Asian J. Dairy Res., 3 (2) : 111-113, 1984.

β-GLUCOSIDASE FROM BUFFALO RUMEN

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ABSTRACT

Properties of β-glucosidase from rumen liquor collected from Murrah buffaloes were studied. Maximum activity of the enzyme was recorded at pH 5.6. The enzyme was observed to be thermolabile since it lost nearly 45% of the activity even when held at 35°C for 10 min. Km value with p-nitrophenyl-β-D-glucopyranoside was 0.25 mM. Cellobiose and Glucono-δ-lactone were observed to be competitive inhibitors of the enzyme. Mn^{2+} and Ba^{2+} stimulated β-glucosidase activity.

Ruminants have an edge over non-ruminants because of their ability to utilise fibrous feeds. Preparation, assay and kinetics of action of β-glucosidase from rumen liquor of sheep were reported by Conchie (1954). The buffaloes have been reported to utilise cellulose better than cattle (Ichhponani *et al.*, 1971). Since the reports on enzymic properties in buffalo rumen are scanty, the investigations were therefore carried out to study the properties of β-glucosidase from buffalo rumen liquor.

Samples of rumen liquor drawn after 6 hr of morning feeding from fistulated adult male buffaloes fed on daily diet consisting of hay and small quantities of concentrate with free access to water were collected in a chilled flask. The liquor was agitated vigorously for 10 min, strained through six layers of cheese cloth and was then centrifuged at 22,000×g for 30 min. The supernatant decanted carefully was used as a source of the enzyme. β-glucosidase activity was assayed by incubating p-nitrophenyl-β-D-glucopyranoside (0.01 M) dissolved in 0.2 M disodium-hydrogen phosphate-0.1 M citric acid buffer (pH 5.6) at 40°C for 30 min. with the enzyme in a final volume of 3.0 ml. At the end of the incubation period the reaction was stopped by addition of 2.0 ml of 1.0 M sodium carbonate and its absorbance was recorded at 400 nm. Enzyme activity was expressed as n moles of p-nitrophenol formed per min at 40°C. Protein concentration was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

β-glucosidase from buffalo rumen recorded a linear relationship with period of incubation upto 100 min. and upto an enzyme concentration of 1.2 mg enzyme protein. Effect of pH on enzyme activity was studied over a pH range 3.8 to 8.2 and the results are presented in Table 1. It may be seen that β-glucosidase activity was maximum at pH 5.6. Different buffers *viz.* acetate buffer (0.1 M), phosphate buffer (0.1 M) and 0.2 M disodium-hydrogen phosphate-0.1 M citric acid buffer did not record any difference in enzyme activity when used to study the enzyme at pH 5.6. Maximum activity recorded at pH 5.6 in the present study is contrary to

Table 1. Effect of pH on β-glucosidase activity

pH	Enzyme activity (n moles p-nitrophenol released/min).
3.8	2.3
4.2	4.56
4.6	6.63
4.8	7.06
5.0	8.1
5.2	8.2
5.4	8.63
5.6	9.03
5.8	8.93
6.0	8.63
6.2	7.73
6.6	7.13
7.0	6.43
7.2	4.16
7.4	3.63
7.8	2.96
8.2	2.06

Table 2. Effect of different compounds on β-glucosidase activity

Test compound (10^{-3} M concentration)	Activity (as % of control)
NaCN	103
$MgSO_4$	104
$BaCl_2$	134
KCl	106
$MnSO_4$	147
$CoCl_2$	101
$NiSO_4$	108
1, 10-phenanthroline	103
Sodium-EDTA	101
Urea	106
α, α'-dipyridyl	100
Cysteine-HCl	115
NH_2OH. HCl	109

the observations made by Conchie (1954) who reported two pH optima, one at pH 5.4 and the other at pH 5.8 for β-glucosidase activity of the enzyme extracted from acetone dried powder of sheep rumen content with triton X-100.

β-glucosidase from buffalo rumen was held at different temperatures ranging from 35°C to 70°C for 10 min. The residual activity (%) was 55.6, 47.9, 29.9, 22.2,

9.4, 8.5 and 1.7 on respective temperatures of 35, 40, 45, 50, 55, 60 and 70°C. The enzyme was observed to lose activity rapidly with increase in temperature. Nearly 45% of the activity was lost even on holding the enzyme at 35°C for 10 min and nearly all the activity was abolished when the enzyme was held at 70°C for the same period. Conchie (1954) however, reported β-glucosidase from sheep rumen liquor to be heat stable.

A Km value of 0.25 mM was calculated for β-glucosidase of buffalo rumen from its Lineweaver-Burk plot. Cellobiose (1.0 mM) and Glucono-δ-lactone (0.2 mM) were observed to be the competitive inhibitors of the enzyme.

Effect of certain activators and inhibitors on β-glucosidase activity are presented in Table 2. None of the compounds tested were observed to inhibit the enzyme. Manganese and Barium ions were however, observed to stimulate β-glucosidase activity.

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