Na^+-DEPENDENT UPTAKE OF AMINO ACIDS BY AN ALKALOPHILIC BACILLUS

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1. Introduction

An alkalophilic *Bacillus* isolated in our laboratory grew best at pH 10.0–10.5, and the incorporation of amino acids and uptake of α-aminoisobutyric acid (AIB) into the bacterium were maximum at pH 9.0–10.5 [1]. The uptake of glucose into the bacterium, on the other hand, showed little variation of rate in the pH 7.0–11.0 region. The alkalophilicity of the bacterium has therefore been suggested to be due to the behaviour towards charged substances admitted into the organisms. It was recently found that the bacterium, when incubated with AIB in the absence of NaCl, exhibited no uptake of AIB at pH 10.0. This result suggests a possible role for Na^+ in the transport mechanism of the bacterium. In the present paper, therefore, the effect of NaCl on the uptake of amino acids and glucose into the organisms was examined.

2. Methods

An alkalophilic *Bacillus* was grown aerobically at 37°C in a peptone medium of pH 10.5, as described previously [1]. Organisms in a mid-logarithmic phase were collected, washed twice with 20 mM potassium phosphate buffer (pH 7.2) containing 0.2 M sucrose and 0.1 M KCl, and suspended in the same buffer.

The reaction mixture for the uptake of amino acids consisted of 0.1 M glucose and either 10 μM AIB with 0.1 μCi of [1-^{14}C]AIB (4.84 mCi/mmol) or 10 μM leucine, with 0.1 μCi of [U-^{14}C]leucine (280 mCi/mmol) in 10 mM potassium phosphate buffer containing 0.2 M sucrose. The reaction mixture for the uptake of glucose consisted of 0.2 mM glucose with 0.1 μCi of [U-^{14}C]glucose (5.0 mCi/mmol) in 10 mM potassium phosphate buffer containing 0.2 M sucrose. The pH and Na^+ concentration was adjusted as indicated in the text. KCl was further added to the reaction mixture such that the total salt concentration was held constant at 0.5 M, except when the reaction was carried out in the presence of 0.5 M NaCl or in the presence of gramicidin and 0.1 M NaCl. When the effects of gramicidin on leucine or glucose uptake were examined in the presence of 0.1 M NaCl, gramicidin was added to the reaction mixture, at concentrations indicated in the text.

To 1.2 ml of the reaction mixture, preincubated at 37°C for 3 min was added 0.3 ml of the cell suspension (5.5 mg dry weight cells/ml). After 1 min of incubation, 1 ml of the mixture was removed and filtered through a Millipore filter (grade HA). The organisms on the filter membrane were washed with 2 ml of the reaction mixture without labelled AIB (leucine) or glucose, respectively. The radioactivity of the organisms was measured as described previously [1]. The rate of uptake of amino acid or glucose into the organism under the conditions examined in the present study was linear for 1 min of incubation.

3. Results and discussion

The experiments measuring AIB uptake of the bacterium were carried out in the presence of 0.9% (0.16 M) NaCl, and a maximum AIB uptake was observed at pH 9.5 [1]. Although the bacterium was unable to grow at pH 7.0, the organism exhibited AIB uptake at pH 7.0 one seventh of the uptake rate at pH 9.5.

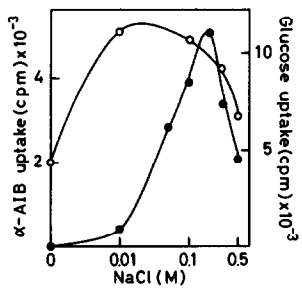

Fig.1. Effect of different concentrations of NaCl on the uptake of AIB (•) or glucose (○) of the alkalophilic *Bacillus*, at pH 9.5.

When the bacterium was incubated with AIB in the absence of NaCl (in the presence of 0.5 M KCl) at pH 9.5 or 7.0, no AIB uptake was observed. (fig.1). AIB was incorporated into the organisms only in the presence of NaCl, at pH 9.5, the rate of AIB uptake increased with increasing NaCl concentration. A maximum rate of AIB uptake was observed at 0.2 M NaCl. A similar effect of NaCl on AIB uptake was observed at pH 7.0. AIB was also incorporated into the bacterium in the presence of 0.2 M sodium acetate, at pH 9.5 or 7.0, but not in the presence of 0.2 M LiCl, RbCl, CsCl or NH_4Cl. Leucine was incorporated into the bacterium at pH 9.5 or 7.0, only in the presence of NaCl or sodium acetate. A maximum leucine uptake was observed at 0.2 M NaCl. The results may indicate that the bacterium requires Na^+ for optimal transport of amino acids into the organism.

In contrast to amino acid uptake, the bacterium exhibited an appreciable rate of glucose uptake into the organism at pH 9.5 and pH 7.0, even in the absence of NaCl (fig.1). The addition of increasing concentrations of NaCl, however, resulted in an increased rate of glucose uptake. A maximum rate (2.7 times that in the absence of NaCl) was observed at 10–20 mM NaCl. The alkalophilic property of the bacterium, which has, been suggested to be due to the behaviour of the membrane towards charged substances [1], may be dependent on Na^+.

Na^+ has been reported to play an important role in the transport of sugars and amino acids in cells of animals and bacteria, and considered to be co-transported with the compounds in some cases [2–4]. 1 nM Gramicidin was found to inhibit the leucine uptake of the bacterium by 30%, in the presence of 0.1 M NaCl; leucine uptake was completely inhibited by 0.1 μM gramicidin. The Na^+ gradient across the membrane seems to be absolutely necessary for the bacterium to incorporate amino acids. 1 μM Gramicidin was found to inhibit the glucose uptake of the bacterium by 60% in the presence of 0.1 M NaCl. The rate of gramicidin-inhibited glucose uptake in the presence of NaCl was similar to that in the absence of NaCl. The glucose uptake of the bacterium may also require an Na^+ gradient for maximum uptake. Attempts to elucidate the mechanism of the role of Na^+ in the transport of amino acids and glucose into the alkalophilic *Bacillus*, together with a study of the relationship between the alkalophilicity of the bacterium and the external concentration of Na^+ are now being undertaken.

References

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