system. Cells were grown in modified Tanaka medium (*16*) supplemented with 1% trypton at 37°C under aerobic conditions. In order to test the effect of IPTG on the production of the His-tagged SstT protein, IPTG (final 1 mM) was added to the culture medium.

Engineering of a His-Tag to the Wild Type SstT—The vector plasmid used was pTrc99A (Pharmacia Biotech, USA). Two oligonucleotides used to generate a unique *Ssp*I restriction site (AATATT) that overlaps the stop codon of the *sstT* gene were SP (GCCTTCTTCACCCGCAGC) and SS (CAAAATATTACGCAGGGCGC). PCR was carried out using a DNA fragment from *Bam*HI–*Eco*RI of pMST298 (*8*) as a template, and the SP primer and SS primer. After PCR, the sequence of the resulting DNA fragment was confirmed by sequencing using an ALF Express DNA sequencer (Pharmacia Biotech, USA). The fragment amplified by PCR was subcloned into the *Eco*RV site of pBluescriptII SK (+). The fragment of this plasmid digested with *Pst*I–*Ssp*I and the *Pst*I–*Eco*RV fragment of pBF4, pBluescriptII SK (+) with an 8× His tag upstream of the *Eco*RV site were ligated. The resulting plasmid was pBPSHis. This plasmid has a C-terminus 8× His tag. Plasmid pTrcBP, a derivative of pTrc99A containing the *Bam*HI–*Pst*I fragment of pMST298, and pBPSHis were digested with *Pst*I and *Hin*dIII, and then ligated. The resulting plasmid was digested with *Nco*I and *Sal*I, treated with Mung bean nuclease and Klenow DNA polymerase, and ligated at the blunt end. The resulting plasmid was pTSTH. The control plasmid, pTST, was constructed according to the same strategy.

Preparation of Membrane Vesicles—Cells were grown to logarithmic phase and induced by adding IPTG (final 1 mM) at 37°C. All subsequent steps were performed at 0–4°C. After harvesting, the cells were washed twice with 0.1 M potassium phosphate buffer (pH 7.0), and resuspended in the same buffer containing 0.2 mM PMSF (1 g wet cells/5 ml). The cells were disrupted by two passages through an Amino French Pressure cell at 20,000 p.s.i. Low-speed centrifugation was carried out for 10 min to remove intact cells, and the supernatant was then centrifuged at 150,000 ×*g* for 1 h. The pellets were resuspended at a concentration of about 10 mg protein/ml in 0.1 M potassium phosphate buffer (pH 8.0), 10% glycerol, 5 mM β-mercaptoethanol, 50 mM serine, and 200 mM NaCl. The resulting membrane sample was stored at –80°C until use.

Solubilization and Purification—All steps were performed at 0–4°C. Membrane proteins were solubilized by adding 1.5% OG to the membrane preparation in the presence of 0.2% *E. coli* phospholipid (50 mg/ml). The samples were kept on ice for 30 min, with vortexing every 10 min. After incubation, the samples were centrifuged at 150,000 ×*g* for 30 min, and the supernatant was incubated for 1 h with gentle shaking with Ni^{2+}-NTA resin pre-equilibrated with 0.1 M potassium phosphate (pH 8.0), 10% glycerol, 1.5% OG, 0.2% *E. coli* phospholipid, 5 mM β-mercaptoethanol, 50 mM serine, 200 mM NaCl, and 10 mM imidazole. The mixture was then applied to a column, and the column was washed three times with washing buffer containing 0.1 M potassium phosphate (pH 7.0), 10% glycerol, 1.5% OG, 0.2% *E. coli* phospholipid, 5 mM β-mercaptoethanol, 50 mM serine, 200 mM NaCl, and 200 mM imidazole. The bound protein was eluted with buffer containing 500 mM imidazol.

Reconstitution and Characterization of SstT—The purified His-tagged SstT was mixed with 0.1 M potassium phosphate buffer (pH 7.0) containing bath-sonicated *E. coli* phospholipid (10 mg/ml) and 1.5% OG. The mixture was kept on ice for 20 min, and then diluted 50-fold into 0.1 M potassium phosphate buffer (pH 7.0) and kept for 20 min at room temperature. The resulting proteoliposomes were collected by centrifugation at 150,000 ×*g* for 1 h. The tubes were drained carefully, and the pellets were resuspended in 0.1 M potassium phosphate buffer (pH 7.0).

Transport Assay—After harvesting, the cells were washed twice with buffer A, consisting of 0.1 M MOPS-Tris, pH 7.0, and 2 mM $MgSO_4$. The cells were resuspended in 0.1 M MOPS-Tris, pH 7.0, chloramphenicol (50 μg/ml) and 10 mM lactate, then diluted 10-fold in the same medium and incubated for 3 min at 25°C. The transport assay was initiated by the addition of [^{14}C]serine (final 0.1 mM, 0.25 μCi/ml). Samples were taken at intervals, filtered through membrane filters (0.45 μm, ADVANTEC Toyo, Tokyo), and washed with buffer A. The assay was performed at 25°C, and radioactivity was measured in a liquid scintillation counter. The serine transport assay for proteoliposome was performed as follows. Proteoliposomes were diluted 50-fold in 0.1 M sodium phosphate buffer (pH 7.0), and incubated for 3 min at 25°C. Transport was initiated by the addition of [^{14}C]serine (final 50 μM, 0.25 μCi/ml). Samples were taken at intervals, filtered through membrane filters (0.2 μm, ADVANTEC Toyo), and washed with 0.1 M sodium phosphate buffer (pH 7.0).

Analytical Methods—Proteins were analyzed by SDS-PAGE according to Laemmli (*17*). For Ferguson plotting (*18*), 8, 10, 12, 15, and 18% polyacrylamide gels were used.

Immunoblotting—To estimate the amount of His-tagged SstT protein in crude detergent extracts, the samples were subjected to SDS-PAGE (12%), transferred to a cellulose nitrate membrane filter (0.45 μm, ADVANTEC Toyo), and the filter was treated with Ni^{2+}-NTA HRP Conjugate (Qiagen) according to protocol provided by the manufacturer.

N-Terminal Amino Acid Sequence—The purified protein was subjected to SDS-PAGE in 12% gels and electroblotted to a polyvinylidene difluoride membrane (Bio-Rad). After staining with Coomassie Brilliant Blue, the N-terminal amino acid sequences were analyzed using an Applied Biosystems Model 477A protein sequencer.

Protein Assay—Protein content was determined by the Lowry method using bovine serum albumin as the standard (*19*).

RESULTS AND DISCUSSION

Overproduction of His-Tagged SstT Protein—The first

step in the purification was the construction of a plasmid carrying a modified *sstT* gene possessing several consecutive His codons. The modified *sstT* gene should be located downstream from a strong promoter. We used pTrc99A as a vector plasmid for the overproduction of the modified SstT protein. Eight successive His residues were attached to the C-terminus of SstT by fusing 8 His codons at the 3′-end of the *sstT* gene. To overproduce the modified SstT protein, the modified *sstT* gene was inserted downstream of the *trc* promoter in pTrc99A. Thus, the gene was inducible with IPTG. Plasmids pTST and pTSTH carried wild type *sstT* and modified *sstT*, respectively, and produced the wild type SstT and His-tagged SstT proteins. In the His-tagged SstT protein, the Asp residue of the original C-terminus was replaced with Ile during the generation of the unique *Ssp*I restriction site at the 3′-end of the *sstT* gene.

At an early stage of this study, we used 6 consecutive His residues as the His-tag. We observed the overproduction of the His-tagged SstT protein in cells harboring plasmid carrying the modified gene downstream from the *trc* promoter. The modified SstT with 6 His residues at the C-terminus showed serine transport activity. Unfortunately, however, we could not obtain highly purified His-tagged SstT protein using this tag. Thereafter we constructed an *sstT* gene with 8 successive His codons.

Serine Transport Activity of His-Tagged SstT—It has been reported that most host cells die in some cases when the overexpression of a target membrane protein is induced (*20*). Therefore, it seems important to overproduce the target protein by induction with a pertinent inducer after growing the cells to an appropriate level. Thus, the modified *sstT* gene was located under an IPTG-inducible *trc* promoter. Upon induction by 1 mM IPTG, serine transport activity in cells harboring plasmid pTSTH increased (Fig. 1). The maximal serine transport activity was observed 3 h after the addition of IPTG, and decreased thereafter.

We also investigated the effect of a His-tag added to the C-terminus of SstT. We compared serine transport activity in WAT9/pTrc99A (negative control possessing no SstT), WAT9/pTST (possessing wild type SstT), and WAT9/pTSTH (possessing His-tagged SstT) (Fig. 2). WAT9/pTST and WAT9/pTSTH cells showed much higher activity compared with WAT9/pTrc99A cells. The His-tagged SstT showed serine transport activity as high as that of the wild type SstT. Thus, we conclude that the His tag at the C-terminus did not significantly influence the functional properties of the SstT protein.

Judging from the results shown in Fig. 2, it is likely that the production of SstT reached a maximal level at around 3 h. We confirmed this to be true by testing the level of SstT in induced cells. We prepared membrane vesicles from cells harvested at various times after induction and subjected them to SDS-PAGE. We observed an increase in the intensity of the band corresponding to 33 kDa after induction with IPTG (Fig. 3). The amount of the 33 kDa protein increased with induction time up to 3 h. No further increase was observed at 5 or 7 h. Since we detected no increase in the 33 kDa band with membrane vesicles prepared from WAT9/pTrc99A cells after IPTG addition, we conclude that the 33 kDa band represents SstT. Thus, it seems that the production of SstT increases for up to 3 h after induction,

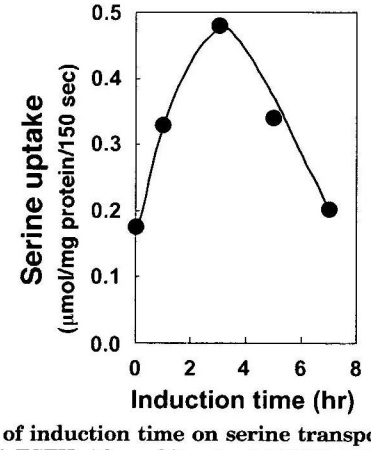

Fig. 1. **Effect of induction time on serine transport activity in *E. coli* WAT9/pTSTH.** After adding 1 mM IPTG to the culture medium, cells were harvested at the indicated times and serine transport activity was measured. The transport assay was performed as described in "MATERIALS AND METHODS."

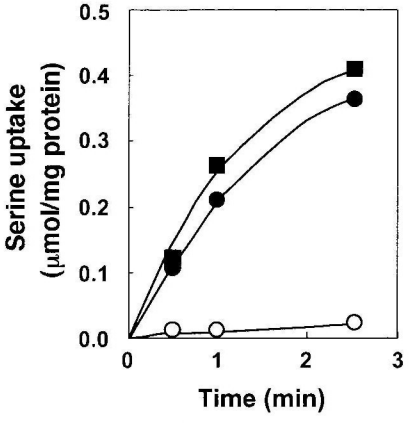

Fig. 2. **Serine transport activity in induced cells.** Cells induced with 1 mM IPTG for 3 h were used for the serine transport assay. Symbols are: ○, *E. coli* WAT9/pTrc99A (no SstT, control); ●, *E. coli* WAT9/pTST (possessing wild type SstT); ■, *E. coli* WAT9/pTSTH (possessing His-tagged SstT).

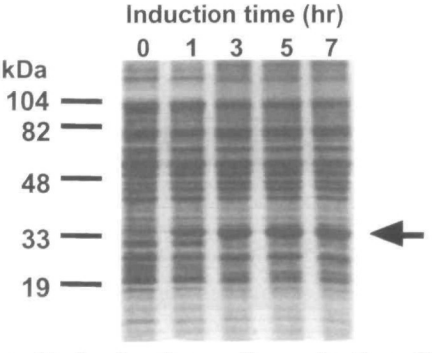

Fig. 3. **Effect of induction time on the production of His-tagged SstT.** *E. coli* WAT9/pTSTH cells were grown in modified Tanaka medium (*16*) supplemented with 1% trypton at 37°C under aerobic conditions. IPTG (1 mM) was added to the culture medium and cells were harvested at the indicated times. Membrane vesicles were prepared as described in "MATERIALS AND METHODS." Membrane samples (40 μg protein) were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue. The arrow indicates the position of His-tagged SstT.

and then, perhaps, the cells gradually lose serine transport activity although SstT is present in the cell membranes. It has been frequently reported that overproduced membrane proteins are not found in the cytoplasmic membrane but instead form cytoplasmic inclusion bodies (*21*). Although we analyzed total cell protein after induction, we obtained similar result. Thus, it is unlikely that SstT forms inclusion bodies after induction. As judged by densitometry, we estimate that the His-tagged SstT accounts for about 7 and 13% of membrane proteins after 1 and 3 h of induction, respectively.

We also detected an increase in the corresponding protein band with cells harboring plasmid pTST after induction with IPTG (data not shown).

Solubilization and Purification of SstT—The protocols of the solubilization and purification of the His-tagged SstT are described in "MATERIALS AND METHODS." The protocols comprise two steps: solubilization of the SstT protein using a detergent, and separation of the solubilized protein by Ni^{2+} chelate affinity chromatography.

The efficient solubilization of the target membrane protein in a functional state is an important requisite for purification. To select an effective detergent, membrane vesicles prepared from *E. coli* WAT9/pTSTH were incubated in the presence of different detergents at final concentrations exceeding their critical micellar concentrations (CMC). To judge the efficiency of the solubilization of His-tagged SstT from the membrane, the solubilized fractions obtained after treatment with each detergent were subjected to SDS-PAGE, and the His-tagged SstT was immunoblotted using Ni^{2+}-NTA HRP Conjugate. It was concluded that OG and nonylthiomaltoside solubilize about 75 and 70% of the protein, respectively. On the other hand, several detergents (Mega 9, CHAPS, Tween 80, and TritonX-100) showed limited solubilization of the His-tagged SstT (data not shown).

We chose OG as the detergent for solubilization and purification, because of its efficiency of solubilization and its high CMC value that enables convenient reconstitution by the detergent dilution method. After solubilization, the solubilized proteins were mixed with Ni^{2+}-NTA resin, and the mixture was subjected to column chromatography. It seemed that most of the His-tagged SstT bound to the resin under our experimental conditions (Fig. 4). The resin was washed with buffer containing 200 mM imidazole to remove weakly bound protein(s). We purified the His-tagged SstT by eluting the column with buffer containing 500 mM imidazole (Fig. 4, lanes 6–8). It seems that the major 33 kDa band is the monomer form of His-tagged SstT. Minor bands corresponding to 66 kDa, 99 kDa and above would be the dimer, trimer or tetramer of the His-tagged SstT. Similar observations have been reported with other membrane proteins (*10, 11, 14, 15*).

Estimation of Molecular Mass of SstT—The deduced amino acid sequence of SstT suggested that the SstT protein consists of 414 amino acid residues, corresponding to a molecular mass of 43 kDa. This value is much higher than the apparent molecular mass of 33 kDa estimated in 12% SDS-PAGE. To determine the molecular mass of the purified His-tagged SstT protein, we performed Ferguson plot (*18*) analysis with the purified His-tagged SstT protein, calculated molecular mass of which is 44.7 kDa. The Ferguson plot shown in Fig. 5 indicates that the molecular mass of the His-tagged SstT protein is about 45 kDa, which is close to the calculated molecular mass of the His-tagged SstT, 44.7 kDa.

Abnormal electrophoretic behavior is often observed with integral membrane proteins (*21–28*), and is explained by increased binding of sodium dodecyl sulphate (SDS) due to the hydrophobic nature of such proteins. It is very likely that a similar phenomenon occurred in the case of the hydrophobic SstT protein.

N-Terminal Sequence of the Purified His-Tagged SstT—Analysis of amino acid residues at the N-terminal end of the purified His-tagged SstT protein showed that the purity of the protein is greater than 95%. The determined N-terminal sequence is T-T-Q-R-S-P-G-L-F-R. This amino acid sequence is identical to the sequence deduced from the *sstT* gene except for Met at N-terminus. Thus, it seems that the N-terminal Met residue of the SstT protein was excised. Hirel and his colleagues (*29*) reported the presence of a methionyl-aminopeptidase that catalyzes the excision of the N-terminal Met in *E. coli*. They suggested that the exci-

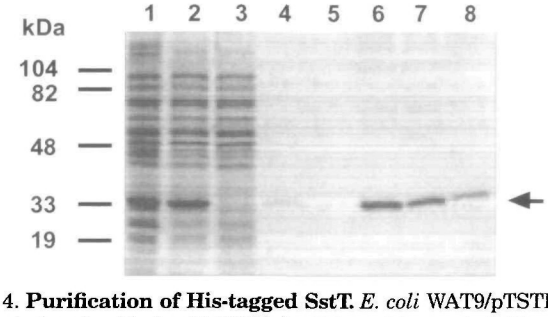

Fig. 4. **Purification of His-tagged SstT.** *E. coli* WAT9/pTSTH cells were induced with 1 mM IPTG for 3 h and harvested. Membrane vesicles were prepared from the cells, and membrane proteins were solubilized with OG. The His-tagged SstT was purified as described in "MATERIALS AND METHODS." The samples were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue. The arrow indicates the position of His-tagged SstT. Lane 1, membrane vesicles (40 μg protein); lane 2, solubilized membrane proteins (40 μg protein); lane 3, unbound fraction (40 μg protein); lanes 4 and 5, wash fraction; lanes 6 to 8, elution fraction.

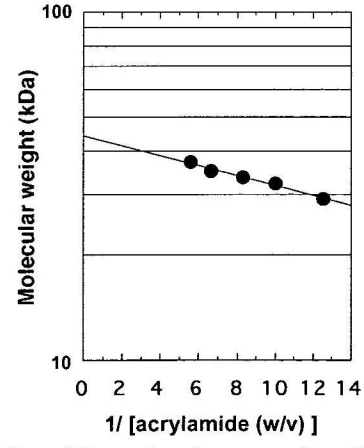

Fig. 5. **Estimation of the molecular mass of purified His-tagged SstT.** His-tagged SstT was subjected to SDS-PAGE at various concentrations of acrylamide. Apparent molecular mass of the His-tagged SstT were calculated from the mobility in each experiment. The apparent molecular masses were plotted against 1/[concentration of acrylamide in the gel (w/v)] as described by Ferguson (*18*).

sion of Met by the enzyme depends mainly on the nature of the second amino acid residue in the polypeptide chain, with Gly, Ala, Ser, Cys, Pro, or Thr in the second position facilitating the excision of the initiation Met residue. The second residue in the SstT is Thr. Thus, it is very likely that the observed N-terminal sequence of the purified His-tagged SstT was generated by the subsequent excision of the Met residue from the original M-T-T-Q-R-S-P-G-L-F-R- sequence by methionyl-aminopeptidase.

Reconstitution and Characterization of the His-Tagged SstT—The His-tagged SstT protein was reconstituted into liposomes by the detergent dilution method (*15*) to characterize the purified SstT protein. In our early studies, we succeeded in reconstituting proteoliposomes possessing serine transport activity using a solubilized membrane protein fraction (also shown in Fig. 6). Thus, it was clear that SstT can be solubilized in an active form. The purified protein was successfully reconstituted into liposomes and the resulting proteoliposomes showed serine transport activity when an inwardly directed Na^+ gradient and membrane potential (inside negative) were established (Fig. 6). On the other hand, no significant serine uptake was observed when Na^+ was not added to the assay medium.

We then tested the effects of ionophores on serine transport with the reconstituted proteoliposomes. Proteoliposomes were preloaded with potassium phosphate and diluted into a solution containing sodium phosphate, thus establishing an inwardly directed Na^+ gradient. This Na^+ gradient drove serine uptake (Fig. 7). The addition of valinomycin, an ionophore for K^+ that generates a membrane potential (inside negative) under these conditions, greatly enhanced serine uptake. On the other hand, serine uptake was inhibited by the addition of monensin, which disrupts the Na^+ gradient. The addition of CCCP, an ionophore for H^+, had no significant effect on serine uptake. These results are fully consistent with our previous report that SstT mediates Na^+/serine symport (cotransport) (*2*).

Table I summarizes the purification of the His-tagged SstT. The specific activity of the purified protein increased 8-fold compared with the solubilized fraction. We obtained 0.13 mg of purified protein from about 5 mg of membrane protein in one day.

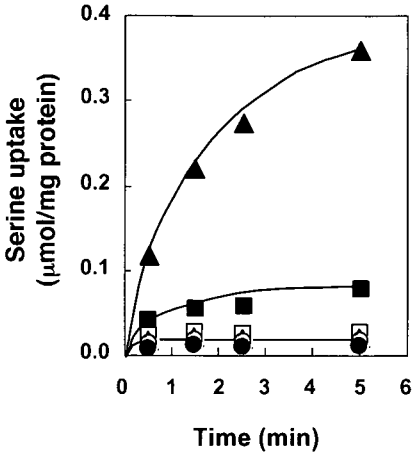

Fig. 6. **Serine transport in reconstituted proteoliposomes.** Proteoliposomes were reconstituted from purified His-tagged SstT and lipid as described in "MATERIALS AND METHODS," and serine uptake was measured. Open symbols indicate that the proteoliposomes were reconstituted in 0.1 M potassium phosphate (pH 7.0) and serine transport was measured in 0.1 M potassium phosphate (pH 7.0) in the presence of 2 μM valinomycin. Closed symbols indicate that proteoliposomes were reconstituted in 0.1 M potassium phosphate (pH 7.0) and serine transport was measured in 0.1 M sodium phosphate (pH 7.0) in the presence of 2 μM valinomycin. Circles, control (liposomes without protein); squares, proteoliposomes prepared with solubilized membrane proteins; triangles, proteoliposomes prepared with purified protein.

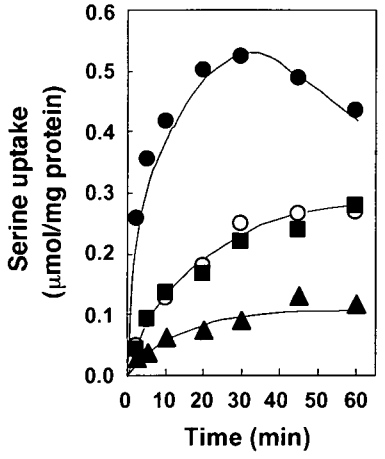

Fig. 7. **Effect of ionophores on serine transport in reconstituted proteoliposomes.** Proteoliposomes were reconstituted using purified His-tagged SstT and serine uptake was measured as described in "MATERIALS AND METHODS." Symbols are: ○, control (no ionophore); ●, +2 μM valinomycin; ■, +2 μM CCCP; ▲, +2 μM monensin.

TABLE I. **Purification of His-tagged SstT.**

Fraction	Protein (mg)	Specific activity (μmol/min/mg protein)	Purification rate (fold)	Yield (%)
Crude extract	1.56	0.05	1	100
Elution	0.13	0.40	8	60

These data are average values from three separate experiments.

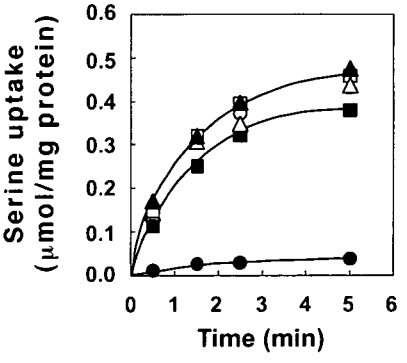

Fig. 8. **Effect of amino acids on serine transport in reconstituted proteoliposomes.** Proteoliposomes were reconstituted using purified His-tagged SstT, and serine uptake was measured as described in "MATERIALS AND METHODS" in the presence of 2 μM valinomycin. In these experiments, the concentration of [^{14}C]serine was 2 μM. Symbols are: ○, control; ●, +0.1 mM threonine; □, +0.1 mM D-serine; ■, +0.1 mM homoserine; △, +0.1 mM valine; ▲, +0.1 mM glycine.

The optimum ratio of protein to lipid for reconstitution was determined. The maximal serine transport activity was observed at a protein to lipid ratio of 1:800 (precise data not shown). Thus, reconstitution was carried out at this ratio.

Kinetic analysis of serine transport in the reconstituted proteoliposomes was performed (data not shown). Serine uptake in the proteoliposomes was saturable with K_m and V_{max} values of 0.82 μM and 0.37 μmol/min/mg protein, respectively.

As reported previously (*2*), SstT from *E. coli* is a serine-threonine transport system. As shown in Fig. 8, serine transport was strongly inhibited when proteoliposomes were preincubated with excess threonine. Other amino acids (D-serine, homoserine, glycine, valine) did not significantly inhibit serine transport.

We tested the effect of temperature on serine transport in the reconstituted proteoliposomes. The activity increased over the range from 15 to 40°C, and the maximal activity was observed at 40°C (data not shown). The activity decreased at higher temperatures. This result is consistent with the report of serine transport in *E. coli* by Lombardi and Kaback (*1*).

The effect of pH on serine transport in the reconstituted system was also investigated. The optimum pH was 7.0 (data not shown), with no big differences in serine transport between pH 6.0 and 7.0.

We also measured serine transport activity by counterflow assay in reconstituted proteoliposomes. We observed counterflow activity with proteoliposomes in the presence of Na^+ (data not shown).

The procedures for the overproduction, rapid and efficient purification of the SstT Na^+/serine symporter in a functional state, and reconstitution into proteoliposomes reported in this paper are useful for investigating the transport mechanism and structure–function relationship of SstT. The procedures also open the way for protein chemical and spectroscopic studies as well as for attempts at crystallization.

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