Role of Peptides in Rumen Microbial Metabolism*
- Review -

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ABSTRACT : Peptides are formed in the rumen as the result of microbial proteinase activity. The predominant type of activity is cysteine proteinase, but others, such as serine proteinases, are also present. Many species of protozoa, bacteria and fungi are involved in proteolysis; large animal-to-animal variability is found when proteinase activities in different animals are compared. The peptides formed from proteolysis are broken down to amino acids by peptidases. Different peptides are broken down at different rates, depending on their chemical composition and particularly their N-terminal structure. Indeed, chemical addition to the N-terminus of small peptides, such as by acetylation, causes the peptides to become stable to breakdown by the rumen microbial population; the microorganisms do not appear to adapt to hydrolyse acetylated peptides even after several weeks exposure to dietary acetylated peptides, and the amino acids present in acetylated peptides are absorbed from the small intestine. The amino acids present in some acetylated peptides remain available in nutritional trials with rats, but the nutritive value of the whole amino acid mixture is decreased by acetylation. The genus *Prevotella* is responsible for most of the catabolic peptidase activity in the rumen, via its dipeptidyl peptidase activities, which release dipeptides rather than free amino acids from the N-terminus of oligopeptides. Studies with dipeptidyl peptidase mutants of *Prevotella* suggest that it may be possible to slow the rate of peptide hydrolysis by the mixed rumen microbial population by inhibiting dipeptidyl peptidase activity of *Prevotella* or the rate of peptide uptake by this genus. Peptides and amino acids also stimulate the growth of rumen microorganisms, and are necessary for optimal growth rates of many species growing on rapidly fermented substrates; in rich medium, most bacteria use pre-formed amino acids for more than 90% of their amino acid requirements. Cellulolytic species are exceptional in this respect, but they still incorporate about half of their cell N from pre-formed amino acids in rich medium. However, the extent to which bacteria use ammonia vs. peptides and amino acids for protein synthesis also depends on the concentrations of each, such that pre-formed amino acids and peptides are probably used to a much lesser extent *in vivo* than many *in vitro* experiments might suggest.

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

INTRODUCTION

Ruminant animals are exceptionally inefficient in their retention of N under conditions where the diet contains protein which can be fermented rapidly in the rumen. Peptides are intermediates in the breakdown process, which results in the over-production of ammonia and its loss by diffusion across the rumen wall. Slowing the rate of breakdown of peptides would lead to more efficient incorporation of dietary amino acid-N by rumen microorganisms and be beneficial to the efficiency of N retention by the ruminant animal. On the other hand, amino acids, in the form of peptides in particular, are important nutrients for rumen microorganisms. It is therefore important to understand how peptides are degraded by rumen microorganisms in order to investigate means of inhibiting peptide breakdown, while being aware that pre-formed amino acids have an important role in microbial growth rate and efficiency (figure 1).

FORMATION OF PEPTIDES BY MICROBIAL HYDROLYSIS OF PROTEIN

Feed protein is often hydrolysed rapidly in the rumen, although the precise rate and extent of breakdown varies

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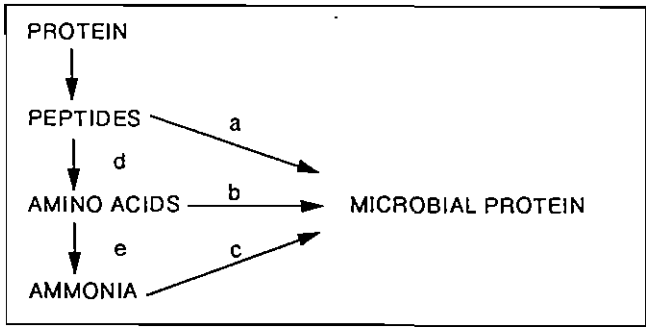

Figure 1. The relative amounts of peptides, amino acids and ammonia (a, b and c) used for microbial protein synthesis depend on the concentration of each, which in ture depends on catabolic rates d and e.

greatly between different proteins. Early work suggested that protein breakdown in the rumen was proportional to solubility (Chalmers & Synge, 1954; Henderickx & Martin, 1963; Henderickx, 1976). However, subsequent research showed that other properties are also important (Kaufmann & Lupping, 1982). For example, some soluble proteins are broken down more slowly than insoluble proteins, depending on the degree of secondary and tertiary structure, and cleavage of disulfide bonds enhances the breakdown of albumin and similarly heavily cross-linked molecules (Mahadevan et al., 1980; Nugent et al., 1983; Wallace & Kopecny, 1983). Conversely, the introduction of artificial cross-links into proteins inhibits their hydrolysis (Friedman & Broderick, 1977; Wallace, 1983). Heating and formaldehyde treatments, affecting both solubility and cross-linking, have been used to protect proteins from rumen degradation and thereby provide bypass protein to the lower tract (Kaufmann & Lupping, 1982). Peptides accumulate in rumen fluid transiently after feeding, and thereafter their concentration declines (Chen et al., 1987a; Kim et al., 1998; Broderick & Wallace 1988; Wallace & McKain 1990; Williams & Cockburn 1991). The hydrolysis of casein is rapid and peptides accumulate (Broderick & Wallace 1988; Williams & Cockburn 1991), but otherwise the extent to which peptides accumulate in rumen contents does not appear to be related to either the rate or extent of degradation of the protein supplement (Williams & Cockburn 1991)

The nature of the diet has a major influence on the proteolytic activity of rumen contents. Fresh herbage promotes an activity up to nine times higher than that found with dry rations, the higher soluble-protein content of the herbage enriching for proteolytic bacteria (Nugent & Mangan, 1981; Hazlewood et al., 1983; Nugent et al., 1983). There has been speculation that endogenous plant proteases may play a significant role in the breakdown of fresh herbage protein in the rumen (Theodorou 1995), largely on the grounds that such enzymes exist and are predominantly responsible for protein breakdown in the silo. Cereal diets also yield higher proteolytic activities than do dry forage diets, probably because proteolytic rumen microorganisms tend to be amylolytic rather than cellulolytic (Siddons & Paradine 1981).

The main type of proteinase which is found in mixed rumen microorganisms is cysteine proteinase, sensitive to inhibition by thiol reagents such as *p*-chloromercuribenzoate (Brock et al., 1982; Kopecny & Wallace 1982; Prins et al., 1983; Attwood & Reilly 1996). The same studies indicated that serine and metalloproteases were also present, judging by the sensitivity of rumen microbial proteinase activity to inhibitors such as phenylmethylsulfonylfluoride (PMSF) and EDTA respectively. Both chymotrypsin and trypsin-like specificities are present in the mixed population (Brock et al., 1982; Kopecny & Wallace 1982; Prins et al., 1983; Attwood & Reilly 1996), although it seems likely that many different types of specificity could be present.

Proteolytic activity is present in many species of ciliate protozoa, bacteria and fungi in the rumen (Wallace et al., 1997a). Many species of proteolytic bacteria have been isolated from the rumen, predominantly from the genera *Prevotella, Eubacterium, Streptococcus* and *Butyrivibrio*, as reviewed by Wallace et al. (1997a). Based on the sensitivity of the *Prevotella ruminicola* isolates to inhibition by protease inhibitors, which had much the same pattern as rumen fluid, it was concluded that *P. ruminicola* played an important role in proteolysis *in vivo* (Prins et al., 1983; Wallace & Brammall 1985). This provides a contradiction, because many of the most active proteolytic isolates have had extracellular serine proteinase activity, atypical of the majority of the mixed population (Wallace & Brammall 1985; Attwood & Reilly 1995, 1996). As in other areas of microbial ecology, molecular phylogenetic techniques have transformed our appreciation of the complexity of natural ecosystems. The New Zealand cattle study carried out by Attwood & Reilly (1995) exemplifies how the relatedness of different isolates can be quantified, and a more rapid and wide-ranging identification of isolates can be carried out. Many of their isolates clustered with *Streptococcus bovis*, and a few were related to *Butyrivibrio*, but 10 of the 212 isolates were classified as *Eubacterium budayi*, not previously isolated from the rumen. Only one strain was identified as *P. ruminicola* A new species, *Clostridium*

proteoclasticum, was also identified (Attwood et al. 1996) and quantified in mixed rumen contents by competitive PCR (Reilly & Attwood 1998), consistent with what appears to an increasing incidence of *Clostridium* spp. being isolated from or recognised in the mixed rumen ecosystem.

As well as there being many different species of proteolytic microorganism present in the rumen, each possibly with several enzyme activities, there appears to be an intrinsic variability in the system. When cell-free extracts were made from rumen fluid and were applied to SDS-PAGE, the resulting proteinase zymograms were different in different animals, even those receiving the same diet (Falconer & Wallace 1998). Some bands appeared to be common to different animals, and the pattern obtained was to some extent diet-dependent, but the differences between samples were much greater than their similarities, and it was concluded that a high variability was present in the predominant proteinase patterns in different animals.

Although oligopeptides result from proteolysis and analysis of the structure of these peptides would reveal the specificities of the predominant proteinases of either pure or mixed cultures, to our knowledge there is no published information in this area. Indeed, different methodologies for determining simply the overall concentrations of peptides in the rumen appear to give different values. Acid hydrolysis followed by ninhydrin estimation of NH_2 groups released (Chen et al., 1987a) gave higher values than fluorescamine, *o*-phthalaldehyde, trinitrobenzenesulfonic acid, or acid hydrolysis followed by chromatographic analysis of the released amino acids (Wallace & McKain 1990). The last method appears to be less subject to systematic error (Wallace & McKain 1990, Williams & Cockburn 1991), although tryptophan is destroyed by the acid hydrolysis (Blackburn 1968).

CHARACTERISTICS OF PEPTIDE BREAKDOWN

Different peptides are broken down at very different rates (Ha et al., 1991), although most would not be expected to survive for more than 1 h or so in mixed rumen contents. The structure of the N-terminus is crucial in determining how rapidly a peptide is degraded. If glycine or proline is present at or next to the N-terminus, or if the peptide has a net negative charge, the peptide tends to be slowly degraded (Wallace & McKain 1989a; Yang & Russell 1992; Wallace 1996). As a result, the peptides which persist in the rumen are enriched for proline, glycine and aspartate (Wallace et al., 1993a). They also have a lower than expected reaction with fluorescamine, which reacts with the N-terminal amino acids of peptides, suggesting that some of these peptides are blocked at the N-terminus, perhaps by N-formyl or N-acetyl groups (Wallace & McKain 1990). Earlier suggestions that hydrophobicity was a major determinant of the rate of peptide breakdown (Chen et al., 1987) have not been sustained in other studies (Williams & Cockburn 1991, Wallace et al. 1993a, Depardon et al. 1995, Depardon et al., 1996, Wallace 1996).

The predominant mechanism of peptide hydrolysis by mixed rumen microorganisms is dipeptidyl peptidase, whereby dipeptides are cleaved sequentially from the N-terminus of peptides (Wallace & McKain 1989b, Depardon et al., 1995, Depardon et al., 1996). The only rumen microorganisms with significant dipeptidyl peptidase activity are the species of the genus *Prevotella*. The species *Prevotella ruminicola* was subdivided into four distinct species on the basis of 16S rDNA sequence analysis (Avgustin et al., 1994), all of which possessed dipeptidyl peptidases (Avgustin et al., 1997). One of these species, *P. albensis*, has at least four distinct enzymes: all are serine proteases, but they have different specificities and sensitivities to inhibitors (Wallace et al., 1997b). The properties of one of these enzymes, similar in its substrate specificity to dipeptidyl peptidase type I (DPP-1) or gingipain, has been studied in detail in *P. ruminicola* B14 (Madeira et al., 1997). Chemical mutagenesis was used to obtain mutants which possessed 1/10 of the DPP-1-like activity of the wild-type. The significance of this enzyme activity was demonstrated in co-culture with the Gram-positive, high-activity ammonia-producing bacteria, *Peptostreptococcus anaerobius* and *Clostridium aminophilum*. The rate and extent of ammonia production was decreased by 25% when the DPP-I mutant replaced wild-type *P. ruminicola* in the mixed cultures. It may therefore be possible to decrease the wasteful metabolism of peptides by controlling the numbers of *Prevotella* in the rumen, or by inhibiting the uptake of peptides into these bacteria, or by blocking peptide hydrolysis by dipeptidyl peptidases. The last may offer the best target for manipulation, because it has proved possible to develop specific inhibitors of dipeptidyl peptidases from other sources (Umezawa & Aoyagi 1983).

Dipeptidase activity, which cleaves the dipeptides resulting from the activity of dipeptidyl peptidases, is present in *Prevotella* (Wallace & McKain 1991; Wallace et al., 1995), but also in a variety of other rumen species, including the ciliate protozoa (Wallace et al., 1990). Rumen dipeptidases are metalloenzymes, subject to inhibition by chelators, such as EDTA and 1,10-phenanthroline, and heavy metal ions (Wallace et al.

1996), but no inhibitor has yet been found which has a sufficiently high specificity for dipeptidase to be an effective rumen manipulating agent *in vivo* (Wallace and McKain 1996).

PROTECTING PEPTIDES FROM DEGRADATION IN THE RUMEN

Feedlot ionophores, including monensin, tetronasin and others, improve feed efficiency in ruminants and as a result they are useful feed additives. Their primary mode of action is unclear, indeed provokes considerable debate, because many influences on rumen fermentation have been recorded, including decreased methane formation and increased propionate production (Russell & Strobel 1988). Among the other effects noted, a decrease in ammonia formation and increased flow of non-ammonia N from the rumen are prominent (Dinius et al., 1976, Hanson & Klopfenstein 1979, Poos et al., 1979, Rowe et al., 1983, Owens et al., 1978). Part of the reason that ammonia formation is inhibited is that a category of ammonia-forming bacteria, mentioned above, are sensitive to ionophores and are therefore suppressed by ionophore addition to the diet (Russell et al., 1991). It has also been observed that peptides accumulate in rumen fluid (Newbold et al., 1990, Wallace 1992a) and in continuous fermenters (Whetstone et al., 1981) when ionophores are present. Part of the reason for this accumulation appears to be an adaptation by *P. ruminicola* which decreases the permeability of the cell envelope to ionophores, and which also inhibits the uptake of larger peptides (Newbold et al., 1992). Thus, ionophores which are presently in use already have some effect on peptide breakdown. It would, however, be beneficial if a more specific inhibitor of *Prevotella* peptidases could be found.

The observation that microbial peptidase activity in the mixed rumen microbial population acts almost exclusively from the N-terminus of small peptides (Wallace & McKain 1989a; Wallace et al., 1990) led to the suggestion that peptides might be protected by chemical modification of N-terminal amino groups (Wallace 1992b; Wallace et al., 1993). Small peptides are protected very effectively from degradation in rumen fluid *in vitro* by this method (Wallace 1992b, Wallace et al., 1993), which involves a direct reaction of peptides with an acid anhydride. Acetic anhydride has been used in several studies done at the Rowett Research Institute. The molecular mass of the peptide is important, because once the peptide can be cleaved by proteinases (endoproteases) rather than peptidases (exoproteases), the protection of the N-terminus is no longer effective. Effective protection can be predicted only for peptides containing up to four amino acid residues, while larger peptides are less predictable in their response to acetylation (Wallace 1992b). Remarkably, the rumen microbial population did not adapt to break down acetylated peptides after 4 weeks of dietary supplementation and the rate of ammonia formation from acetylated peptides did not increase over the same period (Witt et al., 1998), indicating that N-terminal hydrolysis is an obligatory route of peptide breakdown by rumen bacteria. Carboxypeptidase is low in the mixed microbial population (Wallace & McKain 1989a), and is clearly not an option for adaptation by these organisms.

If chemically modified peptides are to be of nutritional value to ruminants, their constituent amino acids must be absorbed from the small intestine and be made available for protein synthesis. There was a possibility that N-terminal acetylation would impair this process. A study (Wallace et al., 1998) was therefore undertaken to determine the intestinal uptake of acetylated peptides and their nutritive value. ^{15}N-labelled peptides were prepared from alfalfa which had been grown with ^{15}N-ammonium sulfate in the absence of *Rhizobium*. Peptides were prepared by enzymic hydrolysis of the extracted protein and unmodified and acetylated peptides were injected into the jejunum of sheep. The uptake of peptides as measured from ileal sampling was high and unaffected by acetylation. Feeding trials were also carried out with rats (Wallace et al., 1998). One trial was carried out with a methionine-free diet to which was added Met, Gly-Met or acetylated Gly-Met. The rats grew equally well on all sources of Met, but failed to grow significantly on a mixture of Met-free amino acids, indicating that the methionine was nutritionally available. In another experiment, acetylated mixed peptides caused a depression of 23% in feed intake. Thus, the rat bioassay indicated that certain specific peptides may well be of high nutritive value following acetylation, but that there may be problems of inappetance and inefficient utilisation with acetylated peptide mixtures.

NUTRITIVE VALUE OF PEPTIDES FOR RUMEN MICROORGANISMS

Excessive breakdown of peptides by rumen microorganisms causes nutritional inefficiency in the ruminant. However, peptides are also important nutrients for rumen microorganisms, and any attempt to alter the availability of peptides may have unwanted consequences in terms of microbial growth. *In vitro* data, from Maeng & Baldwin (1976), Argyle & Baldwin (1989), Fujimaki et al. (1992).

Kaur et al. (1992) and Russell et al. (1983), tend to indicate that microbial growth derives benefit from pre-formed amino acids, and Argyle & Baldwin (1989) noted that, under certain combinations of substrate concentrations, it could be demonstrated that peptides had a stimulatory effect greater than that seen with free amino acids. However, readily fermentable soluble carbohydrate was always present in these experiments, which often does not occur in the rumen and which can have a major influence on the ability of rumen microorganisms to respond to pre-formed amino acids. Cruz Soto et al. (1994) presented evidence which suggested that stimulation by peptides and amino acids would not always occur. Peptides and amino acids benefited even cellulolytic rumen bacteria, but only when they were growing on cellobiose, not cellulose. Subsequent experiments (Chikunya et al., 1996), in which peptides were supplied with diets containing either rapidly or slowly degraded fibre, appeared to confirm that the benefit of peptides would only be evident if the energy source supported a growth rate which enabled the organism to respond. It will be vital to identify at what growth rate that response occurs, because that will determine whether added rumen-degradable protein will be required for maximum productivity of rumen fermentation.

SOURCES OF NITROGEN FOR MICROBIAL GROWTH

Estimates of the contribution of ammonia versus preformed amino acids to protein synthesis by the mixed rumen population have been highly variable. ^{15}N studies using ^{15}N-ammonia or urea (which rapidly releases ammonia) infused into the rumen or added as a single dose indicated values of microbial N derived from ammonia that ranged from 18 to 100% (summarised by Salter et al., 1979). Incorporation of ammonia was greater in the bacterial fraction (50-78%) than in protozoa (31-64%) (Pilgrim et al., 1970; Mathison & Milligan 1971). The composition of the diet was at partly responsible for this variation: lucerne hay gave a lower proportional uptake than wheat hay (Pilgrim et al., 1970), and grass hay gave a lower uptake than barley (Mathison & Milligan 1971). Dietary factors responsible for these differences are the availability of readily fermentable energy (Ben-Ghedalia et al., 1978) and the presence of peptides and amino acids (Argyle & Baldwin 1989).

Recent experiments (Atasoglu 1996) using ^{15}N-NH_3 have confirmed the earlier findings of Salter et al. (1979) in showing that the proportion of microbial N derived from ammonia varies according to conditions. The minimum contribution was 26%, when high concentrations of peptides and amino acids were present, with a potential maximum of 100% when only ammonia was available (table 1). Different amino acids were enriched to different extents (table 1). *De novo* synthesis of proline was abolished when pre-formed proline was available, whereas most glutamate was formed *de novo* even when pre-formed glutamate was available.

In addition to dietary and microbial factors, the general observation that some microbial protein is derived from ammonia and some is derived from other sources must reflect to some extent the heterogeneity of the microbial population. It is commonly believed, based on pure culture studies, that cellulolytic bacteria use ammonia as their main source of nitrogen for growth, while non-cellulolytic species use pre-formed peptides and amino acids. These are the assumptions which are made in the Cornell system for modelling rumen fermentation, for example (Russell et al., 1992). Recent experiments at the Rowett Research Institute indicate that the situation is much more complicated than was appreciated previously.

Ammonia is essential for the growth of celluloytic rumen bacteria Bryant & Robinson (1962). Calculation of N uptake based on changes in concentrations of medium constituents indicated that *Fibrobacter succinogenes, Ruminococcus albus* and *R. flavefaciens* fixed similar amounts of NH_3 in minimal medium and when amino acids and peptides were added (Bryant & Robinson 1961), implying that ammonia was the main source of N in these bacteria. However, more precise information was obtained from the incorporation of ^{14}C-labelled protein hydrolysates (Allison et al., 1962; Bryant & Robinson 1963). When both NH_3 and protein hydrolysate were available, *R. albus* and *R. flavefaciens* used both, with *R. flave- faciens* continuing to use NH_3 to a greater extent than preformed amino acids. Atasoglu (1996) used $^{15}NH_3$ uptake rather than the incorporation of ^{14}C-labelled protein hydrolysate to assess the incorporation of different N sources, and found that, although most rumen bacterial species have very simple N requirements they grow in medium containing ammonia with one or two added amino acids peptides and amino acids are used pre- ferentially when they are available. Among the cellulolytic bacteria, *F. succinogenes* and the *Ruminococcus* spp. used less, but still significant amounts of peptides and amino acids (table 2).

The influence of peptides and amino acids on ammonia assimilation and *de novo* synthesis of amino acids by three predominant non-cellulolytic species of ruminal bacteria, *Prevotella bryantii, Selenomonas*

Table 1. Influence of nitrogen source on incorporation of $^{15}NH_3$ by mixed rumen micro-organisms using a mixture of starch, cellobiose and xylose or a hay/concentrate diet

Energy source	Proportion of new microbial total N or amino acid N formed which was derived from $^{15}NH_3$							
	Starch, cellobiose and xylose				Mixed diet			
N source	NH_4Cl		NH_4Cl + Trypticase		NH_4Cl		NH_4Cl + Trypticase	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total N	0.99	0.19	0.38	0.03	0.34	0.08	0.26	0.05
Ala	1.10	0.23	0.54	0.11	0.64	0.24	0.49	0.26
Gly	0.97	0.21	0.27	0.05	0.42	0.16	0.21	0.10
Val	1.00	0.24	0.20	0.05	0.51	0.20	0.19	0.10
Leu	0.91	0.27	0.27	0.04	0.45	0.17	0.22	0.09
Ile	0.95	0.27	0.28	0.04	0.50	0.19	0.23	0.08
Pro	0.81	0.22	0.05	0.01	0.20	0.09	0.04	0.02
Ser	1.00	0.24	0.39	0.06	0.54	0.20	0.32	0.14
Thr	0.96	0.21	0.18	0.03	0.44	0.17	0.13	0.05
Phe	0.83	0.25	0.36	0.06	0.43	0.13	0.31	0.20
Asp	0.99	0.23	0.49	0.06	0.55	0.20	0.41	0.17
Glu	1.05	0.25	0.60	0.07	0.61	0.23	0.50	0.25
Lys	0.74	0.22	0.18	0.03	0.35	0.14	0.15	0.06
Tyr	0.80	0.26	0.30	0.04	0.46	0.16	0.29	0.16

Results are means and standard deviations from 12 h incubations with rumen fluid taken from four sheep. The NH_4Cl and Trypticase concentrations were 1.33 and 10 g/L respectively. The mixed diet contained protein as a component of barley and of fish meal.

Table 2. Incorporation of $^{15}NH_3$ by rumen bacteria in a rich medium containing 20% rumen fluid and 10 g/L Trypticase

Species	Cell material derived from ammonia	
	Total cell N	Amino acid N
Fibrobacter succinogenes	0.50	0.52
Prevotella ruminicola	0.09	0.03
Ruminococcus albus	0.65	0.69
Ruminococcus flavefaciens	0.62	0.59
Selenomonas ruminantium	0.07	0.05
Streptococcus bovis	0.12	0.01

Results are means for three cultures grown to stationary phase in the liquid form of medium 2 of Hobson (1969) in which 40% of added ammonium chloride was $^{15}NH_4Cl$. The proportion of cell material derived from ammonia was calculated from the enrichment of ^{15}N in cell material or amino acids compared to the average enrichment of ammonia at zero time and at stationary phase.

ruminantium, and *Streptococcus bovis*, was determined in a similar way. All three species used peptides for between 95 and 99% of their protein synthesis in the same rich medium as used above for the cellulolytic species (table 2). In medium containing ammonia and methionine as sole N sources, the proportion of cell N and amino acids formed *de novo* from ammonia increased as the concentration of peptides decreased (figure 2). At 1 g peptides/L, similar to peptide concentrations found in the rumen, 68, 87 and 46% of bacterial amino acid-N was derived from ammonia by *P. bryantii*, *S. ruminantium*, and *S. bovis* respectively.

Thus the dietary variation in *de novo* incorporation of NH_3 by mixed rumen microorganisms reflects the heterogeneity of the microbial population, regulatory changes within individual species according to prevailing substrate concentrations, and differences between individual amino acids. At present, the regulatory mechanisms underlying these observations are unclear. Ammonia uptake into rumen bacteria is thought to be mediated mainly by glutamate dehydrogenase (Hespell 1984), but it is not clear how peptide concentration affects ammonia uptake and vice-versa; it is also unclear what is the mechanistic basis of the difference between Cellulolytic and other bacterial species. Much therefore remains to be

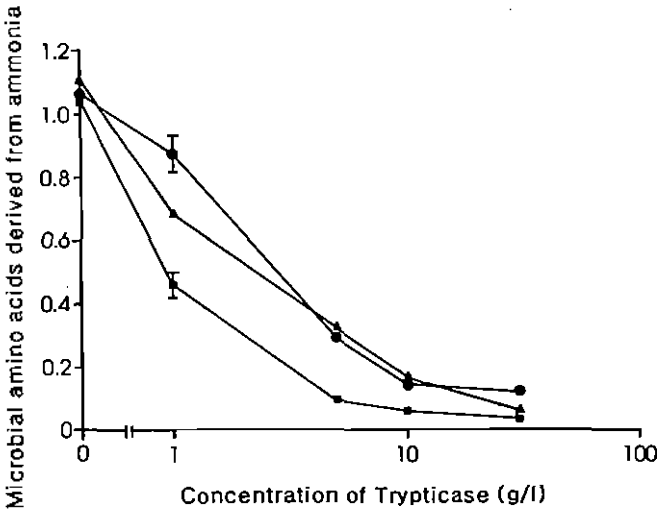

Figure 2. Influence of Trypticase addition on the growth medium on the assimilation of ^{15}N-ammonia by *Prevotella bryantii, Selenomonas ruminantium* and *Streptococcus bovis*. The initial concentration of ammonia in the medium was 13.7 mM.

discovered about the factors affecting peptide, amino acid and ammonia uptake by rumen microorganisms.

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