ACTIVE TRANSPORT OF L-VALINE BY STREPTOCOCCUS DIACETILACTIS

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ABSTRACT

Transport of L-valine by resting cells (16 hr) of Streptococcus diacetilactis strain 18-16 was sensitive to changes in temperature resulting in a temperature coefficient of about 1.8. Moreover, the system was activated by glucose and inhibited by both azide and 2,4-dinitrophenol, indicating the probable involvement of metabolically produced energy. Lipoic acid (oxidized) and pyridoxal phosphate both increased the rate of L-valine uptake and when added together they exerted a synergistic effect. Rate of transport was influenced by the external amino acid and peptide concentrations in the suspending media. Studies at various pH levels revealed the possible presence of two separate uptake systems for L-valine in intact cells of this organism.

The lactic streptococci are known to have an absolute requirement for a number of amino acids for growth (1, 3, 11, 12). Presumably, therefore, these bacteria transport the above nutrients across the cell membrane. Many bacteria have been shown to accumulate amino acids against large concentration gradients (2, 3, 7, 9, 13), but the mechanism involved is not yet understood.

Streptococcus diacetilactis, an organism which plays a vital role in acid and flavor production in dairy product manufacture (15), has been found to transport citrate by an active process (6). The lack of information on amino acid transport in this organism prompted the present study, undertaken to help elucidate the nature of the transport system with respect to L-valine.

EXPERIMENTAL PROCEDURE

Cells of S. diacetilactis strain 18-16 (courtesy of Dr. W. E. Sandine, Oregon State University) were harvested from Elliker's (5) medium after 16 hr of incubation at 30 C in a constant-temperature water bath. The cells were washed twice in NaCl (0.85%) - KH2PO4 - KH2PO4 buffer (0.2 M) at pH 7.0, then resuspended in this buffer. The dry cell weight was determined by oven drying and weighing of a 10 ml aliquot of this suspension, for which the salt content was determined by separately drying aliquots of the buffer.

Determination of valine requirement for growth. Bacto-valine assay medium (Difco Laboratories) was prepared as directed by the manufacturer and dispensed in 5 ml quantities for sterilization. Valine was added aseptically in amounts ranging from 10 to 100 μg per tube. Each 10-ml quantity of medium (adjusted to this volume with sterile water) was inoculated with an approximate number of 500,000 viable cells.

Measurement of valine uptake by the quantitative ninhydrin method of Rosen (14). The experimental mixture contained 100 mg cells, 10 μmoles valine, and 200 μmoles/ml KH2PO4 - KH2PO4 buffer (pH 7.0), used to adjust the volume to 4.0 ml. The effect of pyridoxal phosphate and of lipoic acid (β-6-thioctic acid, oxidized form) was studied by adding 2.0 μmoles of each separately or together. Lipoic acid was purchased from Sigma Chemical Company and pyridoxal phosphate (ammonium salt) from California Foundation for Biochemical Research.

The experimental mixtures were incubated at 2 and at 30 C for 2 hr, then centrifuged at 15,000 X g for 20 min. Aliquots of the supernatant solutions were diluted as necessary and analyzed by a quantitative ninhydrin method (14), for which valine was used as the standard. Appropriate controls (cells alone) were used to correct for ninhydrin-positive material diffusing from cells during incubation in the presence of the buffer.

Millipore filter technique for determination of C14 valine (U) uptake. The uptake mixture was adjusted to contain 500 μg dry weight of resting cells per milliliter and 0.1 μc per milliliter C14 valine (U) (Radiochemical Centre, Amersham, Buckinghamshire, England). In addition, the mixtures contained 85 μmoles NaCl, 25 μmoles KH2PO4, 50 μmoles K2HPO4, 1282
and 5 μmoles of glucose per milliliter. The final total volumes were adjusted to 5 ml with 200 μmoles/ml KH₂PO₄-K₂HPO₄ buffer, at pH 7.0. Incubation was carried out routinely at 30 C, except for the experiment on the effect of temperature. The concentrations of lipoic acid, pyridoxal phosphate, sodium azide, and 2,4-dinitrophenol are given along with the Figures under Results and Discussion. Purified sodium azide and reagent grade 2,4-dinitrophenol were purchased from Fisher Scientific Company.

Several aliquots containing 100 μg of cells were taken during a 12- or 20-min incubation period. The cells were collected and washed with buffer on 0.45-μ Millipore filters, glued lightly to planchets with paper cement, for counting on a gas-flow Nuclear Chicago counter. The amount of C¹⁴ valine incorporated into cells was expressed as per cent of uptake based on the total activity measured at zero-time, using a 0.05-ml aliquot.

RESULTS AND DISCUSSION

Growth requirement. Experiments with the defined medium indicated that valine was an essential amino acid for S. diacetilactis. A minimum of 7 μg valine per milliliter was necessary, to permit turbidity measurements. The keto analog of valine, α-ketoisovalerate, was used also in some experiments, in which case a level of 100 μg per milliliter of medium was required to establish growth. The organism was thus able to aminate the keto compound to valine. In this latter respect, and in its requirement for valine, the bacterium was similar to Streptococcus lactis and Streptococcus cremoris strains investigated by MacLeod et al. (10).

Valine transport. Data in Table 1 and Figure 1 indicate that valine uptake is a temperature-dependent process. By comparing the rates of uptake at 15 and 30 C, a Q₁₀ value of 1.8 is obtained, which indicates that an enzyme-like process may be involved. Values given in Table 1 also indicate a possible involvement of both lipoic acid (oxidized) and pyridoxal phosphate in valine transport. Figure 2 shows the stimulatory effect of pyridoxal phosphate and of lipoic acid on C¹⁴ valine incorporation into cells. The greatest response is obtained when both compounds are added simultaneously. The

<table>
<thead>
<tr>
<th>Incubation temperature (C)</th>
<th>Valine alone (μg)</th>
<th>Valine plus lipoic acid (μg)</th>
<th>Valine plus pyridoxal phosphate (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>112</td>
<td>212</td>
<td>160</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>28</td>
<td>0</td>
</tr>
</tbody>
</table>


TABLE 1

Valine uptake by resting cells of Streptococcus diacetilactis following a 2-hr incubation period

① Represents in each case the average of two experiments. For these 10.0 μmoles of L-valine, 2.0 μmoles of lipoic acid (oxidized form) and 2.0 μmoles of pyridoxal phosphate were incubated with 100 mg (dry weight) of cells suspended in 200 μmoles/ml KH₂PO₄-KH₂PO₄ buffer (pH 7.0), used to adjust the final volume to 4.0 ml.

② Quantity (μg) taken up by cells

Quantity (μg) added initially × 100.
effects of lipoic acid and of pyridoxal phosphate on *S. diacetilactis* remain unexplained at this time. It is conceivable that both substances might function in quite different areas of cellular metabolism and that the latter is reflected, although indirectly, by an increased requirement for valine. This aspect of the problem is currently under investigation.

The rate of valine uptake as shown in Figure 3 increased with increased external valine concentration. Incorporation into the cells was increased only 2.5 times, with a fivefold increase in external valine concentration; hence, the response was nonlinear.

The rate of C\(^{14}\) valine incorporation into cells was also affected by external addition of glycy1-L-valine (Figure 4). A level of about 0.8 \(\mu\)moles of the latter, under the conditions employed, produced a marked stimulation on valine uptake. This phenomenon was noted only after an initial 4-min incubation and did not continue significantly beyond the 12-min samples. Although glycy1-L-valine is enzymatically hydrolyzed under certain experimental conditions, its mode of action is the subject of current investigations. It is perhaps noteworthy that Brock and Wooley (4) found a similar stimulation by glycy1glycine on C\(^{14}\) glycine uptake by *Streptococcus faecium*.

C\(^{14}\) valine uptake in this organism appears to be associated with the availability of energy-yielding substances. This is supported by the
observation (Figure 5) that increased external glucose concentration results in increased valine accumulation by the cells. Under the conditions of this typical experiment a concentration of from 0.1-2.0 μmoles glucose per milliliter was the effective range. A concentration of 1.5 μmoles glucose per milliliter was necessary to maintain the maximum level of C¹⁴ valine accumulated. The marked depression in the rate of C¹⁴ valine uptake (Figure 6) by both azide and 2,4-dinitrophenol provided additional evidence for the possible involvement of energy-dependent processes.

The uptake of valine was also influenced by changes in the pH of the medium (Figure 7). The organism was unable to accumulate C¹⁴ valine at values above pH 7.5, but accomplished this best in the more acid range of pH. The most rapid uptake during the first 4 min occurred at pH 4.75. During the subsequent 8-min period the rate of uptake was most rapid at pH 6.0. Thus, it appears that the organism could have two transport systems for valine: one functioning best at pH 4.75 and the other at pH 6.0. Other criteria will have to be established, however, before this initial suggestion can be regarded as being completely satisfactory. It is conceivable that the ability of this organism to transport valine at acid pH could be related to its capacity to grow and produce diacetil (16) best at pH 4.5, as this may apply also to other amino acids present in the medium.

Even though valine served as a model amino acid in this particular study, it is already evi-
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REFERENCES