SOME OBSERVATIONS ON THE SUSCEPTIBILITY OF PEPTIDES TO DEGRADATION BY RUMEN MICROORGANISMS

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Introduction

The hydrolysis of peptides to amino acids is often the rate-limiting step in the wasteful conversion of protein to ammonia in the rumen (Chen et al., 1987; Broderick and Wallace, 1988). A transient accumulation of peptides occurs immediately after feeding when the diet contains protein that is rapidly degraded, but published experiments differ in their estimates about how much peptide may escape degradation even after prolonged incubation in the rumen. Substantial quantities of ninhydrin-reactive material, thought to be amino acids, were released by acid hydrolysis of cell-free rumen fluid taken 8 h after feeding, implying that nutritionally significant amounts of peptide escaped hydrolysis (Chen et al., 1987). In contrast, a fluorescence technique, in which peptides react with fluorescamine to a much greater degree than amino acids, indicated that peptide concentrations were low in rumen fluid after the post-feeding peak (Broderick and Wallace, 1988). The aim of the present work was to resolve this apparent contradiction by comparing the techniques directly. Structural factors affecting the degradation of peptides were also assessed, by following the rates of breakdown of different individual compounds added to rumen fluid *in vitro*.

Materials and Methods

Two sheep received twice daily 150 g grass nuts, 150 g grass hay and 150 g concentrate (Broderick and Wallace, 1988). Samples were removed 7 h after feeding via rumen cannulae, and kept at 39°C for 10 min before being strained through four layers of muslin. The rumen fluid was centrifuged for 5 min at 125 g and 39 °C to remove particulate material. Procedures were thereafter as described by Chen et al. (1987). Bacteria were removed by centrifugation, the supernatant was extracted with perchloric acid to remove protein, then adjusted to alkaline pH and boiled to remove ammonia, and hydrolysed with HCl. The HCl hydrolysate was neutralized with NaOH.

Ammonia and fluorescamine-reactive peptides were determined as described previously (Broderick and Wallace, 1988). Amino acids were analysed by ion-exchange chromatography (Wallace, 1986) and the Folin reagent was used in the Lowry method. O-Phthalaldehyde (OPA) analysis for amino N was carried out fluorimetrically (Roth, 1971).

The rates of hydrolysis of individual peptides were determined in samples of rumen fluid removed 3 h after feeding. Peptides (0.05 vol of 25 mM solution) were mixed with strained rumen fluid, incubated at 39 °C, and samples were removed into 0.25 vol 1.25 M H_3PO_4. Remaining peptide in the supernatant after centrifugation was determined by reverse-phase HPLC. Heptanesulphonic acid was used as an ion-pairing reagent for the more hydrophilic peptides.

Results and Discussion

The analysis of extracellular, acid-soluble material present in rumen fluid gave the same apparently contradictory results as had been found before (Chen et al., 1987; Broderick and Wallace, 1988). Although the concentration of peptides present in rumen fluid was undetectable using the fluorescamine procedure, large quantities of ninhydrin-reactive material were released by acid hydrolysis (table 1). If this material comprised amino acids similar to casein hydrolysate, the quantity of peptides escaping ruminal degradation would be of the same magnitude as the 400 μg/ml (64 μg N/ml) value suggested by Chen et al. (1987).

One difficulty that was encountered in these experiments was that the removal of ammonia was not as effective as had been claimed. Only 86 % of the ammonia was removed, resulting in falsely high values in the ninhydrin analysis of acid hydrolysates because of the reaction between

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ninhydrin and ammonia. OPA also detects free amino groups, but reacts very much less with ammonia. Even with this source of potential error removed, however, OPA analysis gave a similar conclusion to ninhydrin, that considerable amounts of amino groups were released by acid hydrolysis (table 1). Analysis using the Folin reagent gave even higher apparent concentrations of amino acids in the hydrolysate, presumably because of the presence of phenolic compounds in rumen fluid. Other protein assay reagents, including Coomassie Blue and bicinchoninic acid, also suffered from severe interference.

Direct analysis of the amino acids present in acid hydrolysates finally resolved the anomaly. Only low concentrations of amino acids, as opposed to amino groups, were released by acid hydrolysis, depsite the ninhydrin and OPA analyses (table 1). Thus the released amino groups must be of non-protein origin. It is possible that they are amino sugars released by hydrolysis of N-acetylhexosamines, since amino sugars also react with ninhydrin. This remains to be confirmed, however.

In order to determine whether some individual peptides could be more resistant to degradation than others, and to investigate the enzymatic mechanism of peptide breakdown, the hydrolysis of different oligopeptides was examined by HPLC. Some individual peptides were hydrolysed rapidly and others were remarkably stable in rumen fluid (table 2). Ala_3 and ala_3-p-nitroanilide were rapidly hydrolysed, whereas N-acetyl-ala_3 was relatively stable. Hippuryl-phenylalanine, a carboxypeptidase substrate, was not broken down. Thus rumen peptidase activity appears to be predominantly of the aminopeptidase type, and adding glocking agents to the N-terminal would help prevent the hydrolysis of dietary peptides. The resistance of other compounds, such as gly_3, proline-containing compounds and acidic peptides (table 2), suggests that there may be structural factors, in terms of different N-terminal amino acid sequences, that could be exploited to protect peptides from degradation.

TABLE 1. APPARENT CONCENTRATIONS OF PEPTIDES AND AMINO ACIDS IN PCA-EXTRACTED, CELL-FREE RUMEN FLUID AS DETERMINED BY DIFFERENT ANYTICAL METHODS

Analysis	Apparent amino acid or peptide concentration (mg/l)[a]	
	PCA extract	Hydrolysed PCA extract[b]
Ninhydrin	386	762
O-Phthalaldehyde	61	603
Fluorescamine	10	11
Folin	n.d.	1299
Σ Amino acids, from ion-exchange chromatography	< 8	68

[a] Using casein acid hydrolysate (Oxoid) as standard, except for fluorescamine, in which Trypticase (BBL) was used. Results are means of replicate determinations on each of two sheep.
[b] 6 M HCl, 24 h.

TABLE 2. BREAKDOWN OF SHORT-CHAIN PEPTIDES IN RUMEN FLUID *IN VITRO*

Peptide	Rate of hydrolysis (nmol/ml/h)	
	Sheep 1	Sheep 2
Ala_3	480	369
Phe_3	379	379
Glu_3	23	34
Gly_3	8	20
N-Ac-Ala_3	8	31
Ala_3-pNA	311	371
GluAlaGlu	42	63
GluAlaAla	93	67
GlyHisLys	210	412
$ProGly_2$	29	14
$LeuGly_2$	105	278
Gly_2Arg	42	39
Ala_4	227	492
Phe_4	227	296
ValGlyAspGlu	22	21
$ProLeuGly_2$	67	41

In conclusion, therefore, it appears that few peptides remain undegraded in rumen contents after the post-feeding peak. However, individual oligopeptides vary greatly in their susceptibility to hydrolysis, perhaps offering some potential for controlling the rate of peptide breakdown in

the rumen.

(**Key Words**: Peptides, Nitrogen Metabolism, Rumen Microbiology)

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