Occurrence of independent uptake mechanisms for glycine and glycine peptides in *Lactobacillus casei*

Indirect evidence has been obtained by several workers indicating that there are differences in the mode of uptake or utilization of amino acids and peptides. KIHARA AND SNELL[1] postulated that absorption of L-alanine-containing peptides by *Lactobacillus casei* is by a different pathway than the absorption of L-alanine. They based this postulate on the fact that D-alanine antagonizes the utilization of externally supplied L-alanine during the growth of *L. casei* on a vitamin B_6-deficient medium but does not antagonize the utilization of L-alanine supplied externally in the form of peptides, or of L-alanine synthesized internally in the presence of vitamin B_6. Peptides are quite generally more effective than free amino acids in overcoming inhibitory effects for bacteria of antagonistic amino acids[2,3] and, if separate absorption mechanisms exist, this may frequently result from interference in absorption of the free amino acid, but not of the peptide, by the antagonist. Consistent with this hypothesis are the findings of ROWLANDS *et al.*[4], who showed that certain glutamic acid-containing peptides gave rise to intracellular glutamic acid in *Staphylococcus aureus* faster than free glutamic acid. However, they presented no evidence that the absorption pathway of peptides differs from that of the amino acid. This report describes experiments which show that certain glycine-containing peptides are accumulated by *L. casei* via a different pathway and at a faster rate than glycine and appear to be hydrolyzed to the free amino acids prior to incorporation into protein.

L-Alanyl[$^{14}C_2$]glycine was synthesized from uniformly labelled glycine and was shown to be pure by paper chromatography. *L. casei* 7469 was grown on a synthetic medium and harvested by centrifugation. The cells were resuspended in the salts solution of the growth medium containing 0.1 % glucose and the rate of uptake of radioactive compounds was determined by the membrane-filter technique of BRITTEN, ROBERTS AND FRENCH[5] using "Millipore" filters. Determination of the radioactivity of a sample of cells, obtained by filtration of a 0.5-ml aliquot of cell suspension onto the filter and then washing with 0.5 ml water, gave the total "uptake". A duplicate sample of cells was incubated for 15 min at 37° with 1.0 ml 12 % trichloroacetic acid and the insoluble material filtered onto a "Millipore" filter. Counting of this sample gave the "incorporation" into trichloroacetic acid-insoluble material. The difference between uptake and incorporation is referred to as accumulation or "pool" content. Concentrations of glycine and peptide that gave maximum rates of uptake were determined, and these saturating concentrations used in the experiments reported here.

Fig. 1 shows the curves for uptake, accumulation, and incorporation with [$^{14}C_2$]-glycine and L-alanyl[$^{14}C_2$]glycine as substrates. The rate of accumulation of the peptide is approximately 10 times that of glycine. It is noteworthy that more glycine is accumulated from the peptide than from the amino acid. Such uptake required the presence of an energy source (glucose), and did not occur at 2°. No growth occurs during these experiments, since all of the amino acids and vitamins essential for *L. casei* are missing. When chloromycetin (100 μg/ml) is present during incubation with radioactive substrate, "incorporation" is reduced to approximately 10 % of the normal without affecting either the rate or extent of accumulation.

By centrifuging the cells during the accumulation process and decanting the

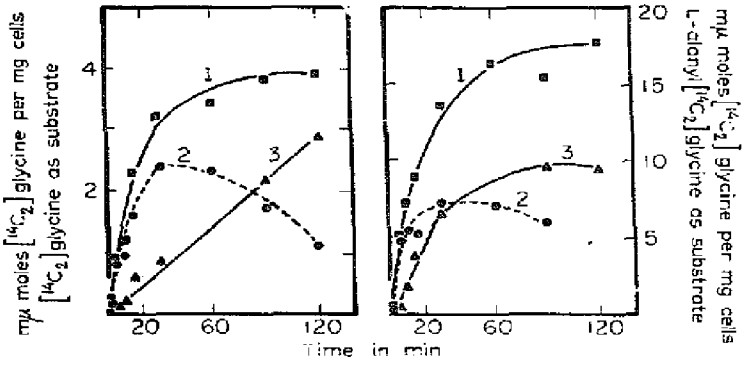

Fig. 1. The time course of uptake, accumulation and incorporation of [$^{14}C_2$]glycine and L-alanyl-[$^{14}C_2$]glycine. Resting cells of *L. casei* in a final vol. of 10 ml were incubated for 30 min at 37° in salts solution, then glucose was added to a final concentration of 0.1 % and incubation continued for another 30 min. L-alanyl[$^{14}C_2$]glycine (140 mμmoles/ml) or [$^{14}C_2$]glycine (42 mμmoles/ml) were then added and samples taken at the indicated times for analysis as described in the text. Each sample contained 780 μg cells. Activity of samples was 3 to 10 times the background counting rate; a total of 1280 counts was measured. Curve 1, uptake; 2, accumulation; and 3, incorporation.

supernatant solution, a sample of cells may be obtained which contains a labelled pool. There is little loss of radioactivity from this pool when the cells are resuspended in salts-glucose medium. When either unlabelled glycine or unlabelled glycyl-L-alanine is added to the suspension medium, substantial loss of label results; this occurs much faster with added peptide than with glycine. Between 90 and 100 % of the label of the pool can be exchanged or displaced by this process. Chromatography of samples of the labelled pool obtained by boiling the cells, centrifuging, and lyophilizing the supernatant solution showed that all of the radioactivity was present as free glycine even when labelled peptide had been the substrate for the accumulation experiments.

That accumulation of peptide and free amino acid occurs by separate processes is shown by the lack of competition of unlabelled glycine during the accumulation of radioactivity from the labelled peptide. There was no effect on either the extent or rate of accumulation; however, the unlabelled glycine lowered the rate of incorporation of radioactive glycine from the peptide. This suggests that the peptide is hydrolyzed to the free amino acids before incorporation occurs. Cell-free extracts of *L. casei* rapidly hydrolyze the peptides used in this study.

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