SUPPLEMENTARY METHODS

Transport Properties of Purified AspT

Proteoliposomes prepared with purified AspT were loaded with 100 mM L-aspartate/KOH and 50 mM potassium phosphate buffer (pH 7) or 50 mM L-alanine/KOH and 50 mM potassium phosphate buffer (pH 7) and suspended in an NMG-based medium (50 mM NMG sulfate; 50 mM NMG-phosphate [pH 7]). 100 μM L-[^{3}H]aspartate or 100 μM L-[^{3}H]alanine, respectively, was added to these prepared proteoliposomes. L-[^{3}H]aspartate or L-[^{3}H]alanine was loaded into the proteoliposomes by L-aspartate self-exchange. The incorporated material was readily expelled after the addition of excess unlabeled L-aspartate or L-alanine, as if L-[^{3}H]aspartate or L-[^{3}H]alanine had been taken up by an exchange reaction and alanine had been the counter-substrate for aspartate.

Temperature Dependence and Thermal Stability of AspT

Proteoliposomes were prepared as described in the materials and methods with loading buffer including 200 mM L-alanine, 50 mM NMG-phosphate, pH 7.0. To avoid heat denaturing of AspT, pre-incubation of liposome was skipped and the reaction was started by addition of AspT reconstituted liposome into pre-incubated buffer; 200 μM L-[^{3}H] aspartate, 50 mM NMG-phosphate, 50 mM NMG sulfate, 1 μM valinomycin (pH 7.0), which is adjusted to pH 7 at each temperature. The reaction was stopped after 10 sec.

Solubilized AspT was incubated at 30 to 60 °C. After the incubation, the solubilized AspT was mixed with cold potassium-phosphate buffer including 40 mM L-alanine and reconstituted into liposome. After reconstitution, proteoliposome loaded with 200 mM L-alanine and 50 mM NMG-phosphate (pH 7.0) were tested for residual transport activity by using simple filtration method. The reaction was initiated with assay buffer; 200 μM [^{3}H] L-aspartate, 50 mM potassium phosphate (pH 7.0), 50 mM K_2SO_4, 1 μM valinomycin and stopped after 15 sec. The membrane potential generated by the L-aspartate:L-alanine exchange reaction was eliminated by addition of valinomycin.

pH Dependence of AspT Transport

The purified AspT was incubated at either acidic or neutral pH, and then reconstituted into proteoliposomes at a lipid-to-protein ratio of 5000:1 (molar ratio) before the initiation of the assay. L-aspartate:L-alanine exchange experiments were carried out with AspT-reconstituted proteoliposomes loaded with 10 mM L-alanine and citrate-phosphate buffer (pH 4.0 to 8.0), the ionic strength of which was adjusted to 75 mM with KCl, whereas the external pH was changed from 4.0 to 8.0 by using citrate-phosphate buffer, the ionic strength of which was adjusted to 80 mM with KCl.

Substrate Protection of Solubilized AspT

The solubilized AspT was incubated with L-aspartate (5 mM, 10 mM, 20 mM) or L-alanine (50 mM, 100 mM, 200 mM) at 37°C for several minutes. After incubation, the solubilized AspT was mixed with cold potassium-phosphate buffer including 40 mM L-alanine and reconstituted into liposomes. After the reconstitution, remaining transport activities of AspT reconstituted into proteoliposomes loaded with 200 mM L -Alanine and 50 mM NMG-phosphate (pH 7.0) were examined by using simple filtration method (Nanatani, 2009). The reaction was initiated with addition of assay buffer; 50 mM potassium phosphate (pH 7.0), 50 mM K_2SO_4, including 1 μM valinomycin and 200 μM L-[^{3}H] aspartate, and stopped after 15 sec.

Kinetic Analysis of AspT Transport

We prepared proteoliposome loaded with 50 mM potassium phosphate (pH 7.0) including 100 mM L-aspartate or 100 mM L-alanine. The reaction was initiated with assay buffer; 50 mM potassium phosphate (pH 7.0), 50 mM K_2SO_4 including several concentration of [^{3}H] aspartate or [^{3}H] alanine, and stopped after 10 sec.

Substrate Screening of AspT

Proteoliposomes including loading buffer (L-aspartate self-exchange: 100 mM L-aspartate/KOH, 50 mM potassium phosphate [pH 7]; L-alanine self-exchange: 100 mM L-alanine/KOH, 50 mM potassium phosphate [pH 7]) were pre-incubated with assay buffer (50 mM potassium phosphate [pH 7] and 50 mM K_2SO_4) at 25°C. After pre-incubation, the self-exchange reaction was initiated with L-[^{3}H] aspartate (0.039 mM) or L-[^{3}H] alanine (2.9 mM) mixed with 15 mM of unlabeled amino acids, and stopped after 10 sec. To obtain clear inhibitory effect, the concentration of external L-[^{3}H] aspartate and L-[^{3}H] alanine was adjusted to which gives 1/10 V_{max}.

SUPPLEMENTAL FIGURES LEGENDS

Supplemental figure 1. Temperature dependence of AspT transport and thermal stability of solubilized AspT. (A) Initial rates of uptake over a temperature range of 25 to 65 °C, as measured by use of a filtration assay. Proteoliposomes were prepared as described in the Experimental Procedures. To avoid heat denaturing of AspT, pre-incubation of the liposome was omitted and the reaction was started by addition of the AspT-reconstituted liposome to the pre-incubated buffer (200 μM L-[^{3}H] aspartate; 50 mM NMG-phosphate; 50 mM NMG sulfate; 1 μM valinomycin; pH 7.0), which was adjusted to pH 7 at each temperature. The reaction was stopped after 10 s by filtration. (B) Thermal stability of AspT. Solubilized AspT was incubated at 30 to 60 °C for the maximum of 10 min. After this incubation, the solubilized AspT was mixed with cold potassium phosphate buffer that included 40 mM L-alanine and was reconstituted into a liposome. After the reconstitution, proteoliposome loaded with 200 mM L-alanine and 50 mM NMG-phosphate, (pH 7.0) was tested for residual transport activity by using the simple filtration method. The reaction was initiated with assay buffer [200 μM L-[^{3}H]aspartate, 50 mM potassium phosphate (pH 7.0), 50 mM K_2SO_4, 1 μM valinomycin] and stopped after 15 s. The electrochemical gradient generated by the aspartate: alanine exchange reaction was eliminated by the addition of valinomycin. Data are from three independent experiments and presented as the average with standard deviation (S.D.).

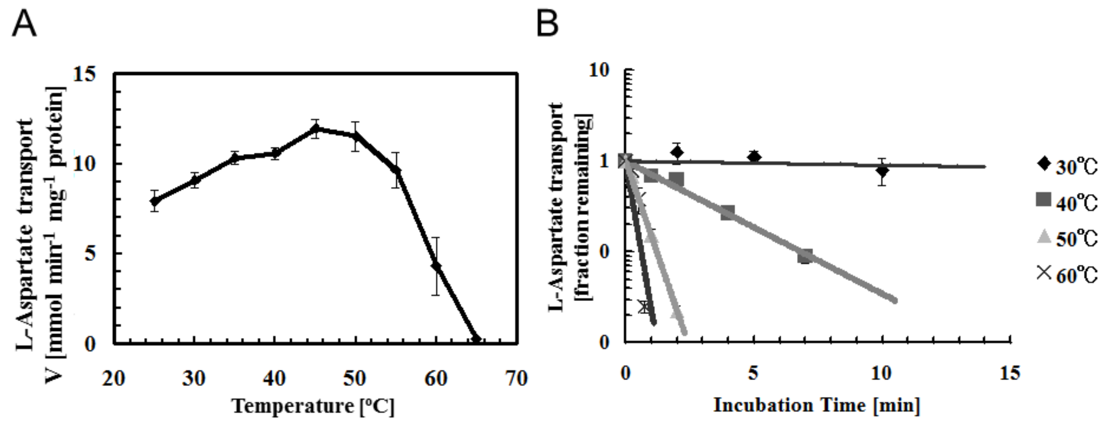
Supplemental Figure 2. Structures of compounds used in the inhibition experiments.

Supplemental figure 3.

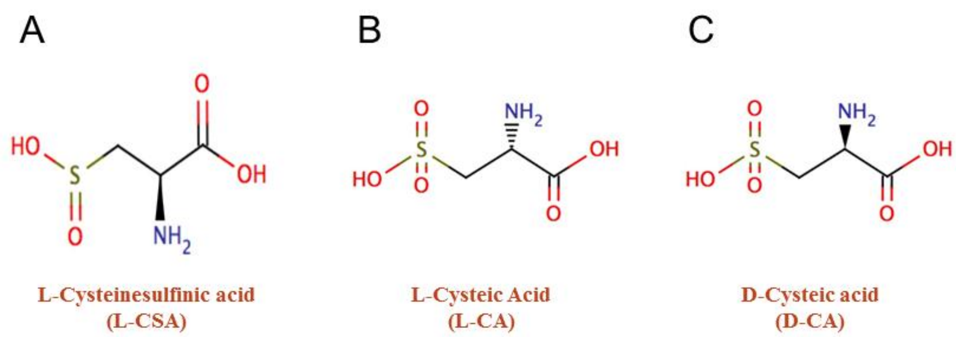
Proteoliposomes were loaded with 100 mM L-aspartate (A) or L-alanine (B) plus 50 mM potassium phosphate (pH 7) and then washed and resuspended as described in the Experimental Procedures. Proteoliposomes (◆, ■, ▲) were placed in 100 mM (A) or 50 mM (B) K_2SO_4 plus 50 mM potassium phosphate (pH 7) at 3.3 μg of protein/ml, at which point 100 μM L-[^{3}H]aspartate (A) or 100 μM L-[^{3}H]alanine (B) was added. To estimate substrate transport, aliquots were taken for filtration and washing at the times indicated. The *arrow* denotes the addition of buffer (◆), 15 mM unlabeled L-CSA (▲), L-CA(×) or D-CA(●).

SUPPLEMENTAL FIGURES

Supplemental figure 1



Supplemental figure 2



Supplemental figure 3