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Characterization of Spermidine Transport System in a Cyanobacterium, *Synechocystis* sp. PCC 6803

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The transport of spermidine into a cyanobacterium, *Synechocystis* sp. PCC 6803, was characterized by measuring the uptake of ^{14}C-spermidine. Spermidine transport was shown to be saturable with an apparent affinity constant (K_m) value of 67 μM and a maximal velocity (V_{max}) value of 0.45 nmol/min/mg protein. Spermidine uptake was pH-dependent with the pH optimum being 8.0. The competition experiment showed strong inhibition of spermidine uptake by putrescine and spermine, whereas amino acids were hardly inhibitory. The inhibition kinetics of spermidine transport by putrescine and spermine was found to be noncompetitive with K_i values of 292 and 432 μM, respectively. The inhibition of spermidine transport by various metabolic inhibitors and ionophores suggests that spermidine uptake is energy-dependent. The diminution of cell growth was observed in cells grown at a high concentration of NaCl. Addition of a low concentration of spermidine at 0.5 mM relieved growth inhibition by salt stress. Upshift of the external osmolality generated by either NaCl or sorbitol caused an increased spermidine transport with about 30–40% increase at 10 mosmol/kg upshift.

Keywords: Cyanobacterium, spermidine transport, osmotic upshift, *Synechocystis* sp. PCC 6803

Polyamines, represented by putrescine, spermidine, and spermine, are widely distributed from bacteria to higher organisms [8, 18, 24, 30]. Polyamines can bind easily to anionic constituents such as nucleic acids and phospholipids, and bind directly to some ion channels [35, 36]. Therefore, they are essential for regulating cell growth, development, and differentiation [28, 32]. Additionally, polyamines are involved in developmental processes, as well as responses of plants to environmental challenges [3]. The cytoplasmic polyamine level is actively regulated by *de novo* synthesis, degradation, and transport [33, 35].

Uptake systems specific for polyamines have been reported in both prokaryotic and eukaryotic cells. Extensive study on polyamine uptake was carried out in *Escherichia coli* where polyamine uptake depends on the proton potential and is energy-dependent [9]. In *Saccharomyces cerevisiae*, it was reported that the polyamine-specific transport system depends on a proton potential in the vacuolar membrane of this organism [12]. Because this pattern is so distinctive, cyanobacteria may become organisms of choice for the study of the metabolism and function of polyamines. The earliest study in *Anacystis nidulans* reported that spermidine and spermine were actively metabolized [23]. Although putrescine was quite inhibitory to growth of *Anacystis nidulans* [6], its presence at a low concentration could stimulate growth of *Synechocystis* sp. PCC 6803 under moderate salt stress [22]. The importance of polyamine uptake by living cells is far from clear, since all organisms contain enzymes necessary for their synthesis and maintain optimum levels of their metabolism. However, there has been substantial evidence suggesting an important role of polyamine uptake in the homeostasis of polyamine levels in the cells, especially under conditions where polyamine biosynthesis is partially or totally inhibited [16, 29].

Synechocystis sp. PCC 6803 appears to be a typical representative of the moderately salt-tolerant cyanobacteria able to accumulate glucosylglycerol as an osmoprotective compound [5]. Previously, we reported that the moderate osmotic stress imposed on *Synechocystis* cells led to the increase of both cellular putrescine contents [11] and putrescine uptake activity [22]. To better understand the polyamine uptake mechanism by living cells, we further studied spermidine transport in *Synechocystis* sp. PCC 6803 and found that the transport was specific for spermidine, with noncompetitive inhibition by putrescine and spermine.

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Its uptake activity depended on pH, electrochemical potential, and substrate concentration. The uptake could be stimulated by moderate osmotic upshift.

Materials and Methods

Organism and Culture Conditions

Axenic cells of *Synechocystis* sp. PCC 6803 were grown photoautotrophically in BG-11 medium [26] at 30°C under continuous illumination (warm white fluorescent tubes) of 50 μmol/m^2/s. The cultivation was done using cotton-plugged 250-ml conical flasks on a rotatory shaker at 160 rpm. The growth rate was monitored by measuring the optical density of the culture at 730 nm (OD730) with a Spectronic Genesys2 spectrophotometer.

Measurement of Polyamines

Polyamine levels in *Synechocystis* cells were determined by high performance liquid chromatography (HPLC model: Hewlett Packard Series 1050) after extraction with 5% perchloric acid and derivatization with benzoyl chloride as previously described [11]. Extraction of the cells by 5% perchloric acid effectively released soluble (free) polyamine into the extract. Repeated extraction of the pellet after centrifugation resulted in no further increase in the soluble polyamine. A C-18 reverse-phase column was used with a UV–Vis detector at 254 nm. Different classes of polyamine (i.e., putrescine, spermidine, and spermine) were identified and quantified using authentic polyamine standards (Sigma Chemicals, U.S.A.). The retention times for putrescine, spermidine, and spermine were 10.9, 14.9, and 17.5 min, respectively, upon elution with a 50–80% methanol gradient at a flow rate of 1 ml/min.

Transport Assays

Cells at late log phase were harvested by centrifugation (8,000 ×*g*, 10 min, 4°C), washed twice with 50 mM Hepes-KOH buffer, pH 7.6, containing 0.4% glucose, and suspended in the same buffer to yield a protein concentration of 0.1 mg cell protein/ml. The cell suspension (95 μl) was preincubated at 37°C for 5 min. The uptake experiment was initiated by the addition of 5 μl of ^{14}C-spermidine (spermidine-8-^{14}C trihydrochloride; Radiochemical Centre, Amersham, England) with a specific activity of 2 mCi/mmol at a final concentration of 50 μM. After incubation, the cells were rapidly collected on membrane filters (cellulose acetate, 0.45 μm pore size; Millipore Corp.). The filters were washed twice with 1 ml of cold buffer containing 1.0 mM spermidine to remove the adsorbed ^{14}C-spermidine. The amount of amine adsorbed to the cell surface and the filter was less than 0.1% of the added amine under these experimental conditions. The radioactivity on the filter was determined with a liquid scintillation counter (Beckman Coulter Multipurpose Scintillation Counter LS6500, U.S.A.). Initial spermidine uptake rates were determined from the linear increase of uptake and are expressed as nanomoles of spermidine taken up per minute per milligram protein, as determined by the method of Bradford [4] using bovine serum albumin as a standard. The osmolality of the uptake assay medium was measured with a Wescor vapor pressure osmometer model 5520. For the assay of inhibition by substrate analogs, cells were added to a mixture of labeled substrate and 20-fold excess of unlabeled analogs. In inhibitory assays, cells were preincubated with the inhibitor at 37°C for 30 min before the addition of ^{14}C-spermidine.

Results

Inhibition of Growth and Protection Against Salt Stress by Spermidine

Synechocystis grown in the medium supplemented with 550 mM NaCl showed a retardation of growth (Fig. 1A).

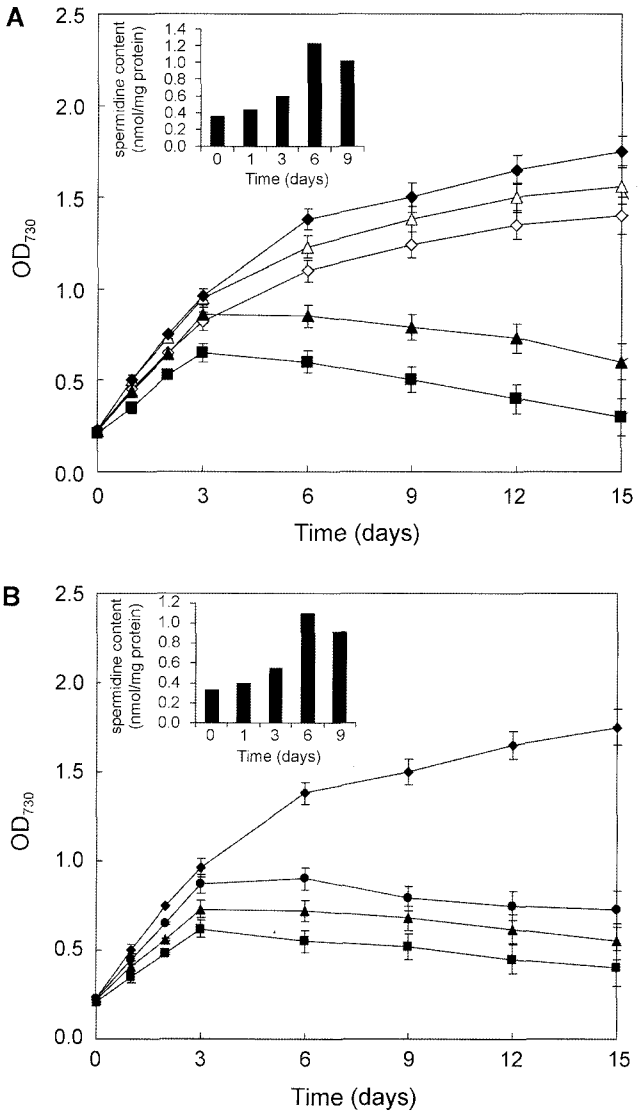

Fig. 1. Effect of exogenous spermidine on growth of *Synechocystis* sp. PCC 6803 under salt-stress (**A**) and non-stress (**B**) conditions.

A. Cells were grown under normal condition (◆), salt-stress condition by the addition of 550 mM NaCl (◇), 550 mM NaCl plus 0.5 mM spermidine (△), 550 mM NaCl plus 1.0 mM spermidine (▲), and 550 mM NaCl plus 2.0 mM spermidine (■). **B**. Cells were grown under normal condition without spermidine (◆), and with 0.5 (●), 1.0 (▲), and 2.0 mM (■) spermidine. The data are means from three independent experiments with vertical bars representing standard errors of the means, n=3. Insets show spermidine contents of cells during 9-day growth under salt-stress (Inset **A**) and non-stress (Inset **B**) conditions with 1.0 mM exogenous spermidine. The data are the average values from duplicate experiments with difference not exceeding 10%.

The addition of 0.5 mM spermidine to the growth medium relieved the inhibition of growth of *Synechocystis* by the 550 mM NaCl during the first 3 days of growth. This release of growth inhibition was not observed at 1.0 mM and 2.0 mM spermidine present in the medium. Moreover, after 3 days, the cessation of growth was noticeable in these cases (Fig. 1A). The intracellular contents of spermidine in *Synechocystis* grown with 550 mM NaCl and 1.0 mM spermidine were also determined. The inset of Fig. 1A shows that spermidine contents were slightly increased until 3 days of growth. Drastic increases in the levels of spermidine were observed at day 6 and day 9. When *Synechocystis* cells were grown in the absence of salt stress but supplemented with spermidine, cessation of growth occurred after 3 days for cells with 0.5, 1.0, and 2.0 mM spermidine (Fig. 1B). The profile of the contents of spermidine in cells grown with 1.0 mM exogenous spermidine (Fig. 1B, inset) was similar to that shown in Fig. 1A, inset.

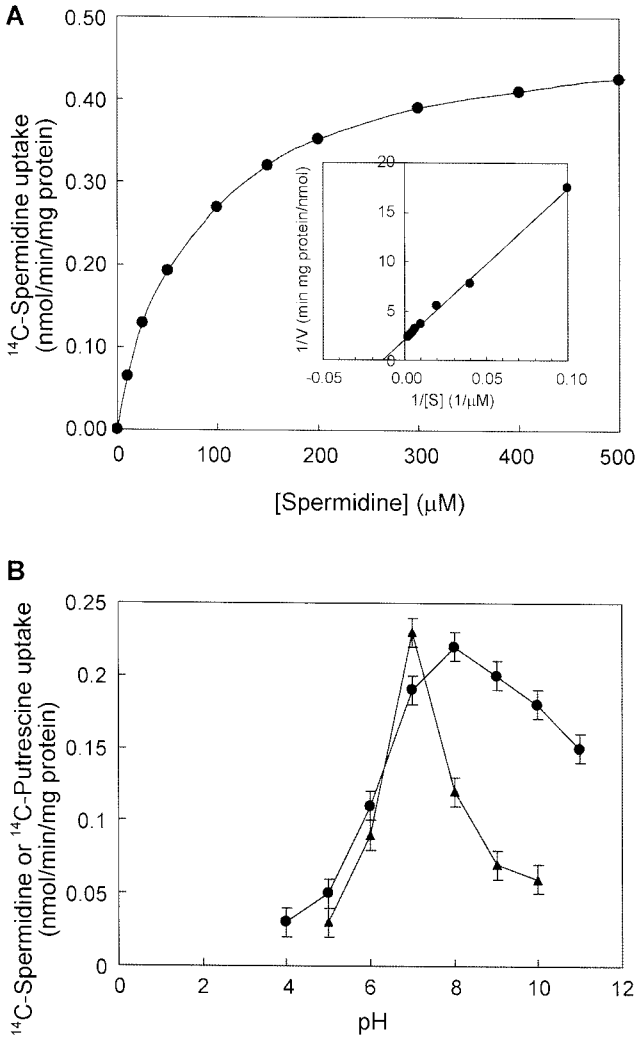

Fig. 2. Effects of spermidine concentration (**A**) and pH (**B**) on spermidine uptake by *Synechocystis* sp. PCC 6803.

A. Spermidine concentration was varied from 0–500 μM. Initial rates were determined with a 5 min incubation time. The inset represents a Lineweaver-Burk transformation of the data. The line drawn is that derived from regression analysis of the data and the points shown are typical of this result from triplicate experiments. **B**. Spermidine uptake (●) was assayed at different pHs, where 100 mM potassium citrate was used for buffer pH 4.0–6.0; 100 mM Tris-HCl for buffer pH 7.0–8.0; and 100 mM glycine-KOH for buffer pH 9.0–11.0. In addition, putrescine uptake (▲) was also done as described by Raksajit *et al.* [22] The data shown are the means of three independent experiments with vertical bars representing standard errors of the means, n=3.

Effects of Spermidine Concentration and pH on Spermidine Uptake

Incubation of *Synechocystis* cells with varying spermidine concentration from 0 to 500 μM resulted in saturable initial uptake (Fig. 2A). A Lineweaver-Burk transformation yielded a straight line (Fig. 2A, inset), indicating that the uptake follows typical Michaelis-Menten kinetics. The apparent affinity constant (K_m) value of 67±9 μM and the maximal velocity (V_{max}) value of 0.45±0.03 nmol/min/mg were obtained.

To investigate whether the extracellular pH influences the activity of spermidine uptake, *Synechocystis* cells were incubated at different extracellular pHs and the uptake of spermidine was determined. Fig. 2B shows changes in the spermidine uptake of *Synechocystis* as a function of extracellular pH, being optimal at around pH 8.0. The uptake of spermidine decreased with the decrease of external pH. Relatively high uptake activity was observed at alkaline pH as high as pH 10.0. The effect of pH on the uptake of putrescine was also tested and the results in Fig. 2B showed a narrow optimum pH of 7.0 for putrescine uptake. In contrast to spermidine uptake, the uptake of putrescine drastically decreased at alkaline pH.

Specificity of the Spermidine Transporter

To establish the specificity of the transporter, spermidine transport was measured in the presence of potential

Table 1. Effect of spermidine analogs on spermidine uptake of *Synechocystis* sp. PCC 6803.

Analogs	Concentration	Spermidine uptake (%)[a]
None	-	100±2
Agmatine	1 mM	89±6
L-Asparagine	1 mM	91±5
Cadaverine	1 mM	87±7
L-Lysine	1 mM	90±5
Ornithine	1 mM	87±3
Putrescine	1 mM	62±5
Spermine	1 mM	51±6

[a]Cells were incubated in the mixture containing 1 mM unlabeled analog and 50 μM ^{14}C-spermidine. The data shown are the means of three independent experiments representing the percent uptake rate relative to the control rate, which was 0.19±0.02 nmol/min/mg protein.

competitive substrates (Table 1). L-Lysine, a molecule comparable in size and charge to spermidine, did not inhibit spermidine uptake. The basic amino acid L-asparagine showed no significant inhibition of spermidine uptake. Cadaverine, a diamine found in plants but not as widely distributed as putrescine, did not inhibit spermidine uptake. Inhibition of spermidine uptake was hardly observed in the presence of ornithine, a precursor of polyamines. Agmatine, a biogenic amine formed by the decarboxylation of arginine did not affect spermidine uptake. In contrast, putrescine and spermine, which are structurally similar to spermidine, showed about 40% and 50% inhibition, respectively, suggesting that the same transport system is likely shared by putrescine, spermidine, and spermine.

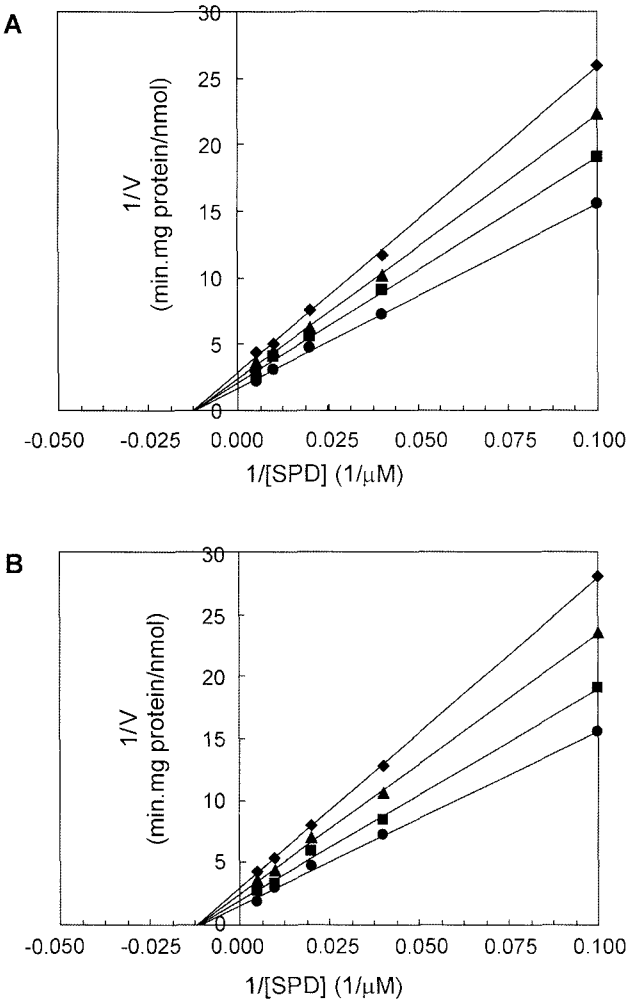

Fig. 3. Lineweaver-Burk plots for the inhibition of spermidine transport in *Synechocystis* sp. PCC 6803 by putrescine (**A**) and spermine (**B**).
The inhibition kinetics was determined without inhibitors (●) or with three different concentrations, 0.5 mM (■), 1.0 mM (▲), and 2.0 mM (◆), of putrescine and spermine. Inhibition was found to be noncompetitive with apparent K_i values of 292 and 432 μM for putrescine and spermine, respectively.

We further assessed the transporter specificity through the competitive inhibition by measuring the spermidine uptake rate. The plot between inhibitor concentrations and the slopes obtained from double-reciprocal plots of spermidine concentrations and uptake rates yielded the approximate value of inhibition constant, K_i (data not shown). The K_i values of putrescine and spermine were 292 and 432 μM respectively. Inhibition of spermidine transport by either putrescine or spermine was found to be noncompetitive (Figs. 3A and 3B), indicating that the binding of putrescine and spermine to the transporter occurred at site(s) distinct from that of spermidine.

Effects of Metabolic Inhibitors, Ionophores, and ATPase Inhibitors on Spermidine Transport

To evaluate the role of ATP and proton motive force in energizing the uptake of spermidine, the effects of some inhibitors on spermidine uptake were investigated. As shown in Table 2, *N*-ethylmaleimide (NEM) and *p*-chloromercurisulfonic acid (PCMS), which are the protein structure modifiers, markedly reduced spermidine uptake. Chloramphenicol inhibited spermidine uptake, indicating that protein synthesis is required for functional spermidine transport. The inhibitors for ATP formation, sodium arsenate and sodium fluoride, also decreased the uptake activity suggesting the requirement of ATP for spermidine uptake. The role played by the electrochemical gradient on transport was assessed by disrupting the transmembrane potential and proton-dependent processes [20]. Potassium cyanide,

Table 2. Effects of metabolic inhibitors on spermidine uptake of *Synechocystis* sp. PCC 6803.

Inhibitors	Concentration	Spermidine uptake (%)[a]
None	-	100±2
Chloramphenicol	1 μg/ml	64±9
Nigericin	10 μM	60±13
Valinomycin	10 μM	48±4
Amiloride	100 μM	57±8
CCCP	100 μM	63±3
DCCD	100 μM	68±7
Gramicidin D	100 μM	56±1
Monensin	100 μM	50±2
Sodium arsenate	1 mM	70±7
KCN	1 mM	43±6
DNP	1 mM	56±6
NaF	1 mM	51±8
NEM	1 mM	48±13
Ouabain	1 mM	53±17
PCMS	1 mM	45±10
Orthovanadate	1 mM	54±7

[a]Cells were preincubated with inhibitors for 30 min before the addition of 50 μM ^{14}C-spermidine. The data shown are the means of three independent experiments representing the percent uptake rate relative to the control rate, which was 0.19±0.02 nmol/min/mg protein.

an inhibitor of the electron transport chain, caused effective inhibition of spermidine uptake. Transport uncouplers such as gramicidin D and dinitrophenol, which dissipate the proton motive force, could significantly inhibit spermidine uptake to a similar extent. Valinomycin, an ionophore that permeabilizes the membrane to potassium and has been proposed to affect the $\Delta\psi$, strongly inhibited spermidine uptake. Strong inhibition of spermidine uptake was also observed with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and nigericin.

N,N'-dicyclohexylcarbodiimide (DCCD), a specific inhibitor for the H^+-ATPase, and orthovanadate, an inhibitor of P-type ATPase, also effectively inhibited spermidine uptake. Furthermore, spermidine uptake was inhibited by amiloride, a potent inhibitor of many Na^+-coupled transport systems including the Na^+/H^- antiporter. Reagents that interfere with the transmembrane sodium ion gradient (monensin, a sodium ionophore; ouabain, an inhibitor of the plasma membrane Na^+/K^+-ATPase) caused an effective reduction of spermidine uptake. Judging from the sensitivity of spermidine transport to various types of inhibitors, this system was ATP-dependent, requiring the proton motive force with the contribution of both ΔpH and $\Delta\psi$.

Effect of Osmotic Upshift on Spermidine Uptake

In order to determine whether the spermidine transport in *Synechocystis* is affected by the osmotic upshift, the effect of external osmolality generated by either NaCl or sorbitol on spermidine uptake was extensively examined. The uptake was very sensitive to osmotic upshift (Fig. 4). A small increase of the osmolality up to 10 mosmol/kg resulted in about 30–40% increase of spermidine uptake. Elevation of osmotic upshift higher than 10 mosmol/kg caused a progressive decline in the extent of the stimulation of the uptake. An upshift higher than 100 mosmol/kg caused a drastic reduction of uptake activity (data not shown). It is noteworthy that the uptake of spermidine responded to changes in osmolality with similar pattern regardless of the source generating the osmotic upshift.

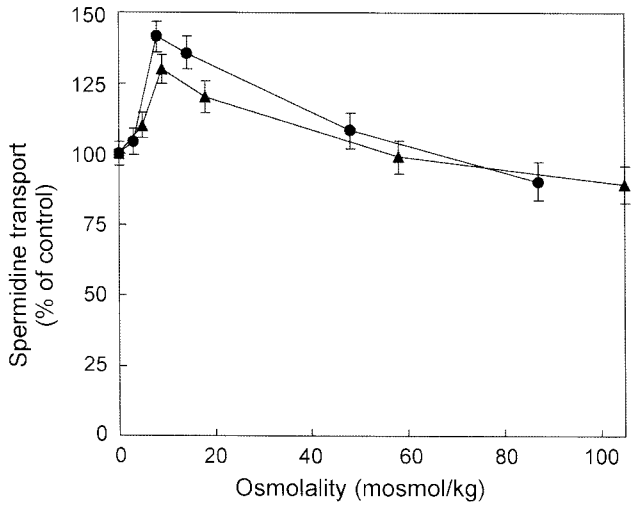

Fig. 4. Effect of external osmolality on spermidine uptake by *Synechocystis* sp. PCC 6803.

Initial uptake rates were determined in the presence of increasing osmolality generated by NaCl (●) or sorbitol (▲). The data are means from three independent experiments representing the percent uptake rate relative to the control rate, which was 0.19±0.02 nmol/min/mg protein.

DISCUSSION

Here, we have demonstrated the existence of a transport system for spermidine in *Synechocystis* sp. PCC 6803. We reported previously that there exists an accumulation of putrescine in *Synechocystis* by biosynthesis [11] and transport from the environment [22]. In the present study, exogenous spermidine could be taken up by *Synechocystis* under non-stress and salt-stress conditions. The data in Fig. 1A indicated that exogenous spermidine was transported into *Synechocystis* cells and could act as a growth promoter in the presence of high salt concentration. This seems to suggest the role of spermidine as a compatible solute in this cyanobacterium. In spite of this, the protective role by spermidine against inhibition of growth under osmotic stress was not as efficient as that of glucosylglycerol, suggesting that spermidine per se is not an osmoprotectant [5]. It was observed that the growth of *Synechocystis* was markedly inhibited by a higher concentration of spermidine, similar to that found in *Anacystis nidulans* [23]. This could be ascribed to the toxic effects caused by the accumulation of polyamines. Polyamines were able to disrupt several metabolic functions within the cells by inhibition of protein synthesis accompanied by an irreversible dissociation of ribosomes [23]. The cytotoxic effects were observed in *Chlamydomonas reinhardtii* when exogenous putrescine was higher than 1.5 mM [31]. Indeed, we found that *Synechocystis* cells grown in the presence of 1.0 mM spermidine could accumulate a high content of spermidine regardless of salt-stress condition (insets of Figs. 1A and 1B). It is noted that spermidine content at day 9 of growth was slightly lower than that at day 6 of growth. There is a possibility that *Synechocystis* cells could excrete spermidine, and this has been recently reported in *Escherichia coli* [7]. During 9-day growth, very low levels of putrescine and spermine could be detected in cells under both non-stress and salt-stress conditions. At present, it is unclear why a low concentration of exogenous spermidine at 0.5 mM afforded protection of growth under salt-stress but not under non-stress conditions (Figs. 1A and 1B). To answer this, further studies concerning the interactions of positively charged spermidine with negatively charged ions such as Cl^- are needed.

Kinetic analysis of spermidine uptake revealed that the uptake was saturable with Michaelis-Menten relationship.

The spermidine transport kinetics of *Synechocystis*, as shown in Fig. 2A, would seem to indicate that the transport is a carrier-mediated process; however, the transport had a low affinity (K_m 67 μM) for spermidine. A similar situation was observed for putrescine transport with the K_m value of 92 μM in *Synechocystis* [22]. The low affinity for spermidine transport indicated the slow uptake of spermidine by *Synechocystis* cells. This was corroborated by the observations that a rather long period of exposure to spermidine was required for growth inhibition and growth stimulation to manifest in the presence of 1.0 and 0.5 mM spermidine, respectively (Fig. 1A).

Importantly, we found that spermidine transport into *Synechocystis* retained high activity at alkaline pH, and a drastic decrease of activity occurred when the pH became more acidic (Fig. 2B). The highest uptake occurred at pH 8.0, similar to that of sea water red alga *Ulva rigida* [1]. The dependence of polyamines uptake on the extracellular pH was previously reported in *Leishmania* [2] and also in Wistar rats [15]. Worth noticing is that, at alkaline pH, *Synechocystis* cells preferentially took up spermidine rather than putrescine (Fig. 2B). The effect of pH on the uptake activity of the transporter is due to changes in the protonation state of amino acids residing at the binding site of the transporter. The different pattern of uptake of putrescine and spermidine, especially at alkaline pH, suggested that these two polyamines bound at distinct sites on the transporter. This was substantiated by the results showing noncompetitive inhibition of spermidine transport by putrescine (Fig. 3A).

The uptake system for spermidine in *Synechocystis* sp. PCC 6803 is an active transport. Spermidine transport was highly inhibited by various energy generation inhibitors (Table 2), suggesting that the transport system is energy-dependent. The coupling mechanism between the Na^+ electrochemical gradient and transport systems has been extensively studied. Partial Na^+-dependency has been interpreted as indicating the presence of two transporters, one Na^+-dependent, and the other Na^+-independent [21, 34]. The Na^+-gradient has been shown to be a major source of energy, coupling with the active transport for polyamine in mouse cells [25]. Moreover, the dependency on Na^+ has been reported for the transport of choline into a halotolerant cyanobacterium, *Aphanothece halophytica* [10]. The addition of sodium ionophores, namely amiloride and monensin, significantly diminished spermidine uptake; however, it is premature to suggest the involvement of a Na^+-gradient in the transport of spermidine into *Synechocystis* cells. DCCD, a H^+-ATPase inhibitor, inhibited spermidine transport by 30%. A similar effect was observed with ionophores; namely valinomycin, CCCP, as well as an electroneutral K^+/H^+ antiporter, nigericin. It was likely that the uptake of spermidine was dependent upon the proton motive force with the contribution of both ΔpH and $\Delta\psi$ for the uptake. Similar evidence has been reported in *Saccharomyces cerevisiae* [12], *Escherichia coli* [14], and *Drosophila melanogaster* [27]. Moreover, preincubation of cells with chloramphenicol (100 μg/ml) resulted in a significant decrease of spermidine transport. This suggests that the transport appeared to be dependent on *de novo* synthesis of a transport protein or protein(s) regulating the activity of preexisting transport protein(s).

In some mammalian cell lines, such as rat pneumocytes [13, 37], polyamine transport has been described to share a highly specific transport system by the different polyamines. However, many different polyamine transport systems have been described in hamster ovary [17] and in *Escherichia coli* [9]. The present study revealed that putrescine and spermine were able to significantly inhibit the uptake of spermidine in *Synechocystis* cells by about 40 % and 50 %, respectively (Table 1). In addition, kinetics study of inhibition of spermidine uptake showed that putrescine and spermine were noncompetitive inhibitors. Taken together, this suggests that both putrescine and spermine share the same transport system as that of spermidine. However, both putrescine and spermine bound to site(s) distinct from that of spermidine. Stronger binding and uptake activities of that PotD protein (a periplasmic substrate binding protein) toward spermidine than putrescine has previously been reported in *E. coli* [8]. It was further shown that spermidine interacted with Glu171, Trp255, and Asp257 on the PotD protein, whereas putrescine was found to bind to PotD at the position occupied by the diaminobutane moiety of the spermidine molecule. Currently, we have isolated PotD from *Synechocystis* sp. PCC 6803, and experiments on polyamine binding revealed that PotD could bind both putrescine and spermidine with a slightly higher activity and affinity toward the latter (Yodsang, P. *et al.*, unpublished data). These results of PotD binding are in line with the K_m and V_{max} values of putrescine transport [22] and spermidine transport [this study] by *Synechocystis* sp. PCC 6803. Many reports suggest that polyamines are involved in plant stress response including ionic homeostasis within cells disturbed by osmotic stress [3]. Upon an upward shift in osmolality, *Synechocystis* sp. PCC 6803 was able to take up spermidine into the cells, reaching a maximal level with approximately 30–40% increase at 10 mosmol/kg (Fig. 4). The increase of spermidine transport in the presence of low concentration of both NaCl and sorbitol was a result of an osmotic effect rather than an ionic effect, supporting a role of spermidine in short-term osmolality stress response (Fig. 4). This was in line with results showing that sorbitol with no ionic effect also produced a similar pattern of stimulation seen for NaCl. It is noted that spermidine uptake could be detected in the absence of osmotic upshift and that Na^+ was not required for spermidine uptake. Without stress, this low level of spermidine taken up might serve as nutrient for metabolic function. Elevation of osmolality led to an increase in the transport of spermidine. Thus, it seems likely that cells require spermidine to better thrive against osmotic upshift.

A decline in spermidine uptake was detected at higher osmolality. This was probably due to less energy available for transport as a consequence of impaired metabolic function. Taken together, all these results indicated that spermidine transport in *Synechocystis* was efficient at moderate osmolalities. Similar observation was reported in *Escherichia coli* [19]. It is worth mentioning that the slight stimulation of spermidine transport by both NaCl and sorbitol could not be ascribed to the osmotic or salt-stress effect. The enhancement of spermidine uptake might reflect a better physiological state of *Synechocystis* cells rather than the direct effect on the uptake system. This observation would not be related to salt or osmotic acclimation since no parallel increase in the uptake was observed with the increase in the external osmolality. Indeed, a progressive decline in spermidine uptake was evident at greater than 100 mosmol/kg osmolality (data not shown). Further work on the characterization of genes as well as the transporters involved in spermidine uptake in *Synechocystis* sp. PCC 6803 is in progress.

Acknowledgments

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