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Taurine Transport in *Staphylococcus aureus* — effect of energy donors —

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Abstract

The effect of energy donors on taurine transport in *Staphylococcus aureus* (*S. aureus*) was studied. Although taurine transport in *S. aureus* was stimulated by the addition of 20 mM glucose, lactate or glycerol to the assay mixture, intracelluar ATP levels were not parallel with the transport activity. It was surprising that the ATP concentration in the cells during incubation with glucose were markedly decreased, and this effect of glucose was not affected by the airation, suggesting that *S. aureus* has a unique system which utilizes glucose or its metabolite as an activator of taurine transport system.

Introduction

Staphylococci is the only bacterial species in which the ability of taurine transport has been shown[1]. The activation of this tranport system by addition of glucose in *Staphylococcus aureus* (*S. aureus*) has been reported[2]. We demonstrated that this transport system was activated by adding energy donors such as lactate or glycerol as well as glucose[3]. Since taurine was taken up by cells in a Na^+ dependent manner and the taurine transport was inhibited by a proton conductor, carbonylcyanide m-chlorophenylhydrazone (CCCP), this transport system is thought to require the intact respiratory chain and the Na^+/H^+ antiporter[3]. But the mechanisms by which these energy donors stimulate taurine transport in *S. aureus* remains unclear. Attempts have been made to examine whether adenosine triphosphate (ATP) can drive taurine transport in this organism or not. I report here the changes of intracelluar ATP level of *S. aureus* during the exposure of cells to various energy donors.

Methods

Preparation of cells

S. aureus 209 P was used in the experiments. Cells were grown in the medium containing 0.5 % beef extract, 1.5 % polypeptone (Daigo Eiyo Co.), 0.5 % NaCl and 0.5 % K_2HPO_4, pH 7.0. Cultures were initiated with a 10 % inoculum from overnight cultures. Cells were

grown with shaking at 37℃ for about 2 hours to an absorbance at 650 nm of about 1.0. Cells were harvested by centrifugation and were washed twice with cold 0.1 M Tris–HCl buffer, pH 7.0. Washed cells were resuspended in the cold 0.1 M Tris–HCl buffer to appropriate concentrations (about 2 mg protein / ml).

Transport assay

Taurine transport into *S. aureus* was measured by a published method [4]. A typical assay mixture contained 50 mM Tris–HCl, pH 7.0, 20 mM glucose, 10 mM NaCl, 9.9 μM ^{14}C–taurine (0.02 μCi) and cells of *S. aureus* (about 0.1 mg protein/ml). Glucose was replaced with lactate–Tris (pH 7.0) or glycerol, when the effect of energy donors on taurine transport was studied. Assays were carried out at 37℃, and transport was initiated by adding radioactive taurine to the assay mixture which was prewarmed for 5 minutes at 37℃.

ATP determination

Washed cells were incubated at 37℃ in 0.1 M Tris–HCl buffer (pH 7.0) with 20 mM of energy donors such as glucose, lactate–Tris (pH 7.0) or glycerol. N_2 or O_2 gas was bubbled in the cell suspension when the effect of airation was examined. Intracellular ATP was extracted by boiling the appropriate amount of cell suspension (about 0.3 mg protein) in 10 mM TES–NaOH, 40 mM $MgSO_4$ (pH 7.0) for 6 minutes. The supernatant from centrifugation was used for the ATP determination by a luciferin luciferase assay [5].

Protein content was determined by the method of Lowry et al [6] with bovine serum albumin as a standard.

Results

Effect of energy donors on taurine transport

Fig. 1 shows the marked stimulatory effect of lactate, glycerol or glucose on taurine uptake. Among the three energy sources tested, lactate was most effective and glucose was less effective than lactate or glycerol. When NaCl was omitted from the assay mixture, almost no taurine uptake was observed even in the presence of these energy donors (data not shown).

Effect of energy donors on intracelluar ATP levels

Fig. 2 shows the changes in intracelluar ATP levels when *S. aureus* was incubated with various energy donors. The addition of lactate or glycerol elevated the intra-

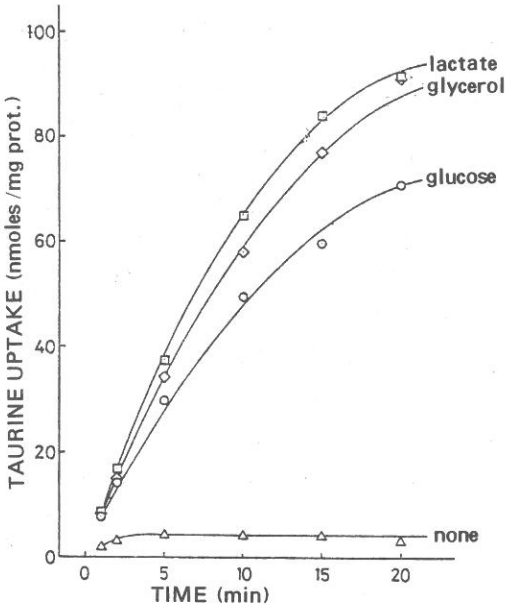

Fig. 1. Effect of energy donors on taurine uptake by *S. aureus*. Taurine transport was assayed as described under "Methods", in the presence of 10 mM NaCl and 20 mM of various energy sources.

celluar ATP levels during the incubation, although the magnitude was not parallel with the transport activities. It was unique that the ATP level was decreased to lower level than that observed in the presence of proton conductor CCCP, when cells were incubated with glucose.

Fig. 3 shows the effect of airation on intracelluar ATP levels during the incubation of cells with energy donors. *S. aureus* synthesized ATP when incubated with lactate under aerobic condition. Surprisingly, the intracellular ATP level was drastically decreased immediately after the addition of glucose to the medium. Furthermore, no increase in the intracelluar ATP level was observed when O_2 was introduced into the assay medium.

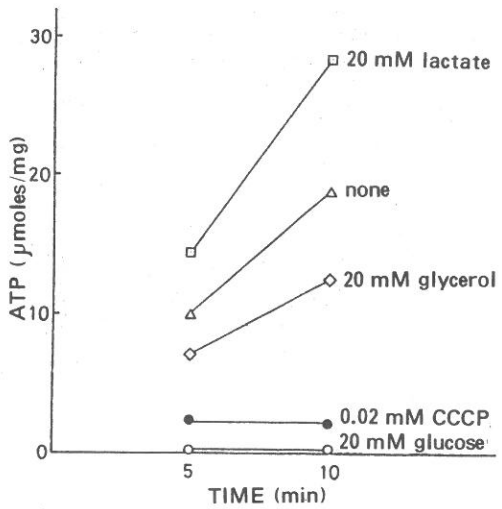

Fig. 2. Effect of energy donors on intracelluar ATP levels in *S. aureus*. ATP concentrations in cells were measured as described under "Methods", in the presence of 20 mM of various energy sources or 0.02mM of CCCP.

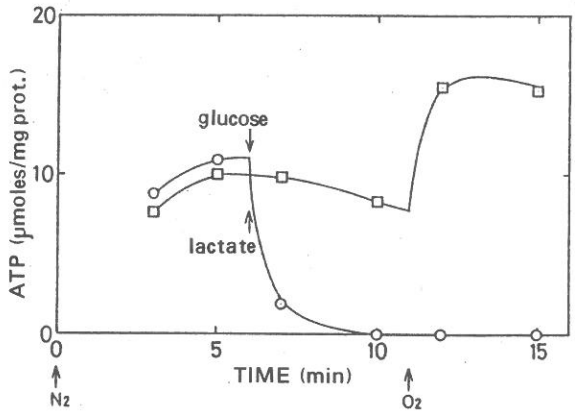

Fig. 3. Changes in intracellular ATP level after the addition of glucose or lactate. ATP concentrations in cells were measured as described under "Method". N_2 gas was introduced into the assay vessel at 0 time, and the N_2 gas was replaced with O_2 gas at 11 min. Glucose or lactate was added to the assay mixture at 20 mM where indicated.

Discussion

Judging from some characteristics of taurine transport in *S. aureus*, it seems reasonable to suppose that this system is an active transport system which requires a sort of driving force[2)3)]. Lactate stimulated taurine tranport and increased intracelluar ATP level under aerobic conditions (Fig. 1, 2, 3), suggesting the following scheme. Lactate metabolism in *S. aureus* would establish electrochemical potential of H^+ by the respiratory chain. This electrochemical potential of H^+ would be replaced with that of Na^+ by the Na^+/H^+ antiporter. The consequently established Na^+ gradient would drive taurine tranport. During the stimulation of taurine tranport, H^+ gradient might drive ATPase to produce ATP in the cell. On the other hand, taking the effect of glucose on *S. aureus* into account, more complicated mechanisms of the regulation of taurine transport, which is affected by glucose, might exist. There are several possibilities to explain the seemingly contradictory effect of glucose, the stimulation

of taurine transport and the drastic decrease of the intracellular ATP level. The first one is that ATP was exhaused during glucose uptake. It has been reported that there existed the phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS) specific for lactose in *S. aureus* [7]. Although PTS for glucose in *S. aureus* has not been demonstrated, it seems reasonable to suppose that this organism consumes 1 mole of ATP to take up 1 mole of glucose like in *Escherichia coli*. The second possibility is that ATP may not be produced immediately after cells take up glucose, because some of the metabolic pathways for glucose in *S. aureus* might be inducible. Glucose or its close metabolite may stimulate taurine transport. The third possibility is that glucose might inhibit ATP synthetase in some way, and therfore the cell might not be able to produce ATP. Thus the electrochemical potential establised by the respitatory chain may not be consumed, and the potential may be maintained at high level. As a result, high level of the electrochemical potential may be established via the Na^+/H^+ antiporter. Thus Na^+-taurine cotransport might be stimulated. I am going to elucidate these possibilities as the next step of this study. Anyway, taurine transport in *S. aureus* is not thought to require ATP as driving force.

Acknowledgments

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