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THE TRANSPORT AND UTILIZATION OF PEPTIDES BY *ESCHERICHIA COLI*: INTERRELATIONS BETWEEN PEPTIDE UPTAKE AND AMINO ACID EXODUS AND BIOSYNTHESIS

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1. Introduction

We recently described [1] a direct procedure for studying peptide transport by *Escherichia coli* that does not require amino acid auxotrophs or radioactive substrates. Disappearance of peptide from the incubation medium, and appearance of peptide (and hydrolysed amino acid residues) within the bacterial cell is monitored by preparing fluorescent dansyl derivatives of the peptides which are then separated by thin-layer chromatography. Peptide concentrations are determined from the fluorescent intensities on the chromatograms. Using this method, we have now found that during transport of certain peptides, not only do the cleaved amino acid residues simultaneously leave the cell [1] but additional, newly synthesized amino acids also appear in the medium. Possible implications of these findings are discussed.

2. Materials and Methods

2.1. Materials

Peptides and sodium lactate were obtained from Sigma. Dansyl chloride and polyamide sheets (25 cm^2) were from BDH (Poole, England). Membrane filters (type HA, 13 mm and 25 mm diam., 0.45 μm pore size), and the corresponding Swinnex filter holders were obtained from Millipore. Other chemicals were of analytical grade.

2.2. Growth of bacteria

A spontaneous wild type revertant of the lysine auxotroph M-26-26 of *E. coli* W (ATCC 9637) was used [2]. Cultures were grown at 37°C in the minimal medium A of Davis and Mingioli [3] supplemented with glucose (0.5%, w/v), or lactate (1%, w/v).

2.3. Transport assays

Exponential phase bacteria (approx. $1 \cdot 10^9$ ml^{-1}) were collected by centrifugation [1], or by filtration through a 25 mm Swinnex system. After washing the cells with 5 ml of 50 mM potassium phosphate buffer (pH 7.2, 37°C), the filter was removed and the bacteria resuspended in the required medium (see Results) using a vortex mixer. The filtration procedure routinely took less than 1 min. The bacteria (final concentration approx. $2 \cdot 10^9$ ml^{-1}) were preincubated for 15 min at 37°C, with required energy source, inhibitor and other additions, as specified, and transport initiated by addition of peptides (100 μM). Periodically, samples (0.2–1.0 ml) were taken with a syringe, rapidly filtered through a 10 mm Swinnex and samples (0.05 ml) of the filtrates were dansylated. When using arsenate (20 mM), bacteria were first washed and then resuspended in 100 mM Tris buffer pH 7.0 (minus phosphate), before incubating 15 min to deplete the intracellular ATP levels [4].

2.4. Extraction of cytoplasmic pool

A bacterial sample was quickly washed on the Swinnex filter with 2 ml of 20 mM potassium phosphate buffer (pH 7.2, 37°C) and the filter immediately added to 1 ml boiling water. Toluene (0.05 ml) was added, the tube capped, and the bacteria resuspended by using a vortex mixer (30 s) and an ultrasonic bath

(20 s). The suspension was boiled 15 min, the cooled extract filtered through a Swinnex system, and the filtrate made to 1 ml.

2.5. Dansylation and chromatography

Fluorescent estimation of amino acids and peptides in incubation media and extracts was achieved by dansylation and chromatography [1]. When required, resolution was improved by finally rerunning the polyamide sheets in the 1st dimension using solvent system 1.

3. Results

Fig. 1 A–G shows that trivaline is rapidly transported by *E. coli* and that consequently it completely disappears from the incubation medium; simultaneously, the intracellularly hydrolysed valine residues leave the bacteria and appear in the medium. Transport was inhibited by arsenate (Fig. 1 H, I), and by several other energy poisons and uncouplers (Payne, unpublished results). Adding lactate to the incubation medium (Fig. 1L) in place of glucose (Fig. 1K) severely diminished uptake (Fig. 1 J–L). These results are typical of those seen with many peptides [1]. However, a novel feature here was the additional exodus of proline (Fig. 1 A–L). The effect is specific for valyl peptides, divaline giving identical results, but free valine gave no similar proline exodus. Examination of extracts from cells transporting valine peptides (Fig. 1, M, N), showed similar intracellular proline concentrations to cells incubated without valine peptides (not shown). Furthermore, the prline pool was calculated

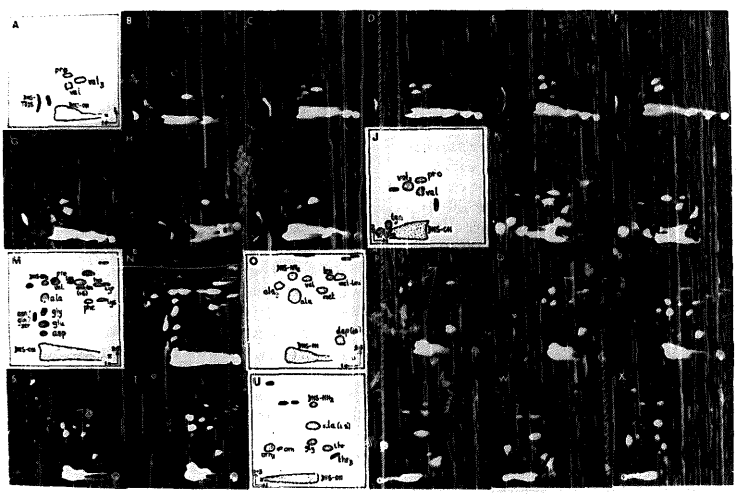

Fig. 1. Peptide uptake and amino exodus in *Escherichia coli.* Chromatograms of dansyl derivatives were photographed under ultraviolet radiation [1]. Diagrams, A, J, M, O, U show identities of derivatives; cross hatched spots are "unknowns"; i.s., internal standard [1]; dap, diaminopimelic acid; ×, origin; 1,2,3 refer to the solvent systems used [1]. 1B, C, D, E, F, G, and H, I are supernatant samples collected after 0, 2, 5, 9, 13, 18, and 2, 13 min incubation, respectively. Cells used in K, L were grown on lactate and the incubation media were sampled at 12 min; P, Q, R, S, T, and V, W, X are supernatant samples taken after 0, 2, 5, 10, 20 and 0, 5, 10 min incubation, respectively.

(either from (*i*) observation of the original pool extracts, or (*ii*) using values for intracellular water volumes and dry wt. of bacteria [6]), to comprise only about 10% of the observed proline exodus. It follows therefore, that much of the proline leaving the cells must have been newly synthesised.

When *E. coli* was incubated with methionyl leucine, the dipeptide was rapidly taken up from the medium, and free methionine and leucine appeared in its place (Fig. 1 O–T). However, after several minutes, exodus of free valine was also observed. Again this effect was peptide-specific, for identical incubations with either free methionine or leucine, or both, failed to cause similar valine exodus. Fig. 1 O–T also show that after complete uptake of its peptide, met-leu, and exodus of methionine and leucine, leucine was quickly reaccumulated, leading to an imbalanced distribution of the amino acids in the medium. These experiments (Fig. 1O–T) also show uptake and cleavage of trialanine, but its presence had no effect on the valine exodus. In a further example, we noted that incubation with trithreonine led to exodus of virtually equivalent amounts of threonine and glycine (Fig. 1U–X); the presence of diornithine in these samples did not influence the observed amino acid exodus. In the above three examples, identification of the effluxed valine, proline and glycine was confirmed by using an automatic amino acid analyser.

4. Discussion

E. coli, which lacks extracellular peptidases, is known to absorb peptides and to hydrolyse them with intracellular enzymes. Recent studies [1] have endorsed an earlier speculation [5] that exodus of the liberated amino acid residues can occur simultaneously with peptide uptake, and that transport continues until peptide uptake is complete. Certain consequences may arise from this situation. Thus, after transport and cleavage of a peptide, if exodus of the released amino acids is not at least as fast as peptide uptake, their concentration in the pool will increase. Through various feedback mechanisms these could regulate uptake by amino acid permeases [6]. This ability to control amino acid uptake provides a possible explanation for the "peptide-specific" nature of the effects reported here. In addition, biosynthetic pathways for these amino acids, which will frequently be end-product regulated, will be switched off, which may lead to the transient switching of metabolic intermediates into alternate pathways. Finally, catabolism of the excess amino acid residues may lead to yet further products. Certain of these speculations may be illustrated by the present results. Thus, with valyl peptides, excess intracellular valine leads to the synthesis and exodus of proline. The appearance of glycine from trithreonine may indicate the action of threonine aldolase (EC 4.1.2.5) which converts threonine to glycine and acetaldehyde [7]; interestingly, trithreonine is reportedly toxic to *E. coli* [8]. The exodus of valine, seen with the peptide met-leu, may reflect the biosynthetic relationship between the branched chain amino acids [6]. The observed exodus is clearly pertinent to the process of "counter-flow", by which a pool amino acid can exchange for an entering amino acid [9], and to other exchange-coupled processes [6,10,11]. The results highlight the interrelations between uptake, exodus and biosynthesis, and point a warning to the problems that could arise when transport events are monitored using only radiotracer techniques. To study these interrelations further we propose using mutants deficient in either the transport, or biosynthesis and metabolism of amino acids.

Acknowledgments

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References

[1] Payne, J.W. and Bell, G. (1977) J. Bacteriol. In press.
[2] Payne, J.W. (1974) J. Gen. Microbiol. 80, 269–276.
[3] Davis, B.D. and Mingioli, E.S. (1950) J. Bacteriol. 60, 17–28.
[4] Klein, W.L. and Boyer, P.D. (1972) J. Biol. Chem. 247, 7257–7265.
[5] Payne, J.W. (1975) in: Peptide Transport in Protein Nutrition (Matthews, D.M. and Payne, J.W., Eds.), pp. 341–344, North-Holland, Amsterdam.
[6] Quay, S.C., Dick, T.E. and Oxender, D.L. (1977) J.

[7] Simmonds, S. and Miller, D.A. (1957) J. Bacteriol. 74, 775–783.
[8] Barak, Z. and Gilvarg, C. (1975) J. Bacteriol. 122, 1200–1207.
[9] Brock, T.D. and Wooley, S.O. (1964) J. Bacteriol. 104, 177–182.
[10] Gross, W., Ring, K. and Heinz, E. (1970) Arch. Biochem. Biophys. 137, 253–259.
[11] Maloney, P.C., Kashket, E.R. and Wilson, T.H. (1975) in: Methods in Membrane Biology (Korn, E., Ed.), Vol. 1 pp. 1–49, Plenum, New York.