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NH₄⁺ transport system of a psychrophilic marine bacterium, Vibrio sp. strain ABE-1

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Abstract

NH₄⁺ transport system of a psychrophilic marine bacterium Vibrio sp. strain ABE-1 (Vibrio ABE-1) was examined by measuring the uptake of [¹⁴C]methylammonium ion ([¹⁴C]CH₃NH₃⁺) into the intact cells. [¹⁴C]CH₃NH₃⁺ uptake was detected in cells grown in medium containing glutamate as the sole nitrogen source, but not in those grown in medium containing NH₄Cl instead of glutamate. Vibrio ABE-1 did not utilize CH₃NH₃⁺ as a carbon or nitrogen source. NH₄Cl and nonradiolabeled CH₃NH₃⁺ completely inhibited [¹⁴C]CH₃NH₃⁺ uptake. These results indicate that [¹⁴C]CH₃NH₃⁺ uptake in this bacterium is mediated via an NH₄⁺ transport system and not by a specific carrier for CH₃NH₃⁺. The respiratory substrate succinate was required to drive [¹⁴C]CH₃NH₃⁺ 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 [¹⁴C]CH₃NH₃⁺ uptake. The [¹⁴C]CH₃NH₃⁺ uptake activity of Vibrio ABE-1 was markedly high at temperatures between 0° and 15°C, and the apparent Kₘ value for CH₃NH₃⁺ of the uptake did not change significantly over the temperature range from 0° to 25°C. Thus, the NH₄⁺ transport system of this bacterium was highly active at low temperatures.

Key words

Psychrophilic bacterium · Vibrio · NH₄⁺ transport system · [¹⁴C]CH₃NH₃⁺ uptake · Nitrogen source for growth

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). NH₄⁺ transport systems therefore should play important roles in bacterial nitrogen metabolism. In fact, energy-dependent NH₄⁺ transport systems have been found in several bacteria (Kleiner 1985, 1993). However, in spite of their significance, bacterial NH₄⁺ transport systems are less well characterized than the transport systems for other cations such as Na⁺ and K⁺.

Since it was demonstrated that methylammonium (CH₃NH₃⁺) could be incorporated by the NH₄⁺ transport systems (Hackette et al. 1970; Stevenson and Silver 1977), [¹⁴C]CH₃NH₃⁺ has been exclusively employed as a very useful radioactive analog of NH₄⁺ in studies of bacterial NH₄⁺ transport systems (Kleiner 1985). Because a psychrophilic marine bacterium Vibrio sp. strain ABE-1 (Vibrio ABE-1) can utilize NH₄⁺ as the sole nitrogen source (Hakeda and Fukunaga 1983), this bacterium is expected to possess an NH₄⁺ transport system. As the first step in characterization of the NH₄⁺ transport system of Vibrio ABE-1, we examined the uptake of [¹⁴C]CH₃NH₃⁺ into intact cells. The NH₄⁺ 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 20mM NH₄Cl as the nitrogen source with vigorous shaking. One milliliter of the preculture was inoculated into fresh Tris-salts medium (100ml) containing 20mM sodium glutamate instead of NH₄Cl as the nitrogen source, and the bacterium was cultured at 15°C for 120h with vigorous shaking. Bacterial growth was monitored by measuring the turbidity at 600nm with a Shimadzu spectrophotometer model UV-100 (Kyoto, Japan).

Preparation of cell suspension

The bacterial cells were harvested at late exponential phase of growth (OD₆₀₀ = 1.5) and washed three times with an assay buffer consisting of 50mM HEPES-NaOH, 0.5M NaCl, 0.1M KCl, 2mM MgCl₂, 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 $^{14}$CH₃NH₃⁺ uptake, 50mM 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 50mM HEPES-NaOH in the assay buffer. KCl was excluded from the assay buffer when the effect of KCl on $^{14}$CH₃NH₃⁺ uptake was examined. The cell suspension was stored on ice until use. $^{14}$CH₃NH₃⁺ uptake into the cells was assayed within several hours after preparation of the cell suspension as described next.

$^{14}$CH₃NH₃⁺ uptake

NH₄⁺ transport activity was determined by measuring $^{14}$CH₃NH₃⁺ uptake into the intact cells (Barns and Zimniak 1981). Unless otherwise stated, $^{14}$CH₃NH₃⁺ uptake was assayed at 15°C. To energize the cells, aliquots of 200μl of the cell suspension were mixed with 200μl 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 5μl of 312.5μM $^{14}$CH₃NH₃⁺ (1.48 GBq mmol⁻¹; final concentration, 14.88μM). After incubation for the desired periods, the reaction was terminated by dilution with 3ml of ice-cold wash buffer [50mM HEPES-NaOH, 0.5M NaCl, 50mM KCl, 2mM MgCl₂, 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; 5ml liquid scintillation fluid [0.4% (v/v) 2,5-diphenyloxazole, and 0.05% (v/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 $^{14}$CH₃NH₃⁺ uptake was examined, 50mM 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 50mM 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

$[^{14}]$CH₃NH₂·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 NH₄⁺ (Kleiner 1985, 1993). Furthermore, several bacteria possess a specific carrier for CH₃NH₂⁺ distinct from the NH₄⁺ transport system and are consequently able to grow using CH₃NH₂⁺ 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 100mM sodium succinate and 20mM NH₄Cl as carbon and nitrogen sources, respectively (succinate-NH₄⁺ 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-NH₄⁺ medium. On the other hand, no growth was observed when 20mM CH₃NH₂·HCl was used as the nitrogen source. To determine whether CH₃NH₂·HCl can be utilized as the carbon and nitrogen source, sodium succinate, a major carbon and nitrogen source, and NH₄Cl were replaced by 100mM CH₃NH₂·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-NH₄⁺ medium, essentially the same pattern of growth was observed. These results suggest that the initial growth observed in the CH₃NH₂·HCl media was probably the result of utilization of a small amount of a chelator, 3mM sodium citrate, but not CH₃NH₂·HCl. Therefore, we concluded that CH₃NH₂⁺ cannot be utilized as a nitrogen and carbon source by Vibrio ABE-1.

$^{14}$CH₃NH₃⁺ uptake by Vibrio ABE-1

The NH₄⁺ transport system of Vibrio ABE-1 grown on glutamate as a nitrogen source was examined at 15°C at pH