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Most of gram-negative and gram-positive bacteria contain 2,6-diaminopimelic acid (DAP) as a component of their peptidoglycans in cell walls. DAP has been studied in the rumen ecosystem both as a marker of bacterial biomass (Hutton et al, 1971, Br J Nutr, 25, 165-173) and as a precursor of lysine production by rumen protozoa (Onodera and Kandatsu, 1973, Nature New Biol, 244, 31-32; Onodera et al, 1974, Agric Biol Chem, 38, 921-926). These were confirmed by Masson and Ling (1986 ,J Appl Bacteriol, 60, 341-349). However, it is not yet clarified which types of stereoisomers of DAP (DAP-SI) can be converted to lysine by rumen protozoa and bacteria. In the present study, we planned to examine the metabolism of the three DAP-SI by mixed rumen protozoa and bacteria to reveal the type of DAP-SI convertible to lysine.

At first, a standard solution of DAP (mixture of three stereoisomers, Sigma) was disolved in buffer solution (MB9) containing no microbes and incubated for up to 12 h at 39°C with antibiotics (0.1 mg/ml each of chloramphenicol, streptomycin sulfate and penicillin G potassium) and with and without sterilization treatment of MB9 and all tools to be used to check the stability of DAP-SI under the cultural conditions. Rumen microorganisms were collected from fistulated goats (Japanese native breed), and the suspensions of mixed protozoa (P) and mixed bacteria (B) were prepared and anaerobically incubated with and without DAP (5 mM, mixed DAP-SI ; less than 1 mM, individual DAP-SI) for up to 12 h at 39°C using sterilized MB9 and tools. All incubations contained 0.5 mg/ml rice starch. Samples (1 ml) of the incubations were collected at 0, 6 and 12 h, deproteinized with trichloroacetic acid (TCA) and centrifuged at 27000 x g for 20 min. The supernatant was extracted three times with diethyl ether in a separating funnel to remove TCA and evaporated to remove diethyl ether. Bacterial sediment was hydrolysed with 6 M HCl. All samples were analysed for DAP-SI and lysine by HPLC (El-Waziry et al, 1994, VIIIth ISRP).

Three DAP-SI dissolved in MB9 showed no changes during 12 h incubation at 39°C under sterile conditions. Under unsterilized conditions, however, 11.6, 6.80 and 6.20% (against total DAP) of meso-, LL- and DD-DAP, respectively, were broken down.

In P suspensions, DAP-SI (mixture) decreased by 0.52 mM (10.59%) and was converted mainly to 0.41 mM lysine (8.35%) after 12 h incubation under sterile conditions. The remainder (0.11 mM, 2.24%) of DAP may have been converted to an undetermined compound like pipecolic acid as an end product of lysine. When meso-, LL- and DD-DAP were added singly to the media, the preliminary results showed that mixed rumen protozoa have an ability to synthesize lysine from meso-DAP and also from DD- and LL-DAP probably via a meso-DAP intermediate. This is the first demonstration showing the synthesis of lysine from three DAP-SI by mixed rumen protozoa.

In B suspensions, the added DAP-SI (mixture) decreased by 0.54 mM (10.84%) and was converted to 0.21 mM lysine (4.22%) after 12 h incubation in supernatant plus hydrolysates. The remaining DAP (0.33 mM, 6.63%) may have been used for constructing cell walls and converted to undetermined compounds such as acetic and butyric acids and ammonia probably via lysine. When meso-, LL- and DD-DAP were added singly to the media, the preliminary results showed that mixed rumen bacteria have the ability to synthesize lysine from LL- and DD-DAP probably via meso-DAP, this is the first discovery of lysine synthesis from DAP by rumen bacteria.