A POSSIBLE TRANSPORT SYSTEM FOR 2,6-DIAMINOPIMELATE AND LYSINE PRODUCTION IN RUMEN CILIATE PROTOZOA

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Introduction

Rumen ciliate protozoa have been known to produce lysine from free 2,6-diaminopimelate (DAP) (Onodera and Kandatsu, 1973; 1974) and this fact has been confirmed by Masson and Ling (1986). The amount of lysine produced from free DAP by rumen protozoa, however, did not coincide between the two reports, where the former was much higher. To know one of the reasons why there was a difference in lysine production, an effect of the concentration of substrate (DAP) was thought to be examined and in this process, a possible existence of some transport systems for DAP was introduced into mixed rumen protozoa.

In the present paper, a transport system for DAP in mixed rumen protozoa will be reported in relation to lysine production at the substrate levels for near the maximum production rate.

Materials and Methods

Rumen ciliate protozoa were collected from the rumen contents of a fistulated goat (Japanese native breed, female, body weight: 45 kg) fed on a ration consisting of haycube (600 g/day) and concentrate feed (200 g/day) twice daily at 09:00 and 17:00. The mixed protozoal suspension (about 4%, v/v) was prepared using MB9 buffer solution (initial pH: 6.8) (Onodera and Henderson, 1980), which always contained three kinds of antibiotics (0.1 mg/ml each of dihydrostreptomysin sulfate, penicillin G potassium and chloramphenicol sodium succinate), in the same manner as described previously (Onodera and Kandatsu, 1974). In the experiment to examine the effect of substrate levels on the lysine production by the mixed protozoal cell suspension, 3-ml portions of the suspension containing 0.1 to 8.0 mM of DAP (three incubation test tubes for one substrate level) were incubated at 39°C for 6 hr. In the experiment to examine the effect of the addition of single amino acids other than DAP on the lysine production, 3-ml portions of the protozoal suspension containing 3 or 5 mM of DAP were incubated with the same concentration of single other amino acids as DAP (using three incubation test tubes for each amino acid) at 39°C for 6 hr. Sonicated protozoal homogenate in MB9 buffer solution was also incubated similarly at 39°C for 1 hr. Incubation was carried out once or twice for each amino acid tested. After incubation, the incubation mixture was mixed with sulfosalicylic acid so as to be 3% (w/v) in final concentration to stop the propozoal activity and left for more than 3 hr in a refrigerator. The mixture was then centrifuged (27,000 x g, 15 min at 4°C) and the supernatant fluid was submitted for analysis of lysine. Lysine was analyzed by an automatic amino acid analyzer (AA-100, Sibata Chemical App. Manufacturing Co., Ltd., Japan).

Results

At first, an effect of substrate (DAP) levels on the lysine production by mixed rumen diliate protozoa was examined and as a result, the maximum lysine production was obtained at the level of 5 mM DAP. At the levels of over 6 mM, lysine production decreased.

In the next experiment, inhibitory and stimulative effects of addition of single amino acids other than DAP upon the lysine production from DAP by intact cell suspensions of rumen protozoa were observed (table 1). Though all data are expressed as percentages in table 1, the actual amount of lysine production, for example, was 292.1 nmol/ml in the medium containing only DAP (5 mM), while it decreased to the level of 132.8 nmol/ml (45.5%) in the medium containing DAP (5 mM) and L-alanine (5 mM). On the other hand, single amino acids tested (alanine, glycine, 2-aminobutyrte, valine and leucine) did not have any effect on the lysine production by sonicated protozoal homogenate (table 2). In these experiments, the

TABLE 1. INHIBITION OR STIMULATION OF LYSINE PRODUCTION FROM 2,6-DIAMINOPIMELATE (DAP) BY ADDITION OF SINGLE OTHER AMINO ACIDS IN RUMEN PROTOZOAL SUSPENSION DURING 6-HR INCUBATION AT 39°C

Amino acid added	Lysine[a] yield (%)	Amino acid added	Lysine[a] yield (%)
L-Alanine (3 mM)	69.2, 80.6	MAIB[b] (20 mM)	95.4
(5 mM)	45.5, 76.1	L-Aspartate (3 mM)	117.5
Glycine (3 mM)	82.6, 91.4	(5 mM)	91.2
(5 mM)	92.3	L-Glutamate (3 mM)	125.7
L-Serine (3 mM)	48.2	(5 mM)	121.7
L-Cysteine (3 mM)	46.0	L-Valine (3 mM)	118.2, 324.7
2-Aminobutyrate (3 mM)	79.6	(5 mM)	117.0, 141.0
(5 mM)	75.1	L-Leucine (3 mM)	126.2, 207.6
2-Aminoisobutyrate (3 mM)	59.4	(5 mM)	142.1, 176.1
MAIB[b] (1 mM)	109.0	L-Isoleucine (3 mM)	172.7
(10 mM)	105.7	(5 mM)	148.7

[a] Percentage of lysine production in the medium containing DAP (3 or 5 mM except for the case of MAIB) and the same concentration of other single amino acids as DAP to that in the medium containing only DAP.
[b] 2-Methylaminoisobutyrate (MAIB) at the levels shown in ( ) was incubated with 1 mM DAP.

TABLE 2. EFFECT OF ADDITION OF SINGLE AMINO ACIDS OTHER THAN 2,6-DIAMINOPIMELATE (DAP) ON LYSINE PRODUCTION FROM DAP BY SONICATED PROTOZOAL HOMOGENATE DURING 1-HR INCUBATION AT 39°C

Amino acid added	Lysine yield (%)[a]	
	3 mM DAP	5 mM DAP
L-Alanine	105.2	101.0
Glycine	99.0	99.0
2-Aminobutyrate	99.0	95.0
L-Valine	101.0	95.2
L-Leucine	95.8	86.9

[a] Percentage of lysine production in the protozoal homogenate containing DAP (3 and 5 mM) and the same concentration of other single amino acids as DAP to that in the homogenate containing only DAP.

compositions of rumen protozoa (percentages in number) were as follows: Entodiniinae, 93-99%; Diplodiniinae, 0.6-1.5%; Isotrichidae, 0-6.6%.

Discussion

The present experiments were carried out standing on a hypothesis that intact cells of rumen protozoa can produce lysine from DAP only after absorbing DAP inside their cells and so there may be some transport systems for the amino acid. This seemed to be partially supported by some of the results obtained in the present experiments that lysine production increased with increasing substrate level up to 5 mM and decreased at the levels over 6 mM and that inhibitory and stimulative effect of single amino acids on lysine production from DAP was observed not in cell homogenate (table 2) but in only intact cell suspension (table 1).

At present, there have been known to be three distinct transport systems (Systems A, L and ASC) for neutral amino acids in many eukaryotic cells (Shotwell et al., 1981). Namely, System A (alanine) serves mainly for the uptake of L-alanine, glycine and 2-aminoisobutyrate, System L (leucine) for L-leucine, L-valine, L-isoleucine and L-phenylalanine and System ASC (alanine, serine and cysteine) for L-alanine, L-serine and L-cysteine. System ASC can be distinguished from System A by its inability to accept N-monomethylated analogs like 2-methylaminoisobutyrate (MAIB) which is known to specifically inhibit a transport of neutral amino acids by System A.

In the present experiments, lysine production, namely the uptake of DAP, tended to be inhibited by neutral, linear, short chain amino acids and stimulated by neutral branched chain and acidic amino acids, though the effect of aspartate was negligible. Remarkable facts were that inhibition rate by glycine was not so high and MAIB did not

have any inhibitory effect even in high concentration. According to these findings, a transport system for DAP in mixed rumen protozoa and suggested to be System ASC.

At the same time, one of the causes of a diffeence in lysine production between the two reports, Onodera and Kandatsu (1974) and Masson and Ling (1986), seemed to be the differences of the substrate (DAP) concentration and other amino acids levels due to different incubational conditions.

(**Key Words**: Rumen Protozoa, Amino Acid Transport System, Lysine, 2,6-Diaminopimelate)

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