IN VIVO METABOLISM OF 2,2'-DIAMINOPIMELIC ACID AND ITS ACCURACY AS A MARKER OF BACTERIAL BIOMASS IN DUODENAL DIGESTA OF SHEEP

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Introduction

The amino acid, 2,2'-diaminopimelic acid (A_2pm, also known as DAP) is still the most popular marker for estimating the bacterial biomass content of digesta within the gastro-intestinal tracts of ruminant animals. Yet doubts about its accuracy continue, not least because of its known *in vitro* metabolism by rumen microorganisms (Onodera et al., 1974; Masson and Ling, 1986; Denholm and Ling, 1989). The work presented here is part of a study of the *in vivo* metabolism of A_2pm.

Materials and Methods

Three mature, wether sheep were each fitted with a permanent cannula in the rumen and re-entrant cannulas in the proximal duodenum. The daily dietary allowance was 600 g concentrate plus 500 g chopped hay (fresh weight basis) offered in two equal feeds at 09:00 and 16:00 h. Water was available at all times. The animals were housed in metabolism cages under conditions of constant light and temperature.

The experimental period lasted 4 days. On days 1 and 4, at 15:00 h, samples of rumen digesta were removed and isolates of mixed bacteria were prepared as described by Ling and Buttery (1978). Cells of the bacterial mutant, *Bacillus magaterium* GW1 were radiolabelled with [^{3}H] A_2pm and harvested as detailed by Denholm and Ling (1989). On day 2 at 09:00 h, 3.4 g DM [^{3}H] A_2 pm-labelled *B. megaterium* GW1 cells were infused, as single shots, into the rumen of each animal. Throughout day 2, total duodenal digesta was collected by methods similar to those of Ling and Buttery (1978).

Samples of duodenal digesta were mixed with equal volumes of picric acid (20 g per l) for 12 h at 4°C before centrifugation (30,000 g for 20 min at 5°C) produced deproteinised supernatants. Picrate was removed from these by column chromatography and they were further processed by the methods of Masson and Ling (1986). Supernatants were stored at −20°C; rumen bacterial isolates and samples of unfractionated duodenal digesta were freeze-dried and stored in desiccators at 4°C.

Samples were acid-hydrolysed by refluxing with 6 M-HCl for 22 h and subsequently prepared according to the methods of Ling and Buttery (1978). Concentrations of A_2pm were measured by using either a standard amino acid analyser elution system with the addition of a sodium citrate buffer (pH 3.80) to separate A_2pm from methionine and detection by ninhydrin, or by elution at 62.5°C with a single buffer (pH 3.66) and detection by acid ninhydrin. Total N was determined by the Kjeldahl method.

Results

The A_2pm content of the rumen bacterial isolates produced on days 1 and 4 varied from 2.83 to 5.21 g A_2pm-N per kg total N, but there were no significant differences ($p > .05$) due to either animals or times of sampling.

The total A_2pm contents of duodenal digesta samples were determined from unfractionated, acid-hydrolysed, freeze-dried digesta; analysis of acid-hydrolysed, deproteinised supernatants gave measures of A_2pm as peptidyl plus free acid forms and deproteinised supernatant samples were used to assess the free A_2pm concentrations. The differences between total A_2pm and peptidyl plus free A_2pm, namely the A_2pm contents of the picrate pellets, were assumed to be measures of A_2pm associated with either whole or large fragments of bacterial cells.

Figure 1 shows the patterns of mean A_2pm concentrations in these digesta fractions; no signi-

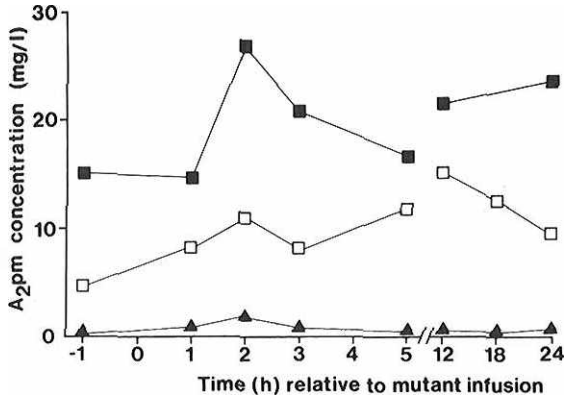

Figure 1. Mean concentrations of total A_2pm (■), peptidyl plus free A_2pm (□) and free A_2pm (▲) in duodenal digesta of sheep.

ficant differences ($p > .05$) due to animals were evident. The 2 h post-infusion maxima were probably due in part to the rapid passage of some *B. megaterium* GW1 related material as well as a 2 h post-feeding effect. Nevertheless, A_2pm contributions originating from rumen bacteria (as opposed to those from the mutant) must have been substantial because A_2pm from *B. megaterium* GW1 cells would be either nil or minimal in the pre-infusion (that is, −1 h) and the 12 to 24 h measurements, yet the patterns of A_2pm-containing compounds persisted.

Peptidyl plus free forms of A_2pm, expressed as a proportion of the total A_2pm varied from 29.9 to 71.0% throughout the sampling period. Free A_2pm constituted 0.1 to 5.6% of the total A_2pm.

Discussion

Denholm and Ling (1989) have demonstrated that the *in vitro* degradation of A_2pm-containing bacteria by rumen microorganisms can be extensive. This present study confirms a similar process occurring *in vivo*. Thus A_2pm contained in duodenal digesta is not all closely associated with bacterial cellular material. Indeed, based on the difference between the total A_2pm and the peptidyl plus free A_2pm data reported here, an average of only 50% is.

These observations have implications for the use of A_2pm as a marker of bacterial biomass. Firstly, if some fractionation of duodenal digesta, such as solvent extraction or centrifugation, occurs prior to acid-hydrolysis and A_2pm analysis, then underestimates of A_2pm passage are likely to occur. Secondly, since marker accuracy depends upon a close relationship between the A_2pm bound in whole bacterial cells (as measured in the rumen bacterial isolates) and the A_2pm contained in digesta, estimates of bacterial biomass calculated from total duodenal A_2pm measurements that include (from 30 to 71%) soluble A_2pm *in both peptidyl and free forms, will inevitably* lead to inaccuracies.

Conventional calculations relation A_2pm and N in rumen bacterial isolates to A_2pm and N in duodenal digesta, showed that in these animals, the mean ratio of bacterial N: total N in the digesta was 0.77 ± 0.153 (mean ± SE of 12 observations). The fact that this value seems 'reasonable' should not necessarily be interpreted as being 'realistic'. The considerable metabolism of bacterial cells that occurs between the rumen and proximal duodenum with the production of numerous A_2pm-containing complexes, should give cause to question the accuracy of A_2pm as a bacterial biomass marker.

(Key Words: Diaminopimelic Acid, Bacterial Biomass Marker)

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