Correlation between the respiration-driven Na\(^+\) pump and Na\(^+\)-dependent amino acid transport in moderately halophilic bacteria

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(Received 8 March 1993; revised 18 May 1993; accepted 21 June 1993)

Introduction

Halophilic bacteria are divided into three categories based on their salt requirement for optimal growth (Kushner, 1978). Slightly halophilic marine bacteria grow best in media containing 0.2–0.5 M-NaCl, moderately halophilic bacteria have a growth optimum at 0.5–2.5 M-NaCl, and extremely halophilic bacteria at 2.5–5.2 M-NaCl. Marine bacteria (Niven & MacLeod, 1980; Tokuda et al., 1982) and extreme halophiles (MacDonald et al., 1977; Lanyi, 1979) are known to require a sodium-motive force (ApNa\(^+\)) for the active uptake of amino acids. The moderate halophile *Vibrio costicola* requires a ΔµNa\(^+\), as the driving force of amino acid uptake. Recently, we have demonstrated that among moderately halophilic bacteria from diverse origins, Gram-negative, but not Gram-positive, halophiles have a respiration-driven Na\(^+\) pump specifically required for AIB uptake. On the other hand, the two Gram-positive bacteria that have no respiration-driven Na\(^+\) pump showed no requirement for Na\(^+\) for AIB uptake. Thus, the mode of energy coupling to amino acid transport was quite different between the Gram-negative and Gram-positive moderate halophiles tested: the former, but not the latter, utilized Na\(^+\) circulation for the active uptake of AIB.

Methods

Bacterial species and culture conditions. The moderately halophilic bacteria used in this study and their culture conditions were described previously (Unemoto et al., 1992). Each bacterium was grown in the presence of 1.0 M-NaCl and harvested at the late exponential phase of growth by centrifugation. The cells were washed twice with a medium containing 25 mM-HEPES/Tris (pH 7.5), 1.0 M-KCl and 10 mM-MgSO\(_4\), and suspended in this medium at a concentration of 5–10 mg cell protein ml\(^{-1}\). The cell suspension was stored on ice until use. Protein was determined by the Lowry method with bovine serum albumin as a standard.

Amino acid uptake. AIB was used as a nonmetabolizable amino acid analogue. To 100 µl of the reaction mixture containing 25 mM-buffer, 0.1 mM-[\(^{3}H\)]AIB [20 µCi µmol\(^{-1}\); (740 kBq µmol\(^{-1}\ ]); New England Nuclear] and 0–2.0 mM-NaCl or KCl, 2 µl of the cell suspension was added at 37°C. At time intervals, the reaction was terminated by the addition of 1.0 ml 1.0 M-NaCl and by immediate filtration on a MF Millipore filter (pore size 0.45 µm). The filter was washed once with 1.0 ml 1.0 M-NaCl within 10 s. The radioactivity was determined with 3 ml of Bray's scintillation liquid. The radioactivity retained on the filter in the absence of cells served as the blank. To prevent the lysis of cells at low salt concentrations, 10 mM-MgSO\(_4\) was included in the reaction mixture. The following buffers were used at different pH values: 25 mM-MES/Tris (pH 6.0–6.5), 25 mM-HEPES/Tris (pH 7.0–7.5), 25 mM-Tricine/Tris (pH 8.0–8.5) and 25 mM-Tris/CHES (pH 9.0).

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Abbreviations: AIB, α-aminoisobutyric acid; CCCP, carbonyl cyanide m-chlorophenylhydrazone; ΔµH\(^+\), electrochemical potential gradient of H\(^+\); ΔµNa\(^+\), electrochemical potential gradient of Na\(^+\).
Results

Effect of NaCl on the active uptake of AIB

Optimal conditions for the assay of AIB uptake were examined using Halobivrio variabilis (DSM 3051T). In the presence of 1·0 M-NaCl, AIB uptake increased linearly with incubation time at least for 5 min. Therefore the reaction was terminated at 3 min to measure the initial velocity of AIB uptake. As shown in Fig. 1, H. variabilis required Na⁺ for the active uptake of AIB with an optimal concentration of 0·6–1·2 M-NaCl. The rate of AIB uptake decreased at higher concentrations. No significant AIB uptake was observed with KCl as a replacement for NaCl at any concentrations. In the marine moderate halophiles, the six Gram-negative bacteria (H. variabilis, Deleya halophila (NCMB 701T), Pseudomonas beijerinckii (ATCC 19372), Ps. halosaccharolytica (ATCC 29423) and an unidentified halophile (NRCC 41227) required Na⁺ for the active uptake of AIB. In our previous paper (Unemoto et al., 1992), the above six Gram-negative, but not the two Gram-positive, halophiles were demonstrated to have a respiration-driven Na⁺ pump. Therefore, these results strongly suggest that moderate halophiles that have a respiration-driven Na⁺ pump require Na⁺ for the active uptake of AIB.

Effect of a proton conductor on the active uptake of AIB

In the marine V. alginolyticus, the respiratory-driven Na⁺ pump extrudes Na⁺ against its electrochemical gradient and generates a proton-conductor-resistant membrane potential especially at alkaline pH (Tokuda & Unemoto, 1982). At acidic pH, the ΔµNa⁺ is mainly generated by a ΔµH⁺-driven Na⁺/H⁺ antiporter. If amino acid uptake is driven by ΔµNa⁺, it may be inhibited by a proton conductor at acidic, but not at alkaline, pH. In the absence of CCCP, the rate of AIB uptake was almost completely inhibited by 5 µM-CCCP at pH 6·5, whereas at pH 8·5 more than 20 µM-CCCP was required to inhibit AIB uptake by 50%. In the presence of CCCP, the rate of AIB uptake was almost unaffected by medium pH. However, the AIB uptake was strongly inhibited by 10 µM-CCCP at acidic, but not at alkaline, pH values. These results were essentially the same as those obtained with the marine V. alginolyticus (Tokuda & Unemoto, 1981). Since H. variabilis has the same type of respiration-driven Na⁺ pump as V. alginolyticus (Unemoto et al., 1992), the CCCP-resistant AIB uptake at alkaline pH was probably due to the direct generation of ΔµNa⁺ by the

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**Fig. 1** Effects of NaCl and KCl on the active uptake of AIB by H. variabilis. The initial rate of AIB uptake was assayed at pH 7·5 in the presence of NaCl (○) or KCl (●). The reaction was terminated at 3 min to measure the specific activity with either NaCl or KCl, exhibiting no requirement for Na⁺ for the active uptake of AIB. In our previous paper (Unemoto et al., 1992), the above six Gram-negative, but not the two Gram-positive, halophiles were demonstrated to have a respiration-driven Na⁺ pump. Therefore, these results strongly suggest that moderate halophiles that have a respiration-driven Na⁺ pump require Na⁺ for the active uptake of AIB.

**Fig. 2** Effects of CCCP and pH on the AIB uptake of H. variabilis. (a) Initial rate of AIB uptake assayed in the presence of 1·0 M-NaCl and various concentrations of CCCP at pH 6·5 (○) and 8·5 (●). (b) AIB uptake assayed in the presence of 1·0 M-NaCl at various pH values. ○, No addition; ●, 10 µM-CCCP.
Table 1. Effects of 10 mM-NaCl and KCl on the AIB uptake of moderate halophiles at pH 6.5 and 8.5

<table>
<thead>
<tr>
<th>Moderate halophile</th>
<th>pH 6.5</th>
<th>pH 8.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl</td>
<td>KCl</td>
</tr>
<tr>
<td>Halobibrio variabilis (DSM 30517)</td>
<td>14.9</td>
<td>0.89</td>
</tr>
<tr>
<td>Deleya halophila (CCM 36627)</td>
<td>10.3</td>
<td>1.09</td>
</tr>
<tr>
<td>Vibrio costicola (NCMB 7017)</td>
<td>1:34</td>
<td>0.03</td>
</tr>
<tr>
<td>Pseudomonas beijerinkii (ATCC 19372)</td>
<td>1:36</td>
<td>0.06</td>
</tr>
<tr>
<td>Ps. halococcaurotica (ATCC 29423)</td>
<td>3:03</td>
<td>0:03</td>
</tr>
<tr>
<td>Unidentified halophile (NRCC 41227)</td>
<td>0:50</td>
<td>0:04</td>
</tr>
<tr>
<td>Marinococcus halophilus (CCM 27067)</td>
<td>2:12</td>
<td>2:22</td>
</tr>
<tr>
<td>Micrococcus varians subsp. halophilus (ATCC 21971)</td>
<td>2:44</td>
<td>2:97</td>
</tr>
</tbody>
</table>

The initial velocity was determined from the linear part of AIB uptake and results presented are mean values of three to four separate experiments.

respiration-driven Na⁺ pump. With respect to V. costicola, detailed studies on the generation of ΔµNa⁺ have already been reported (Udagawa et al., 1986). For the other Gram-negative moderate halophiles examined in Table 1, it was also confirmed that AIB uptake was resistant to 10 μM-CCCP at alkaline, but not acidic, pH (data not shown). These results strongly suggest that moderate halophiles that have a respiration-driven Na⁺ pump utilized ΔµNa⁺ for the active uptake of AIB. In this connection, it is interesting to note that the pH-dependent resistance curve of AIB uptake against CCCP is very similar to the pH-dependent resistance curve of growth rate of V. alginolyticus against CCCP (see Fig. 3 in Tokuda & Unemoto, 1983).

Discussion

The Na⁺-dependent active transport of various nutrients has been reported in the marine Alteromonas haloplanktis and V. fischeri (Droniuk et al., 1987), and Deleya aesta (Berthelet & MacLeod, 1991). The marine V. alginolyticus also requires Na⁺ for the active uptake of 20 amino acids including AIB (Unemoto et al., 1990). Using AIB as the substrate, ΔµNa⁺ was shown to act as the direct driving force in A. haloplanktis (Niven & MacLeod, 1980) and in V. alginolyticus (Tokuda et al., 1982). The moderately halophilic V. costicola was also shown to require ΔµNa⁺ for AIB uptake (Hamaide et al., 1984). Thus, the Na⁺-coupled transport systems were generally considered to be common in the marine and moderately halophilic bacteria. However, in this paper it was found that all moderate halophiles do not necessarily require Na⁺ for the active uptake of AIB. Indeed, the two Gram-positive moderate halophiles that have no respiration-driven Na⁺ pump showed no requirement for Na⁺ for the active uptake of AIB. Thus, these moderate halophiles are unlikely to utilize ΔµNa⁺ for the active uptake of AIB.

Except for the concentration of NaCl required for optimal growth (1.0–2.0 M), the six Gram-negative moderate halophiles examined are very similar to the marine bacteria in the following properties: (1) the NaCl-washed cells quickly lyse in a hypotonic medium; (2) they have Na⁺-dependent NADH-quinone reductase in the respiratory chain that functions as an electrogenic Na⁺ pump; (3) the active uptake of AIB is dependent on Na⁺ and thus driven by ΔµNa⁺. These three properties are characteristic of marine bacteria and discriminate between marine and terrestrial bacteria. Thus, the Gram-negative moderate halophiles examined are closely related to the marine bacteria, suggesting that these moderate halophiles originated from marine environments. However, the two Gram-positive moderate halophiles examined are resistant to hypotonic lysis, do not have any respiration-driven Na⁺ pump, and exhibit no requirement for Na⁺ for AIB uptake. Since these Gram-positive halophiles have none of the properties characteristic of marine bacteria, they are unlikely to originate from marine environments. Gram-positive halophiles constitute a minor proportion of the bacteria found in marine and high salt environments and only two species were examined in this study. Recently, Bejar et al. (1992) classified 23 strains of moderately halophilic endospore-forming Gram-positive rods into two groups, which were different from those of other previously described Bacillus species. Therefore, it is interesting to further investigate other Gram-positive moderate halophiles from the bioenergetic point of view to generalize our results.

References


