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Plant enzyme mediated proteolysis in herbage incubated anaerobically in the presence and absence of rumen microorganisms

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Cysteine endopeptidases and other proteolytic enzymes in plants reside predominantly in vacuoles. They are extremely robust enzymes, with broad spectrum pH and temperature optima and broad substrate specificities. Although their role in plants is not entirely understood, vacuolar proteases are intimately involved in controlled cell-death during plant senescence and in the perhaps analogous processes caused by cutting grass for silage. They are also involved in plant defence strategies and in the recycling of redundant cytoplasmic proteins after their transport to the cell vacuole.

The phenomenon of plant enzyme mediated proteolysis in the silo is well documented (McDonald et al, 1991, in: The Biochemistry of Silage, Chalcombe Publications, United Kingdom) and is a normal consequence of plant cell death. Protease activity results in the conversion of plant proteins to lower molecular weight peptides and amino acids which are then degraded by the enveloping silage micro flora. To our knowledge, no one has considered the possibility that proteolysis in the rumen of grazing animals may occur under the influence of proteases of plant origin. Ruminants ingest largely intact fragments of plant biomass and these are incubated for many hours in the rumen following ingestion, and prior to rumination. It is during this period that we propose a role for plant enzyme mediated proteolysis in the rumen. Factors in favour of our hypothesis include the elevated temperature of ingested herbage (39°C), a relatively high and constant ruminal pH and the anaerobic conditions in the rumen, all of which may contribute to cell death and plant protease activity.

As a fore-runner to more detailed animal-based studies, we are investigating plant enzyme mediated proteolysis in vitro in fresh grass and a legume (*Lolium perenne* and *Medicago sativa*) incubated under anaerobic, rumen-like conditions in the presence and absence of rumen microorganisms. In these studies, herbage proteins were extracted prior to and after incubation and single dimensional SDS-PAGE gel electrophoresis (Laemmli, 1970, nature, 277, 680-685) used to visualize them and their degradation products. In the gel, lane 0 contained the molecular size markers ; lanes 1-3 were loaded with extracts from herbage incubated in anaerobic buffer for 0, 6 and 24 h respectively and lanes 4-6 contained extracts from herbage incubated as above but with buffer and rumen fluid. With regard to the dominant protein band, Rubisco (fraction 1 leaf protein ; MW ca 55 kDa), the degradation profiles were similar for both treatments, all Rubisco having disappeared within 24 h. Of note in this experiment was the fact that a lack of rumen microorganisms in the buffer treatment did not inhibit the removal of Rubisco, but did lead to the accumulation of lower molecular weight protein breakdown products (at ca 14.7 kDa, lane 3). These low molecular weight products failed to accumulate in herbage incubated with buffer and rumen fluid, presumably because they were metabolized further by the rumen microorganisms.