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NH_4^+ transport system of a psychrophilic marine bacterium, *Vibrio* sp. strain ABE-1

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Abstract NH_4^+ transport system of a psychrophilic marine bacterium *Vibrio* sp. strain ABE-1 (*Vibrio* ABE-1) was examined by measuring the uptake of [^{14}C]methylammonium ion ($^{14}CH_3NH_3^+$) into the intact cells. $^{14}CH_3NH_3^+$ uptake was detected in cells grown in medium containing glutamate as the sole nitrogen source, but not in those grown in medium containing NH_4Cl instead of glutamate. *Vibrio* ABE-1 did not utilize $CH_3NH_3^+$ as a carbon or nitrogen source. NH_4Cl and nonradiolabeled $CH_3NH_3^+$ completely inhibited $^{14}CH_3NH_3^+$ uptake. These results indicate that $^{14}CH_3NH_3^+$ uptake in this bacterium is mediated via an NH_4^+ transport system and not by a specific carrier for $CH_3NH_3^+$. The respiratory substrate succinate was required to drive $^{14}CH_3NH_3^+$ uptake and the uptake was completely inhibited by KCN, indicating that the uptake was energy dependent. The electrochemical potentials of H^+ and/or Na^+ across membranes were suggested to be the driving forces for the transport system because the ionophores carbonylcyanide *m*-chlorophenylhydrazone and monensin strongly inhibited uptake activities at pH 6.5 and 8.5, respectively. Furthermore, KCl activated $^{14}CH_3NH_3^+$ uptake. The $^{14}CH_3NH_3^+$ uptake activity of *Vibrio* ABE-1 was markedly high at temperatures between 0° and 15°C, and the apparent K_m value for $CH_3NH_3^+$ of the uptake did not change significantly over the temperature range from 0° to 25°C. Thus, the NH_4^+ transport system of this bacterium was highly active at low temperatures.

Key words Psychrophilic bacterium · *Vibrio* · NH_4^+ transport system · $^{14}CH_3NH_3^+$ uptake · Nitrogen source for growth

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Introduction

Nitrogen is one of the most abundant elements in cells and a major constituent of various biological molecules including proteins and nucleic acids. Thus, living organisms require this element as an essential nutrient. Bacterial cells utilize various forms of nitrogen in compounds such as amino acids, ammonium and nitrate ions, and nitrogen gas as nitrogen sources. Among these, ammonium ion is known to be available as the sole nitrogen source for many bacteria (Brock and Madigan 1991). NH_4^+ transport systems therefore should play important roles in bacterial nitrogen metabolism. In fact, energy-dependent NH_4^+ transport systems have been found in several bacteria (Kleiner 1985, 1993). However, in spite of their significance, bacterial NH_4^+ transport systems are less well characterized than the transport systems for other cations such as Na^+ and K^+.

Since it was demonstrated that methylammonium ($CH_3NH_3^+$) could be incorporated by the NH_4^+ transport systems (Hackette et al. 1970; Stevenson and Silver 1977), $^{14}CH_3NH_3^+$ has been exclusively employed as a very useful radioactive analog of NH_4^+ in studies of bacterial NH_4^+ transport systems (Kleiner 1985). Because a psychrophilic marine bacterium *Vibrio* sp. strain ABE-1 (*Vibrio* ABE-1) can utilize NH_4^+ as the sole nitrogen source (Hakeda and Fukunaga 1983), this bacterium is expected to possess an NH_4^+ transport system. As the first step in characterization of the NH_4^+ transport system of *Vibrio* ABE-1, we examined the uptake of $^{14}CH_3NH_3^+$ into intact cells. The NH_4^+ transport system of this bacterium was shown to be energy dependent and exhibited psychrophilic properties.

Materials and methods

Bacterial strain and growth conditions

The psychrophilic marine bacterium *Vibrio* sp. strain ABE-1 (*Vibrio* ABE-1) (Takada et al. 1979) was precultured at 15°C for 48 h in a synthetic Tris-salts medium (pH 7.5)

(Hakeda and Fukunaga 1983) containing 20 mM NH_4Cl as the nitrogen source with vigorous shaking. One milliliter of the preculture was inoculated into fresh Tris-salts medium (100 ml) containing 20 mM sodium glutamate instead of NH_4Cl as the nitrogen source, and the bacterium was cultured at 15°C for 120 h with vigorous shaking. Bacterial growth was monitored by measuring the turbidity at 600 nm with a Shimadzu spectrophotometer model UV-100 (Kyoto, Japan).

Preparation of cell suspension

The bacterial cells were harvested at late exponential phase of growth ($OD_{600} \cong 1.5$) and washed three times with an assay buffer consisting of 50 mM HEPES-NaOH, 0.5 M Nacl, 0.1 M KCl, 2 mM $MgCl_2$, and 10% (v/v) glycerol (pH 7.5). The washed cells were resuspended in the same buffer at a concentration of 1 mg protein ml^{-1}. To examine the pH dependence of $^{14}CH_3NH_3^+$ uptake, 50 mM Tricine-NaOH (pH 8.0 and 8.5), HEPES-NaOH (pH 7.0, 7.5, and 8.0) or MES-NaOH (pH 6.0, 6.5, and 7.0) was used instead of 50 mM HEPES-NaOH in the assay buffer. KCl was excluded from the assay buffer when the effect of KCl on $^{14}CH_3NH_3^+$ uptake was examined. The cell suspension was stored on ice until use. $^{14}CH_3NH_3^+$ uptake into the cells was assayed within several hours after preparation of the cell suspension as described next.

$^{14}CH_3NH_3^+$ uptake

NH_4^+ transport activity was determined by measuring $^{14}CH_3NH_3^+$ uptake into the intact cells (Barns and Zimniak 1981). Unless otherwise stated, $^{14}CH_3NH_3^+$ uptake was assayed at 15°C. To energize the cells, aliquots of 200 μl of the cell suspension were mixed with 200 μl of 0.2 M disodium succinate, and the assay mixture was incubated for 10 min at 15°C. The mixture was dispensed in 100-μl portions into test tubes, and the reaction was started by addition of 5 μl of 312.5 μM $^{14}CH_3NH_3^+$ (1.48 GBq $mmol^{-1}$; final concentration, 14.88 μM). After incubation for the desired periods, the reaction was terminated by dilution with 3 ml of ice-cold wash buffer [50 mM HEPES-NaOH, 0.5 M NaCl, 50 mM KCl, 2 mM $MgCl_2$, and 10% (v/v) glycerol (pH 7.5)], and the cells were immediately collected by filtration with a nitrocellulose filter (Advantec, Tokyo, Japan; pore size, 0.45 mm). The cells on the filter were washed twice with 3 ml ice-cold wash buffer, dried, and transferred to vials; 5 ml liquid scintillation fluid [0.4% (w/v) 2,5-diphenyloxazole, and 0.05% (w/v) 2,2′-*p*-phenylenebis(5-phenyloxazole) in toluene] was then added to each vial and the radioactivity was determined with an Aloka liquid scintillation system LSC-3500 (Mitaka, Japan).

When the effect of pH on $^{14}CH_3NH_3^+$ uptake was examined, 50 mM Tricine-NaOH (pH 8.0 and 8.5), HEPES-NaOH (pH 7.0, 7.5, and 8.0), or MES-NaOH (pH 6.0, 6.5, and 7.0) was used instead of 50 mM HEPES-NaOH in the wash buffer.

Protein determination

Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Chemicals

[^{14}C]$CH_3NH_2 \cdot HCl$ (2.2 GBq $mmol^{-1}$) was obtained from New England Nuclear (Wilmington, DE, USA); MES, HEPES, Tricine, and KCN were from Nacalai Tesque (Kyoto, Japan); and carbonylcyanide *m*-chlorophenylhydrazone (CCCP) and monensin were from Sigma (St. Louis, MO, USA). All other reagents used were of analytical grade.

Results

Effect of nitrogen or carbon source on the growth of *Vibrio* ABE-1

It has been reported that the synthesis of most bacterial ammonium transport systems is repressed in cells grown on high concentrations of NH_4^+ (Kleiner 1985, 1993). Furthermore, several bacteria possess a specific carrier for $CH_3NH_3^+$ distinct from the NH_4^+ transport system and are consequently able to grow using $CH_3NH_3^+$ as the sole carbon or nitrogen source (Bellion et al. 1980; Bellion and Wayland 1982; Brooke and Attwood 1984; Glenn and Dilworth 1984). Therefore, the growth of *Vibrio* ABE-1 in synthetic media containing various nitrogen or carbon sources was examined.

As described previously (Hakeda and Fukunaga 1983), this bacterium grew well in Tris-salts medium containing 100 mM sodium succinate and 20 mM NH_4Cl as carbon and nitrogen sources, respectively (succinate-NH_4^+ medium) (Fig. 1). In addition, it was also able to utilize glutamate as a nitrogen source, but the growth rate was considerably lower than that in succinate-NH_4^+ medium. On the other hand, no growth was observed when 20 mM $CH_3NH_2 \cdot HCl$ was used as the nitrogen source. To determine whether $CH_3NH_2 \cdot HCl$ can be utilized as the carbon and nitrogen source, sodium succinate, a major carbon source, and NH_4Cl were replaced by 100 mM $CH_3NH_2 \cdot HCl$. *Vibrio* ABE-1 showed only poor growth for the initial period of incubation in this medium. When sodium succinate was eliminated from the succinate-NH_4^+ medium, essentially the same pattern of growth was observed. These results suggest that the initial growth observed in the $CH_3NH_2 \cdot HCl$ media was probably the result of utilization of a small amount of a chelator, 3 mM sodium citrate, but not $CH_3NH_2 \cdot HCl$. Therefore, we concluded that $CH_3NH_3^+$ cannot be utilized as a nitrogen and carbon source by *Vibrio* ABE-1.

$^{14}CH_3NH_3^+$ uptake by *Vibrio* ABE-1

The NH_4^+ transport system of *Vibrio* ABE-1 grown on glutamate as a nitrogen source was examined at 15°C at pH

6.5, 7.5, and 8.5 by measuring the uptake of the NH_4^+ analog $^{14}CH_3NH_3^+$ into the intact cells (Fig. 2). At all pH values tested, $^{14}CH_3NH_3^+$ uptake into the cells was observed, and the amount of incorporated $^{14}CH_3NH_3^+$ increased linearly with time for at least 10 min. On the other hand, no $^{14}CH_3NH_3^+$ uptake was observed in the cells grown on NH_4Cl as the sole nitrogen source. The uptake activity at pH 8.5 was less stable than that at pH 6.5 and 7.5, and about 15% of the activity was lost by storage for 8 h at 0°C. Therefore, the $^{14}CH_3NH_3^+$ uptake was assayed immediately after preparation of the cell suspension.

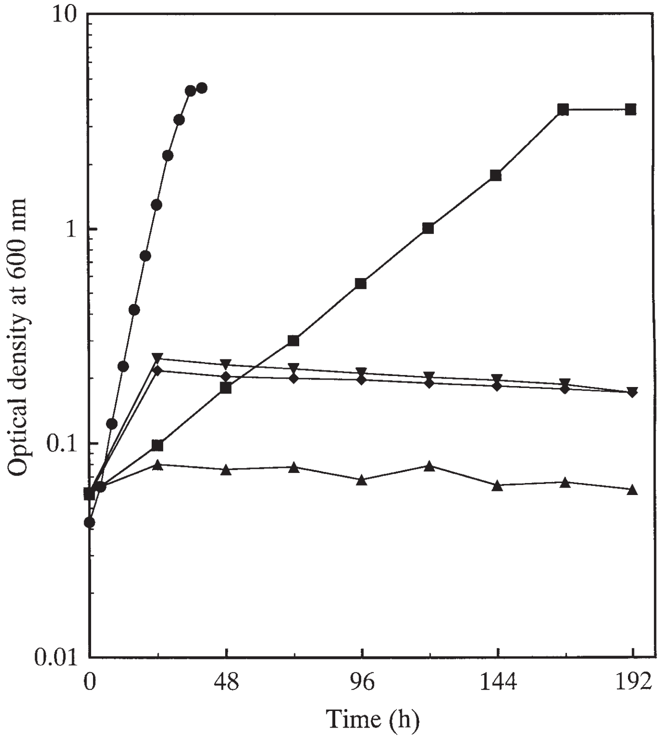

Fig. 1. Growth of *Vibrio* ABE-1 on various nitrogen and carbon sources. *Vibrio* ABE-1 was grown at 15°C in Tris-salts medium containing 20 mM NH_4Cl (*circles*, *inverted triangles*), 20 mM glutamate (*squares*), or 20 mM (*triangles*) and 100 mM (*diamonds*) of $CH_3NH_2 \cdot HCl$ as the nitrogen source. For *inserted triangles* and *diamonds*, 100 mM succinate was removed from the medium

No $^{14}CH_3NH_3^+$ uptake was detected unless the cells were preincubated with the respiratory substrate succinate (Fig. 2). Furthermore, despite the low concentration (0.1 mM), a respiratory inhibitor, KCN, completely blocked the uptake activity at pH 6.5 and 8.5 (Table 1).

These results indicate that the uptake is energy dependent. The energization of the cells necessary for $^{14}CH_3NH_3^+$ uptake was completed by brief incubation of the cell suspension with succinate for 5 min, and further incubation until 30 min did not accelerate the uptake rate (data not shown). On the other hand, the H^+ ionophore CCCP, even at a low concentration (1 μM), completely inhibited uptake activity at pH 6.5 (Table 1). At pH 8.5, the uptake activity seemed to be somewhat more resistant to CCCP than that at pH 6.5. In contrast, a Na^+ ionophore, monensin, blocked the uptake activity more strongly at pH 8.5 than at pH 6.5. These results imply that the electrochemical potentials of H^+ and/or Na^+ across membranes ($\Delta\tilde{\mu}_{H^+}$ and/or $\Delta\tilde{\mu}_{Na^+}$) drive the uptake.

The $^{14}CH_3NH_3^+$ uptake was stimulated by 50 mM KCl, in particular at pH 8.5 (Fig. 2). The effect of KCl on the $^{14}CH_3NH_3^+$ uptake was further examined by addition of KCl to the cell suspension prepared with KCl-free assay buffers (Fig. 3). Preincubation of the cell suspension with KCl for 10 min at 0°C was required for full uptake activity at pH 8.5 but not at pH 6.5 (data not shown). Therefore, KCl was added to the suspension at a suitable time point for the assay at the respective pH (Fig. 3). The maximum activities

Fig. 2A–C. $^{14}CH_3NH_3^+$ uptake by the intact cells of *Vibrio* ABE-1 at 15°C. The cell suspension was prepared by using either of the assay buffers (pH 6.5, **A**; pH 7.5, **B**; pH 8.5, **C**) as described in Materials and methods except that for *triangles* the harvested cells were washed with and suspended in assay buffers excluding KCl. After the cell suspension was incubated at 15°C for 10 min with 100 mM succinate (*circles*, *triangles*) or for 30 min with 0.4 M NaCl (*squares*), the reaction was started by addition of $^{14}CH_3NH_3^+$

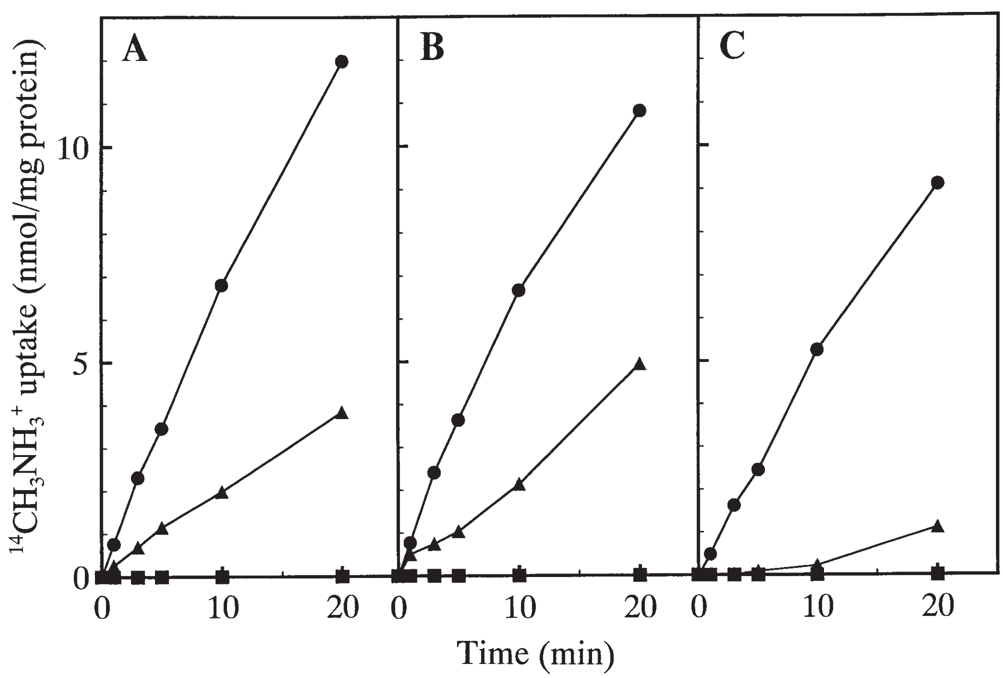

Table 1. Effects of KCN and ionophores on $^{14}CH_3NH_3^+$ uptake

Addition	Concentration	Residual uptake activity at	
		pH 6.5 (%)	pH 8.5 (%)
None		100	100
KCN	0.1 mM	0	0
	1 mM	0	0
	10 mM	0	0
CCCP	1 μM	0	90
	5 μM	0	0
	10 μM	0	0
Monensin	1 μM	83	3
	5 μM	48	1
	10 μM	22	0

KCN or CCCP was added to the cell suspension together with succinate. Before energization by succinate, monensin was added to the cell suspension and the reaction mixture was preincubated for 30 min at 0°C

of the uptake at pH 6.5 and 8.5 were obtained at KCl concentrations of 10 and 100 mM, respectively. In the absence of KCl, the cells exhibited 70% and 23% of the maximum uptake at pH 6.5 and 8.5, respectively.

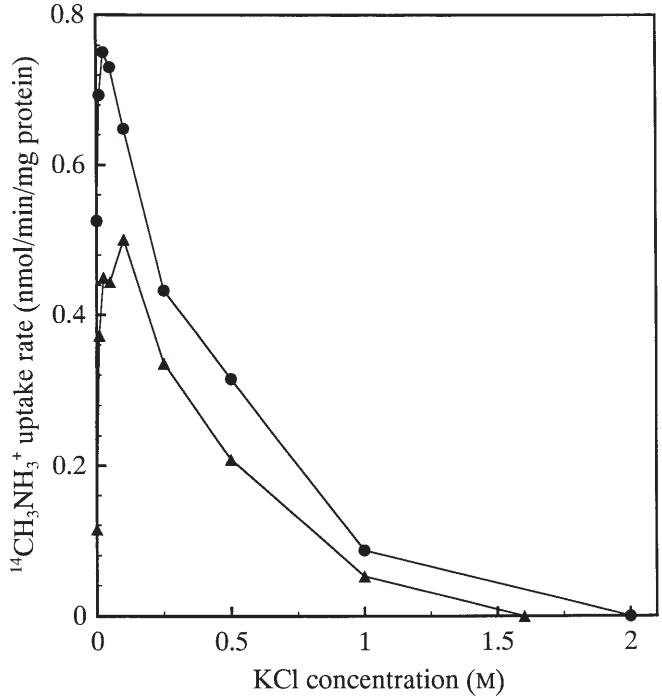

Fig. 3. Effect of KCl on $^{14}CH_3NH_3^+$ uptake. The harvested cells were washed with and suspended in assay buffer excluding KCl. In the assay at pH 6.5 (*circles*), the indicated concentration of KCl was added to the cell suspension at the time of energization by succinate. At pH 8.5 (*triangles*), before energization, the indicated concentration of KCl was added to the cell suspension and the mixture was incubated for 10 min at 0°C. The respective buffers containing the same concentration of KCl as the assay buffers were used for termination of the reaction and subsequent washes

Effect of pH on $^{14}CH_3NH_3^+$ uptake

The optimum pH for $^{14}CH_3NH_3^+$ uptake was between 7.5 and 8.0 (Fig. 4). However, the pH dependence of the uptake activity was not particularly strong, and activities comparable to the maximum uptake were retained over a wide pH range, between 6.0 and 8.5. This characteristic is consistent with the pH dependence of the growth of *Vibrio* ABE-1 in synthetic medium (Takada et al. 1988).

Effects of NH_4^+ and amino acids on $^{14}CH_3NH_3^+$ uptake

To clarify the substrate specificity of the $^{14}CH_3NH_3^+$ uptake system in this bacterium, the inhibitory effects of NH_4^+ and amino acids on uptake activity were examined at pH 7.5 (Fig. 5). The activity was completely blocked without any lag time by addition of 1 mM NH_4Cl or nonradiolabeled $CH_3NH_3^+$. Conversely, 1 mM glutamine or glutamate did not inhibit the uptake at all. Such concentrations of these amino acids have been reported to barely inhibit the $^{14}CH_3NH_3^+$ uptake by several bacterial NH_4^+ transport systems (Barns and Zimniak 1981; Kleiner and Castorph 1982; Kleiner and Fitzke 1981; Mazzucco and Benson 1984). Although hydroxylamine has been reported to be an inhibitor of the NH_4^+ transport system in *Azotobacter vinelandii* (Barns and Zimniak 1981), it had no effect on the uptake activity of *Vibrio* ABE-1.

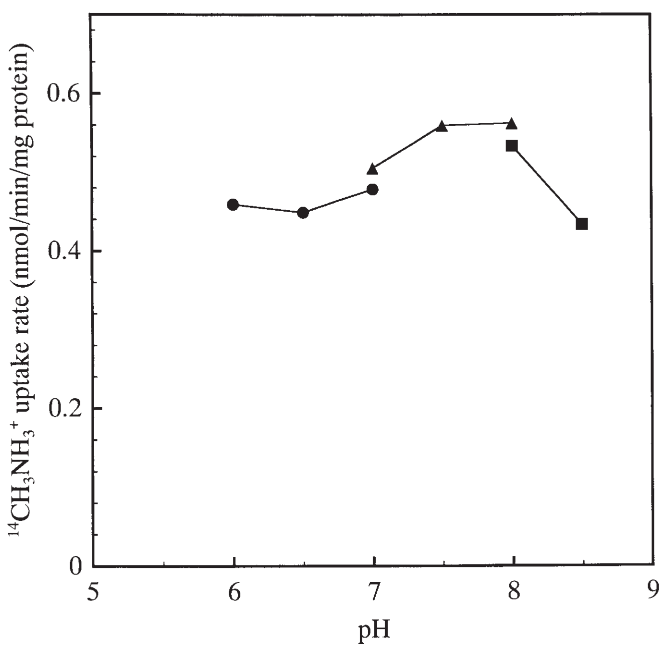

Fig. 4. Effect of pH on $^{14}CH_3NH_3^+$ uptake. $^{14}CH_3NH_3^+$ uptake was assayed at various pH values as described in *Materials and methods*. The following buffers were used: *circles*, MES-NaOH; *triangles*, HEPES-NaOH; *squares*, Tricine-NaOH

Effect of temperature on $^{14}CH_3NH_3^+$ uptake

$^{14}CH_3NH_3^+$ uptake was assayed at pH 7.5 at various temperatures between 0° and 35°C (Fig. 6). The markedly high activity was observed at low temperatures such as 0° and

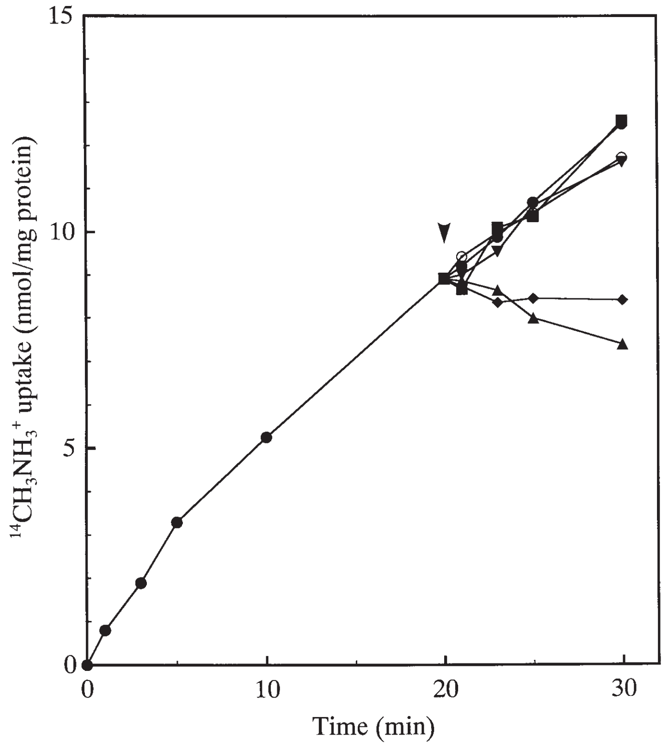

Fig. 5. Effects of NH_4^+, $CH_3NH_3^+$, and amino acids on $^{14}CH_3NH_3^+$ uptake. $^{14}CH_3NH_3^+$ uptake was assayed at pH 7.5 as described in Materials and methods. At the time point indicated by the *arrowhead*, the following analogs were added singly to the assay mixture; *closed circles*, no addition; *triangles*, 1 mM NH_4Cl; *diamonds*, 1 mM $CH_3NH_3^+$; *squares*, 1 mM sodium glutamate; *inverted triangles*, 1 mM glutamine; *open circles*, 3 μM hydroxylamine

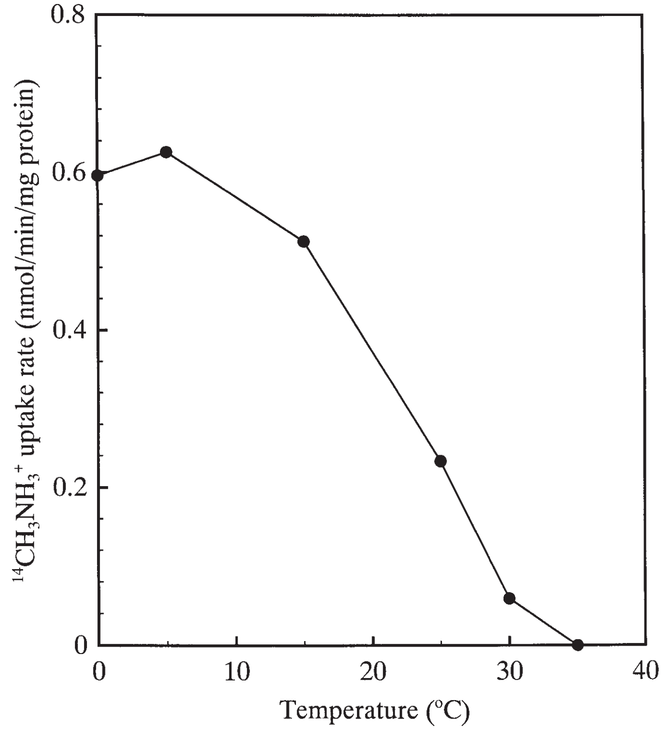

Fig. 6. Temperature dependence of $^{14}CH_3NH_3^+$ uptake. After energization by succinate for 10 min at 15°C, the reaction mixture was further incubated for 5 min at the indicated temperatures and the assay was carried out at pH 7.5 at the same temperatures. $^{14}CH_3NH_3^+$ was added to the mixture to a final concentration of 60 μM to start the reaction

Table 2. V_{max} and apparent K_m values for $^{14}CH_3NH_3^+$ of the uptake at various temperatures

Assay temperature (°C)	V_{max} (nmol mg^{-1} protein min^{-1})	Apparent K_m for $^{14}CH_3NH_3^+$ (μM)
0	1.29 ± 0.30	14.72 ± 0.43
5	0.96 ± 0.12	14.44 ± 2.42
15	1.39 ± 0.30	11.70 ± 0.72
25	0.67 ± 0	12.16 ± 0.53
30	0.22 ± 0.08	5.08 ± 2.20

The uptake activities at various concentrations of $^{14}CH_3NH_3^+$ were assayed at pH 7.5 at the indicated temperatures, and V_{max} and K_m values for $CH_3NH_3^+$ of the uptake were estimated by Lineweaver–Burk plot of the uptake activities. Data are means ± standard deviations of three independent experiments

5°C, and the maximum activity was obtained at 5°C. Activity was decreased with increasing temperature above 15°C (the activity at 25°C was 40% of the maximum) and was completely inactivated at 35°C. Furthermore, the V_{max} and the apparent K_m value for $CH_3NH_3^+$ of the uptake were determined at various temperatures (Table 2). The apparent K_m value for $CH_3NH_3^+$ uptake did not change significantly over the temperature range from 0° to 25°C at which the uptake system was functional.

$^{14}CH_3NH_3^+$ uptake of *Vibrio* ABE-1 cells at various growth stages

The *Vibrio* ABE-1 cells grown at 15°C in Tris-salts medium containing 20 mM glutamate as the sole nitrogen source were harvested at various growth stages and their $^{14}CH_3NH_3^+$ uptake activities were assayed (Fig. 7). No activity was detected in early-log phase cultures. The activity was detectable in cultures after the mid-log phase and reached a maximum at the early stationary phase. The uptake activity then decreased continuously to an undetectable level during the stationary phase.

Discussion

In this study, the NH_4^+ transport system of the psychrophilic marine bacterium *Vibrio* ABE-1 was examined by measuring the uptake of $CH_3NH_3^+$ into the cells. We confirmed that this bacterium is able to transport $CH_3NH_3^+$. There are two types of $CH_3NH_3^+$ uptake system in bacteria: one is a specific carrier for $CH_3NH_3^+$ found in several bacteria that can utilize $CH_3NH_3^+$ as a carbon or nitrogen source, and the other is $CH_3NH_3^+$ uptake mediated by an NH_4^+ transport system (Kleiner 1985, 1993). The two transport systems are

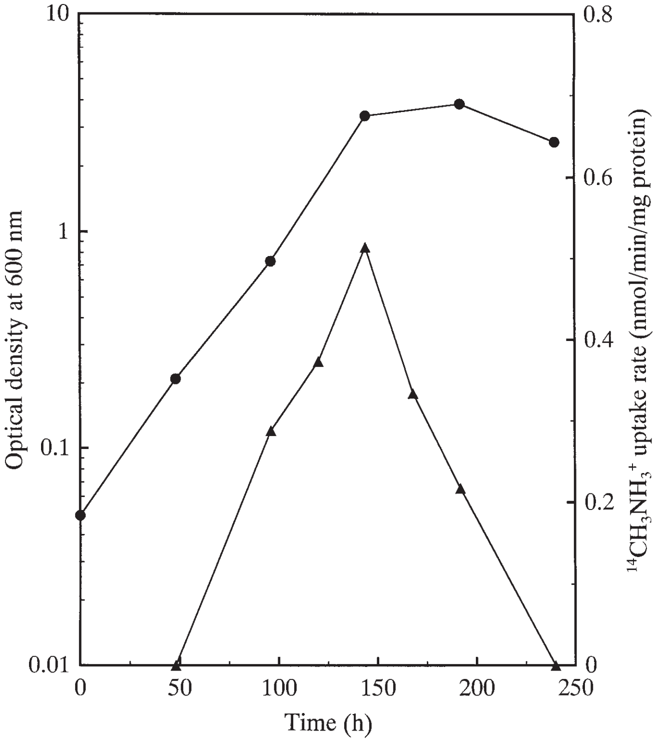

Fig. 7. $^{14}CH_3NH_3^+$ uptake by *Vibrio* ABE-1 cells at various growth stages in batch culture. $^{14}CH_3NH_3^+$ uptake was assayed at pH 8.5 using cells harvested at various growth stages. *Circles*, growth of *Vibrio* ABE-1 on the Tris-salts medium containing 20 mM glutamate as the nitrogen source; *triangles*, $^{14}CH_3NH_3^+$ uptake activity

clearly distinguishable from each other with respect to inhibition of uptake by NH_4^+ in that the latter is strongly inhibited by NH_4^+ whereas the former is insensitive. Because *Vibrio* ABE-1 was unable to utilize $CH_3NH_3^+$ as a nitrogen or carbon source (Fig. 1) and the $^{14}CH_3NH_3^+$ uptake of this bacterium was completely inhibited by NH_4^+ (Fig. 5), we concluded that $^{14}CH_3NH_3^+$ uptake is attributable to an NH_4^+ transport system and that $^{14}CH_3NH_3^+$ can be used as a suitable nonmetabolizable substrate for studying the NH_4^+ transport system of this bacterium.

Synthesis of the components for the bacterial NH_4^+ transport system is known to be generally repressed by growth under NH_4^+-abundant conditions (Kleiner 1985, 1993). In fact, no $^{14}CH_3NH_3^+$ uptake was observed in *Vibrio* ABE-1 cells grown in medium containing 20 mM NH_4Cl as the sole nitrogen source. On the other hand, cells grown on 20 mM glutamate (NH_4^+-limited condition) exhibited uptake activity. These results further support the idea that the $^{14}CH_3NH_3^+$ uptake of this bacterium is mediated by an NH_4^+ transport system. However, the growth rate under the NH_4^+-limited condition was much less than that under the NH_4^+-abundant condition, indicating that glutamate is not a good nitrogen source for this bacterium (Fig. 1). The $^{14}CH_3NH_3^+$ uptake activity of *Vibrio* ABE-1 appeared in batch cultures during the mid-log phase (Fig. 7). It was reported that the $^{14}CH_3NH_3^+$ uptake activity of *Escherichia coli* was recovered by incubating NH_4^+-grown cells for 3 h in NH_4^+-free medium containing glutamate (Jayakumar et al. 1986). As we cultivated *Vibrio* ABE-1 in medium containing glutamate as the nitrogen source by inoculating cells precultured in the presence of 20 mM NH_4Cl, defective uptake activity in the cells of a culture at an early growth stage may result from repression of synthesis of the components of the NH_4^+ transport system by a trace amount of NH_4^+ still remaining in the cells. After bacterial growth reached the stationary phase, a decrease in $^{14}CH_3NH_3^+$ uptake activity was observed. This decrease probably occurred because synthesis of the transport system was repressed by the increase in intracellular free NH_4^+ as a result of cellular metabolism or degradation of some cellular components or by a decrease in protein synthesis itself after the stationary phase of growth.

Because the $^{14}CH_3NH_3^+$ uptake system of *Vibrio* ABE-1 depended on preincubation of the cells with succinate (Fig. 2) and was completely inhibited by KCN (Table 1), it can be concluded that the NH_4^+ transport system of this bacterium is energy dependent. Furthermore, the inhibition of $^{14}CH_3NH_3^+$ uptake by CCCP and monensin implies that the driving force for the NH_4^+ transport system is $\Delta\tilde{\mu}_{H^+}$ and/or $\Delta\tilde{\mu}_{Na^+}$ (Table 1). We previously reported that *Vibrio* ABE-1 has a respiration-dependent primary Na^+ pump to generate $\Delta\tilde{\mu}_{Na^+}$ in addition to an ordinary respiration-dependent primary H^+ pump and synthesizes ATP by $\Delta\tilde{\mu}_{Na^+}$ at alkaline pH (Takada et al. 1988, 1989, 1991). Thus, it is feasible that $\Delta\tilde{\mu}_{Na^+}$ may drive the transport system. In addition, $^{14}CH_3NH_3^+$ uptake was activated by KCl at both pH 6.5 and 8.5 (Fig. 3). The concentrations of KCl necessary to maximize the $^{14}CH_3NH_3^+$ uptake activities at the respective pH scarcely activated the membrane-bound NADH oxidase of this bacterium at pH 6.5 and inhibited it at pH 8.5 (Takada et al. 1989). Therefore, the activation of $^{14}CH_3NH_3^+$ uptake by KCl is not attributable to stimulation of the respiratory chain by this salt.

It has been reported that *E. coli* has an NH_4^+/K^+ antiporter and that intracellular accumulation of K^+ is required for $^{14}CH_3NH_3^+$ uptake by this bacterium (Jayakumar et al. 1985). KCl activated the $^{14}CH_3NH_3^+$ uptake of *Vibrio* ABE-1 more strongly at pH 8.5 than at pH 6.5, and preincubation with KCl was required for full activation of uptake at pH 8.5. These results suggest that, particularly at pH 8.5, an electrochemical potential of K^+ across membranes ($\Delta\tilde{\mu}_{K^+}$) might provide the energy for $^{14}CH_3NH_3^+$ uptake. However, our preliminary studies indicated that the K^+ ionophore valinomycin had no inhibitory effect on $^{14}CH_3NH_3^+$ uptake. Furthermore, attempts to drive $^{14}CH_3NH_3^+$ uptake at pH 8.5 by an artificially imposed $\Delta\tilde{\mu}_{K^+}$ have not been successful. Although both ATP and $\Delta\mu_{H^+}$ have been found to be necessary to drive the NH_4^+/K^+ antiport system of *E. coli* (Barns and Jayakumar 1993), it is not clear whether ATP and/or $\Delta\tilde{\mu}_{H^+}$ is used directly to drive the antiport system or indirectly to generate and retain $\Delta\tilde{\mu}_{K^+}$. Further studies are required to clarify the role of KCl in the NH_4^+ transport system of *Vibrio* ABE-1.

The NH_4^+ transport system of *Vibrio* ABE-1 exhibited high activity at low temperatures such as 0°C (Fig. 6). Thus, the transport system shows a low Q_{10} value and is less dependent on temperature. Because a Q_{10} value of 1.5 was reported for the $^{14}CH_3NH_3^+$ uptake of *E. coli* at pH 7 (Stevenson and Silver 1977), bacterial NH_4^+ transport systems may have relatively low Q_{10} values. In addition, the Q_{10} value of the cold-adapted urocanase from the psychrotrophic bacterium *Pseudomonas putida* is also low (Hug and Hunter 1974). Furthermore, the NH_4^+ transport system of *Vibrio* ABE-1 was markedly thermolabile (Fig. 6). However, about 60% of the maximum $CH_3NH_3^+$ uptake activity was still retained at 20°C, which is the maximum temperature for the growth of *Vibrio* ABE-1. This result implies that this uptake activity at 20°C may be sufficient to sustain growth at this temperature, and that such thermolability of the NH_4^+ transport system may be an important factor determining the maximum growth temperature of this bacterium. In addition, in growing cells this thermolability might be complemented by the newly synthesized transport system, consequently ensuring a steady-state level of the transport activity required for growth.

On the other hand, the V_{max} and the apparent K_m value for $CH_3NH_3^+$ of uptake were substantially constant over the temperature ranges from 0° to 15°C and from 0° to 25°C, respectively (Table 2). These V_{max} and apparent K_m values of the *Vibrio* ABE-1 NH_4^+ transport system are lower than those of other mesophilic bacteria [e.g., apparent K_m and V_{max} values are 25 μM and 3.8 nmol mg^{-1} protein min^{-1} at 25°C in *Azotobacter vinelandii* (Barns and Zimniak 1981) and 36 μM and 4 nmol mg^{-1} protein s^{-1} at 25°C in *E. coli* (Jayakumar et al. 1985), respectively]. These characteristics of the NH_4^+ transport system in *Vibrio* ABE-1 are consistent with the psychrophilic nature of this bacterium; that is, the ability to grow at 0°C with an optimum temperature for growth of about 15°C (Takada et al. 1979), and indicate that this transport system is well adapted to supporting growth at low temperatures.

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