DEGRADATION OF NUCLEIC ACIDS BY CELL-FREE EXTRACT OF MIXED RUMEN PROTOZOA OF BUFFALO RUMEN

P.R. Sinha[1] and S.M. Dutta

Animal Biochemistry Division
National Dairy Research Institute, Karnal-132001
Haryana, India

Summary

Degradation of deoxyribonucleic acid(DNA) and ribonucleic acid(RNA) by cell-free extract of mixed rumen protozoa of buffalo rumen was investigated. DNA was observed to be degraded rapidly during an initial incubation period of 2 hr with simultaneous appearance of degradation products. RNA on the other hand recorded a rapid degradation during an initial incubation period of 1 hr. RNA degradation products appeared upto an incubation period of 2 hr. DNA was observed to degrade into oligo- and mononucleotides, pyrimidine nucleosides, purine nucleoside adenosine and bases xanthine, hypoxanthine and thymine. Degradation products of RNA comprised of pyrimidine nucleosides, purine nucleoside, adenosine and bases xanthine, hypoxanthine and uracil besides oligo- and mononucleotides.
(Key Words: DNA, RNA, Protozoa, Rumen Degradation, Buffalo)

Introduction

Degradation of nucleic acids which form a quantitatively important part of nitrogen ingested by ruminants with special reference to cow and sheep has been delineated by McAllan and Smith (1973). Sinha and Dutta(1986a) investigated the degradation of these nucleic acids in buffalo rumen and observed the pathway of degradation to be similar to that in cow and sheep. Degradation of nucleic acids in rumen essentially is carried out by the bacterial and protozoal population inhabiting the rumen as well as the enzymes released into the rumen fluid(Sinha, 1982). Whereas the pathway of degradation of nucleic acids in rumen as a whole has been investigated, no attempts have been made to fractionate the rumen contents to investigate the individual roles of bacterial and protozoal populations of the rumen in degradation of nucleic acids. The present paper deals with studies on degradation of nucleic acids by intracellular enzymes of mixed protozoal population of buffalo rumen.

[1]Address reprint requests to Dr. P.R. Sinha, Animal Biochemistry Division, National Dairy Research Institute (Indian Council of Agricultural Research) KARNAL-132001, Hyryana, India.
Received August 26, 1988
Accepted December 27, 1989

Materials and Methods

Animals:

Two fistulated Murrah buffalo bulls weighing about 530 kg were fed berseem(*Trifolium alexandrinum*) *ad lib* for 3 weeks before starting the experiment and were maintained on the same feed subsequently. The animals were fed twice daily at 8 a.m. and 4 p.m.

Processing of rumen fluid:

Processing of rumen fluid was according to earlier reports(Sinha and Dutta, 1986b).

Preparation of cell-free extract of mixed rumen protozoa:

It was according to the procedure described earlier(Sinha and Dutta, 1986c). The pellet obtained after centrifugation of rumen fluid at 41xg for 2 min was suspended in minimal quantity of phosphate acetate buffer(0.1M, pH 7.2) containing 0.05% cysteine hydrochloride and frozen at $-20^{\circ}C$. The frozen cells were then thawed at $40^{\circ}C$ with intermittent stirring for 3 to 4 hr. The process was repeated two to three times and the thick suspension obtained was centrifuged at 25,000xg for 30 min. It was dialysed against the same buffer and used as a source of enzyme.

***In vitro* degradation of nucleic acids:**

In vitro degradation of nucleic acids was carried out according to procedure of McAllan and Smith(1973).

Twenty four ml samples of cell-free extract of mixed rumen protozoa were transferred to 100 ml flat bottom flasks containing 55mg either yeast RNA or calf thymus DNA dissolved in 1 ml water. The flasks were immediately transferred to a water bath maintained at 39°C and CO_2 was passed into the fluid for 2 min after 30 min intervals.

At the end of the incubation period nucleic acids were precipitated according to the method described by Munro and Fleck(1966). To 10 ml of the reaction mixture, 2.5ml of 1N perchloric acid was added and the contents were kept in ice for 10-15 min. The contents were then centrifuged at 25,000xg for 20 min and washed with 0.2N perchloric acid.

Estimation of residual nucleic acids:

Residual nucleic acids were estimated according to the method of McAllan and Smith(1969).

RNA was analysed by recording absorption of eluant at 260nm. Yeast RNA subjected to the procedure described above was used as the standard.

DNA was estimated colorimetrically by diphenylamine reagent method of Burton(1956). Calf thymus DNA was used as the standard.

Identification of the degradation products:

Degradation products of DNA and RNA obtained as a result of incubation of these nucleic acids with cell-free protozoal extract were separated from the reaction mixture by passing it through Amicon ultrafiltration membrane(PM-10). The ultrafiltrate was then introduced into a Sephadex G-10 column (70x1.5cm) and was eluted with ammonium acetate(0.01M, pH 7.0). Three millilitres of eluant were collected and their absorbance recorded at 260nm.

Standard preparations of nucleotides, nucleosides and purine and pyrimidine bases were used to identify the degradation products.

Confirmation of degradation products:

The pooled fractions of Sephadex G-10 peaks were applied to cellulose MN-300 chromatographic plates(20x20cm) which were than developed in pre-equilibrated jars by ascending technique using a solvent system comprising of water in one direction for 1 hr and the solvent system of n-Butanol: Water in the ratio 86:14 parts by volume in the second direction for 4 hr. The developed plates were dried in the air and the spots were detected under short range uv-lamp. They were identified by comparing their Rf values with those of known Rf values.

Results

Rate of disappearance of calf thymus DNA incubated with cell-free extract of mixed rumen protozoal population and simultaneous appearance of ultrafiltrable material (exclusion limit approximately 10,000) showing uv absorption spectrum characteristic of degradation products as nucleic acids is presented in figure 1. A progressive increase in degradation of DNA with simultaneous appearance of degradation products at the same rate was observed during first 2 hr of incubation. Further incubation did not record appreciable degradation of DNA. Incubation of RNA on the other hand recorded a rapid degrada-

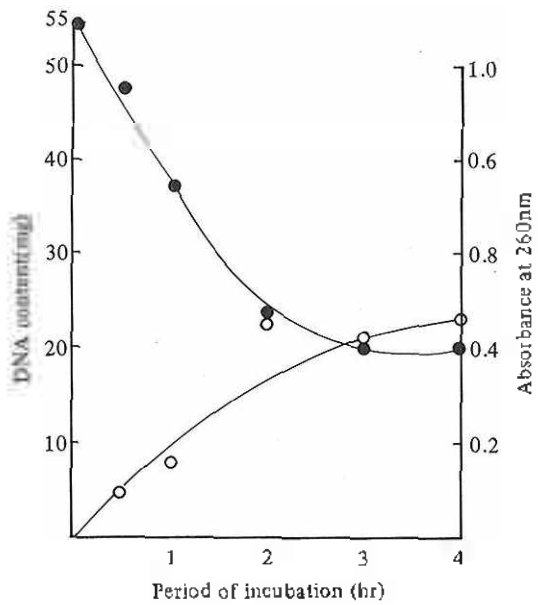

Figure 1. Degradation of DNA with cell free extract of rumen protozoa.
- ● Degradation of DNA
- ○ Appearance of degradation products of DNA

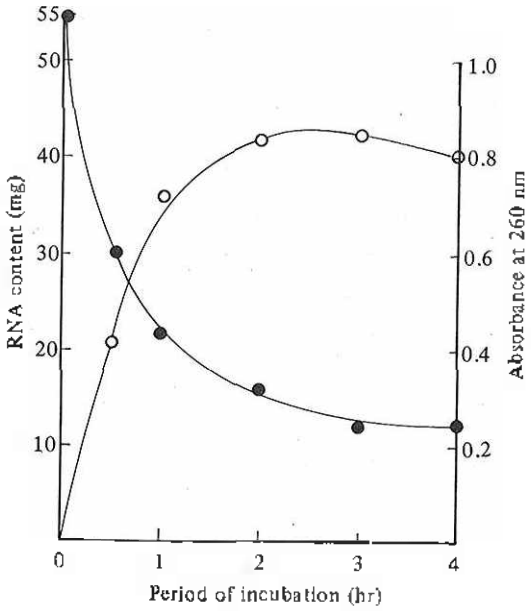

Figure 2. Degradation of RNA with cell free extract of rumen protozoa.
- ● Degradation of RNA
- ○ Appearance of degradation products of RNA

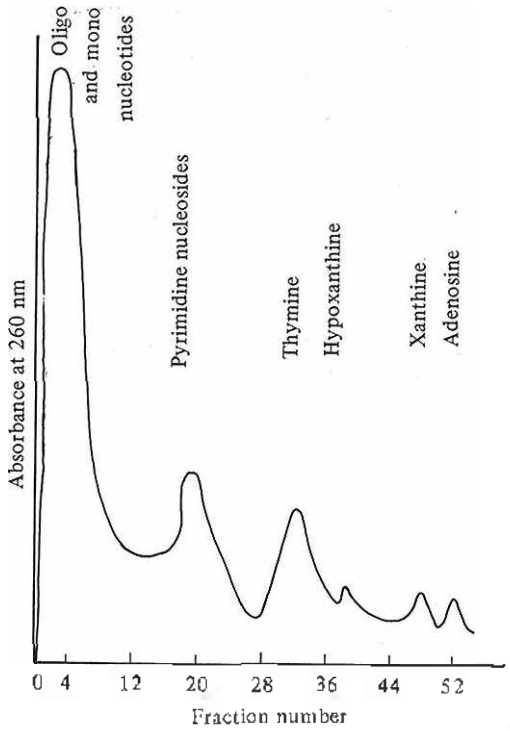

Figure 3. Pattern of degradation products of DNA incubated with cell free extract of rumen protozoa.

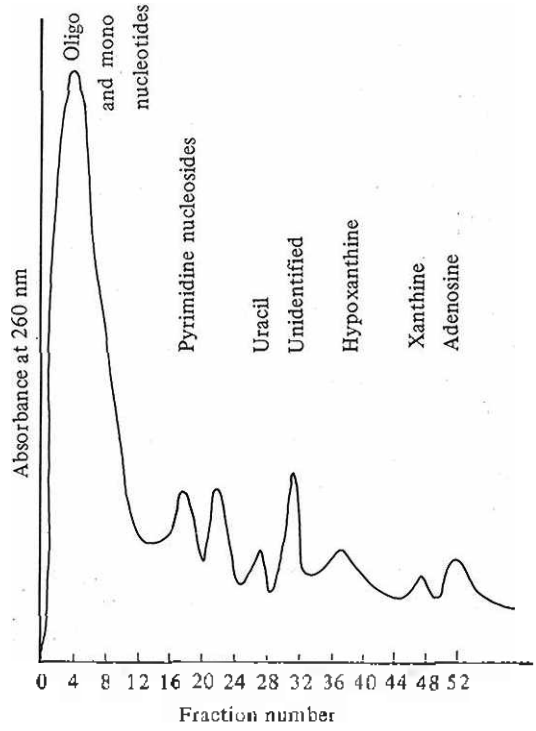

Figure 4. Pattern of degradation products of RNA incubated with cell free extract of rumen protozoa.

tion during an initial incubation period of 1 hr, whereafter degradation was observed to be slow. Degradation products appeared quite rapidly upto an incubation period of 2 hr but thereafter no further increase in the level of degradation products was observed (figure 2).

Results on identification of ultrafiltrable degradation products of DNA and RNA identified by gel filtration on Sephadex G-10 column(70x 1.5cm) and eluted with ammonium acetate(0.01 M, pH7.0) are presented in figure 3 and 4. DNA was observed to be degraded into oligo- and mononucleotides, pyrimidine nucleosides, purine nucleoside adenosine and bases xanthine, hypoxanthine and thymine. RNA on the other hand was observed to degrade into oligo and mononucleotides, pyrimidine nucleosides, purine nucleoside adenosine and bases xanthine, hypoxanthine and uracil. A peak corresponding to one of the degradation products could not be identified.

Discussion

A large number of different types of bacteria and protozoa are known to comprise the mixed microbial population of the rumen(Hungate, 1966; Hobson, 1971; Bryant, 1977). The importance of protozoa at least in degradation of proteins was recognised earlier by Blackburn and Hobson(1960) who reported that rumen protozoa are actively proteolytic. Degradation of nucleic acids in rumen was investigated by McAllan and Smith(1973) but they reported that whole rumen contents of calves used by them contained little or no protozoa. The authors observed that whereas degradation of DNA was carried out predominantely by rumen bacteria and the extracellular enzymes released into rumen fluid, rumen protozoa were as effective in degradation of RNA as rumen bacteria (Sinha and Dutta, 1988). In the present study maximum degradation of RNA was achieved much faster (1hr) as compared to DNA(2hr) which confirms our earlier observation. However, when degradation of RNA and DNA was studied using whole rumen contents, DNA was observed to be degraded faster(1hr) as compared to RNA(2hr) (Sinha and Dutta, 1986a). This presumably is due to faster degradation of DNA by mixed bacterial population of the rumen as compared to the mixed protozoal population.

Analysis of degradation products obtained as a result of incubation of DNA and RNA with cell-free extract prepared from mixed rumen protozoal population gave rise to similar degradation product as in case of mixed bacterial population of rumen except that purine nucleoside, adenosine was detected as one of the degradation products in case of degradation of nucleic acids by rumen protozoa. This would suggest a pathway of coversion of adenylic acid to hypoxanthine involving its conversion to adenosine followed by dephosphorylation and deamination.

Literature Cited

Blackburn, T.H. and P.N. Hobson, 1960. Proteolysis in the sheep rumen by whole and fractionated rumen contents. J. Gen. Microbiol. 22: 232-281.

Bryant, M.P. 1977. In "Dukes physiology of domestic animals" (M.J. Swanson, Ed), Cornell Uni. Press, Ithaka and London, pp.287-304.

Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62: 315-323.

Hobson, P.N. 1971. Rumen microorganisms. Industrial Microbiology 9: 41-47.

Hungate, R.E.(Ed). 1966. In "The rumen and its microbes" 92-147. Academic Press, New York and London.

McAllan, A.B. and R.H. Smith. 1969. Nucleic acid metabolism in the ruminants (1) Determination of nucleic acids in digesta. Br. J. Nutr. 23: 671-682.

McAllan, A.B. and R.H. Smith. 1973. Degradation of nucleic acids in the rumen. Br. J. Nutr. 29: 331-345.

Munro, H.M. and A. Fleck. 1966. Recent developments in the measurements of nucleic acids in the biological material. Analyst 91: 78-88.

Sinha, P.R. 1982. Ph. D. Kurukshetra University, India.

Sinha, P.R. and S.M. Dutta. 1986a. Degradation of nucleic acids by buffalo rumen. Ind. J. Dairy Sci. 39(2): 160-164.

Sinha, P.R. and S.M. Dutta. 1986b. Nuclease activity of whole rumen contents from buffalo (Bubalus bubalis). Ind. J. Dairy Sci. 39: 137-140.

Sinha, P.R. and S.M. Dutta. 1986c. Nucleolytic activity of mixed protozoal population from buffalo rumen. Ind. J. Dairy Sci. 39(4): 415-419.

Sinha, P.R. and S.M. Dutta. 1989. Distribution of nuclease activity in different fractions of rumen fluid. Int. J. Anim. Sci. 11:27-29.