SHORT COMMUNICATIONS

Leaf Fraction 1 Protein as a Nitrogen Source for the Growth of a Proteolytic Rumen Bacterium

By G. P. HAZLEWOOD AND J. H. A. NUGENT*

Department of Biochemistry, A.R.C. Institute of Animal Physiology,
Babraham, Cambridge CB2 4AT

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INTRODUCTION

Fraction 1 protein (ribulosebiphosphate carboxylase, EC 4.1.1.39) is the major soluble protein of the leaves of green plants (Lyttleton & T'so, 1958), and therefore forms a large part of the total protein entering the rumen of the grazing ruminant. The ability of rumen contents to degrade protein has long been established by experiments both *in vivo* (e.g. Blackburn & Hobson, 1960*b*; Mangan, 1972) and *in vitro* (Annison, 1956; Blackburn & Hobson, 1960*a*), and numerous reports have described the isolation and characteristics of proteolytic bacteria from the rumen (e.g. Blackburn & Hullah, 1974). However, the widespread use of casein as a substrate for the rumen proteolytic enzymes has meant that the fate of fraction 1 protein in the rumen has been largely neglected, although it is known to be rapidly degraded by rumen contents both *in vivo* and *in vitro* (Nugent & Mangan, 1978). The problem of obtaining purified fraction 1 protein in quantity has recently been overcome (Jones & Mangan, 1976) making it possible for this protein to be studied as the physiological substrate of the rumen proteases.

This communication reports preliminary results showing that an anaerobic rumen bacterium hydrolyses fraction 1 protein.

METHODS

Growth of organism. The low-nitrogen basal liquid medium had the following composition (g l⁻¹): K₂HPO₄, 6.37; KH₂PO₄, 5.0; NaCl, 0.64; CaCl₂·2H₂O, 0.046; MgSO₄·7H₂O, 0.091; resazurin, 0.001; haemin, 0.001; NaHCO₃, 4.0; dithiothreitol, 0.10; glucose, 4.0. Wherever possible, reagents were analytical grade, and the medium, which had a final pH of 6.8, was made up to volume with deionized water. Dithiothreitol, glucose and NaHCO₃, and a mixture of 14 vitamins (Roché *et al.*, 1973) were prepared as two separate solutions and filter-sterilized before addition to the remaining components of the medium which were sterilized by autoclaving (115 °C; 20 min). Further additions made to the medium in the course of the work were also filter-sterilized. The preparation, distribution and inoculation of the medium were carried out by the open-tube technique under an atmosphere of sterile O₂-free CO₂ (Latham & Sharpe, 1971).

Proteins. Both universally ¹⁴C-labelled [specific activity 1.4 µCi (mg protein)⁻¹] and unlabelled fraction 1 protein were prepared from lucerne (*Medicago sativa* L.) by the procedure of Jones & Mangan (1976). The radioactive lucerne was cultivated in a growth chamber similar to the one described by Grossbard & Barton (1963). Purified protein was stored at 4 °C as an (NH₄)₂SO₄ suspension and was desalted as required by gel filtration on a column (2.6 × 70 cm) of Sephadex G-200, eluted with 0.05 M-phosphate buffer, pH 7.5. When necessary, eluates containing protein were concentrated by ultrafiltration (Centriflo CF50 Membrane Cones; Amicon Corp., Lexington, Massachusetts, U.S.A.). Values of A₂₈₀ were compared with a calibration curve, prepared using fraction 1 protein as standard, to determine protein concentrations. Bovine serum albumin (fraction V) was obtained from Sigma, and casein from Hopkin & Williams.

* Present address: Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT.

Fig. 1. Utilization of ^{14}C -labelled fraction 1 protein. The bacterium was cultured in the absence ($\square$) or presence ($\circ$) of fraction 1 protein (1 g l^{-1} plus $50\text{ }\mu\text{Ci } [^{14}\text{C}]\text{protein l}^{-1}$). Samples of culture were centrifuged (10000 g ; 15 min) and radioactivity ($\bullet$) in the soluble protein of the culture supernatant was determined by sampling before and after acid deproteinization.

Analytical procedures. Total nitrogen, non-protein nitrogen and amino acids were determined as described by Mangan & West (1977).

Radioactivity. A Unilux II scintillation counter (Nuclear Chicago) was used.

Bacterial growth. Culture turbidity (A_{450}) was measured with an EEL colorimeter.

RESULTS AND DISCUSSION

The proteolytic organism is an obligately anaerobic, Gram-negative bacterium of somewhat variable morphology, and was isolated in medium containing rumen fluid (Hobson, 1969) from a 10^8 dilution of the rumen contents of a pasture-fed sheep. It is non-motile, requires haemin (0.001 g l^{-1}) for growth and produces acetic acid from the fermentation of glucose. On the basis of these characteristics it is tentatively assigned to the genus *Bacteroides* (Holdeman & Moore, 1972).

In a large number of experiments the isolate failed to grow in the low-nitrogen basal liquid medium which contained no amino acids or peptides and only a small amount (about $6\text{ }\mu\text{g ml}^{-1}$) of contaminant nitrogen as NH_4^+ . There was no growth with added $(\text{NH}_4)_2\text{SO}_4$ ($200\text{ }\mu\text{g N ml}^{-1}$), but supplementation of the medium with fraction 1 protein (1 g l^{-1}) resulted in heavy growth (Fig. 1). The nitrogen requirement of the isolate could also be satisfied by bovine serum albumin (1 g l^{-1}) or a combination of Bactocasitone (10 g l^{-1}) and cysteine (0.5 g l^{-1}). Casein, cysteine, Bactocasitone or amino acid mixtures added alone did not support comparable growth, even though casein (up to 10 g l^{-1}) was substantially degraded.

The growth response to added fraction 1 protein was concentration-dependent over the range 0 to 2 g l^{-1} . Even at the highest fraction 1 concentration tested (2.2 g l^{-1}), protein still appeared to be growth-limiting. Using fraction 1 protein as substrate, no proteolytic activity was detected in the cell-free culture supernatant at any stage of batch culture.

Utilization of ^{14}C -labelled and unlabelled protein was followed during growth (Fig. 1). The initial drop in radioactivity associated with the trichloroacetic acid-precipitable soluble protein fraction was presumably caused by binding of protein to cells of the inoculum, but the subsequent loss of soluble protein from the culture medium was due to degradation of the fraction 1 protein. A concomitant increase in non-protein nitrogen was accounted for by the production of amino acids, but a comparison of the amino acid pattern of the culture supernatant with that which one would expect from fraction 1 protein alone revealed anoma-

lies. The recovery of amino acids amounted to only 35 to 40% of those initially present in the added fraction 1, indicating substantial incorporation into bacterial protein. Less than 10% of the aspartic acid, serine, glutamic acid and cysteine contained in the original protein was found in the culture supernatant, and methionine, threonine and glycine were also present in lowered amounts, having presumably also been incorporated into bacteria.

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