SC 2207

The effect of prior incubation with glycyl-L-alanine on the uptake of peptides by *Lactobacillus casei*

Specific systems have been demonstrated in bacteria for the absorption of peptides (LEACH AND SNELL[1,2]; LEVINE AND SIMMONS[3]) and amino acids (COHEN AND RICKENBERG[4,5]; BRITTEN, ROBERTS AND FRENCH[6]; LEACH AND SNELL[2]). COHEN AND MONOD[7] postulate that specific "permeases" are responsible for the absorption of nutrients (amino acids and carbohydrates), and these systems are able to concentrate the nutrient inside the cell.

Exchange diffusion also results in transport of inorganic ions or amino acids into the cell interior[8,9] but without concentrating them relative to the internal concentration. In this process, an amino acid in the cell pool exchanges with the corresponding amino acid (or other amino acids within the limits of biological specificity) in the external medium through an active process. MANDELSTAM[10] interprets his results with *Escherichia coli* as showing specific intracellular binding sites for the amino acids. What appears as uptake of amino acids when unstarved, resting cells are exposed to a labeled amino acid would in reality be partly an exchange of an incoming radioactive amino acid for an unlabeled amino acid already present in the cell, possibly attached to some intracellular site, and partly a process which permits net uptake of amino acids in growing cells. If an exchange process is important in absorption and if this process is rapid as compared with that which permits net uptake of these compounds, treatment of cells with cold peptide prior to the addition of labeled peptide should saturate the intracellular sites and result in an apparent increased uptake of subsequently added radioactive peptide due to the combined processes of exchange and net uptake. Experiments reported here demonstrate this effect of prior incubation with unlabeled peptide. Thus, systems both for active carrier transport (uphill concentration) and exchange diffusion are present in bacteria.

Lactobacillus casei 7469 was grown as previously described[2]. The cells were harvested by centrifugation and suspended in a salts solution[2]. After 15 min at 37°, glucose (0.1 %, 5.5 mM) was added and incubation was continued for 15 min. To this (Sample A) unlabeled peptide was added and incubated for 60 min. Sample B (starved cells) was incubated for 15 min, glucose was added and the cells were incubated for a further 15 min. Both samples were centrifuged for 15 min in the cold. The cells were suspended in warmed (37°) salts–glucose and labeled peptide was added. At the indicated intervals, an aliquot from each sample was filtered onto a Millipore filter and washed with water. Determination of the radioactivity of these cells gave total uptake. A duplicate sample was added to 1 ml of 12 % trichloroacetic acid and incubated for 15 min at 37°. The insoluble material was filtered onto a Millipore filter. Determination of the radioactivity of this sample gave the incorporation into protein, cell wall, and other insoluble material. The difference between these two values was designated as accumulation or "pool content". Uptake of label by the two cell samples at various times during this first experimental period (Period I) is shown in Fig. 1. After 60-min exposure to the labeled peptide, cells of both samples were centrifuged for 15 min in the cold, then suspended in salts–glucose solution at 37° containing unlabeled peptide. Samples were prepared for counting during this period (Period II, Fig. 1) as described above. After 60-min incubation with the unlabeled peptide, the cells of both samples were centrifuged once more and suspended in salts–glucose

solution (37°) with radioactive peptide. Samples were again taken at intervals during this 60-min period (Period III, Fig. 1).

The results (Fig. 1) show that during Period I those cells which were treated with unlabeled glycyl-L-alanine prior to the addition of the radioactive peptide show a more rapid uptake and accumulated radioactivity to a higher level than those cells

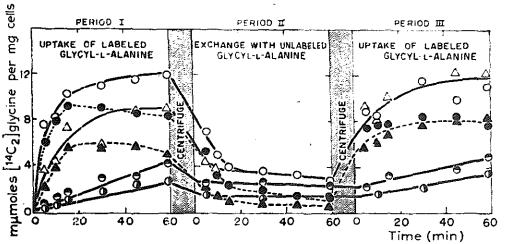

Fig. 1. The effect of prior incubation on glycyl-L-alanine uptake by *Lactobacillus casei*. The cells were divided into two samples, A and B. Sample A was incubated 15 min at 37°, glucose was added and 15 min later 166 μM of unlabeled glycyl-L-alanine was added. Sample B (started 30 min after the addition of peptide to Sample A) was incubated 15 min at 37° and glucose was added for 15 min more incubation. Both samples were centrifuged and suspended in warm salts–glucose containing 180 μM radioactive glycyl-L-alanine. Samples were taken as described in the text, Period I. After 60-min incubation, the cells were centrifuged and suspended in warm salts–glucose containing 190 μM of unlabeled glycyl-L-alanine. Samples were again taken, Period II. At the end of 60 min the cells were harvested by centrifugation and suspended in warm salts–glucose with 180 μM labeled glycyl-L-alanine. The 60-min incubation is Period III. Sample A: ○—○, uptake; ●—●, accumulation; ◑—◑, incorporation. Sample B: △—△, uptake; ▲—▲, accumulation; ⊖—⊖, incorporation.

exposed to the radioactive peptide without previous treatment. The incorporation of label into protein and other insoluble material was greater in those cells not previously exposed to unlabeled peptide, presumably because other amino acids of the pool essential for such incorporation had not been depleted, and possibly because "empty" sites were present that participate in subsequent exchange reactions. Both samples of cells rapidly lost radioactivity during Period II when unlabeled glycyl-L-alanine was subsequently added. During Period III, when both samples of cells were allowed to take up labeled glycyl-L-alanine, the rates and extents of uptake and accumulation were almost equal.

These results show that prior exposure of cells to unlabeled peptide does increase the apparent rate of uptake of labeled peptide in subsequent experiments, in a manner which might be interpreted as an exchange. In its simplest form, however, exchange alone at a site specific for each amino acid or peptide cannot account entirely for these results since it is known[2] that (a) glycyl-L-alanine does not remain intact in these cells, but is rapidly hydrolyzed to the free amino acids, (b) cells supplied this peptide accumulate glycine to a greater extent than do cells supplied free glycine, and (c) the accumulation of alanine and glycine by such cells is not equal. The possible induction of an increased level of "permease" by prior exposure to glycyl-L-alanine appears unlikely (although it is not eliminated) because of the absence in these experiments of all but internal pools of many essential amino acids.

Although the occurrence of exchange reactions independent of and in addition to the reactions that lead to net uptake of amino acids and peptides by bacteria complicates measurement of the kinetics of accumulation with radioactive tracers, previous conclusions that transport of these substances into the cell occurs by an active, energy-dependent process with structural specificity characteristic of enzymic processes remain unchanged.

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Leucine biosynthesis in higher plants

There is considerable evidence from labelling experiments that in microorganisms leucine arises from the condensation of 2-ketoisovaleric acid (ketovaline) and acetate[1–3]. Support for such a pathway, involving the intermediate formation of α-isopropylmalate, has recently been obtained at the enzyme level in *Salmonella typhimurium* and *Torulopsis utilis*[4,5] and α-isopropylmalate has been isolated from cultures of several microorganisms[6]. In the course of an investigation of the biosynthesis of linamarin in flax, some evidence for the presence of this pathway for leucine biosynthesis was obtained incidentally and the results are briefly reported here.

Flax seeds (*Linum usitatissimum* L. var. *Imperial*) were germinated and grown for 72 h in the dark on gauze moistened with dilute nutrient solution. They were next exposed to artificial light of intensity approx. 2000 ft-candles from an incandescent lamp for 16 h. Uniformly labelled L-[^{14}C]valine was administered by either of the following methods:

(a) 20 seedlings selected for uniformity were placed in a 5-ml beaker containing 5 μmoles L-[^{14}C]valine (1 μC) in 2 ml water. This was administered for 48 h with aeration and continuous illumination.

(b) The stems of 20 seedlings were cut with a sharp razor blade 2 cm below the cotyledon leaves and placed in a 1-ml beaker containing 5 μmoles L-[^{14}C]valine (1 μC) in 0.2 ml water. Successive amounts of 0.1 ml water were added as required during a 7-h absorption period in continuous light.