ON THE NATURE OF ANIMAL LACTASE

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The rate of hydrolysis of disaccharides by enzymes has been found to be considerably influenced by the presence of the products of hydrolysis. The cases of invertase and emulsin by Tammann in 1892,¹ maltase by Croft Hill² in 1898, and lactase by Armstrong³ in 1904. V. Henri,⁴ in 1901, showed that the retarding effect on invertase was mainly due to the fructose, an observation which was confirmed in 1902 by Adrian Brown,⁵ who proved that it was not due to the increased viscosity of the solution. E. F. Armstrong found that the lactase present in Kefir grains was retarded in its action by galactose, but that the lactase present in emulsin was retarded by glucose. He thus showed the existence of two kinds of lactase—a galacto-lactase, inhibited only by galactose and a gluco-lactase inhibited only by glucose. As no data as to the nature of animal lactase at present exist, it was of some interest to ascertain whether the lactase in the intestines of animals was of the type present in Kefir grains or of the type present in emulsin.

The procedure for examining for the existence of animal lactase which was adopted in these experiments was essentially the same as that of Plimmer.⁶ The enzyme solution was prepared by extracting the ground-up mucous membrane of the intestine of the dog for forty-eight hours with water containing toluene as antiseptic, and then filtering through cambric.

A definite volume of this extract was mixed with an equal volume of (a) 5 per cent. lactose solution, (b) 5 per cent. lactose solution to which glucose or fructose, in varying concentrations were added, to both mixtures 5 per cent. toluene was then added as antiseptic.* Samples of 50 c.c. were withdrawn immediately after mixing; the remainder was kept at 37° C.; and at intervals of one to four days, so as to give ample time for the enzyme to act, further samples of 50 c.c. were withdrawn. Each sample was treated with 5 c.c. mercuric nitrate solution to

* In all the experiments the same volume of extract was added to 5 per cent. solutions of glucose and galactose, to ascertain if there were any disappearance of reducing carbohydrate. No disappearance was obtained in any of the experiments here recorded.

precipitate protein; 30 c.c. of the filtrate from this precipitate were treated with 2 c.c. of caustic soda; after again filtering 20 c.c. of the solution were treated with hydrogen sulphide, excess of the gas was removed with copper sulphate, and the filtrate and washings from the sulphides made up to 100 c.c.

The reducing carbohydrate was then estimated by Bertrand's method, instead of gravimetrically. This method is as accurate as the gravimetric method and more rapid; the precipitate of cuprous oxide is filtered off, dissolved in acid ferric sulphate solution, and the ferrous salt titrated with permanganate. A solution of glucose was found to contain by the gravimetric method 4.63 per cent., by Bertrand's method 4.67 per cent.

The reducing power of completely hydrolysed lactose calculated from Bertrand's table is raised from 6.7 to 10. These figures were verified.

Lactose Solution	Hydrolysed Lactose Solution	
7.9 c.c. KMnO_4	11.8 c.c. KMnO_4	$\frac{7.9}{11.8} = 0.670$

With the concentration of lactose employed in these experiments, complete hydrolysis corresponds to a difference of 2.8 c.c. of permanganate. Differences in the titration of two solutions are generally less than 0.2 c.c.; a difference of 0.3 c.c. for experimental error has been allowed for. This is equivalent to 10 per cent. hydrolysis. Less than 10 per cent. hydrolysis of the lactose is therefore neglected in the results. The following data have been obtained; for comparison a similar experiment was made with the lactase in emulsin.

Experiment I.

ANIMAL LACTASE

Concentration of carbohydrate solution	Time of action of enzyme	No. of c.c. KMnO_4 for 10 c.c. of sugar solution	Difference in c.c. of KMnO_4	Percentage of hydrolysis
2.5 per cent. lactose	0	5.6 } 5.8 }	0	0
	36 hours	6.9 } 7.0 }		
	4 days	7.5 } 7.5 }	1.2	43
		7.5 }		
		7.5 }		
2.5 per cent. lactose + 2.5 per cent. glucose	0	15.0 } 15.0 }	0	0
	36 hours	15.2 } 15.0 }		
	4 days	15.2 } 15.0 }	0.1	0
		15.2 }		
		15.0 }		

Concentration of carbohydrate solution	Time of action of enzyme	No. of c.c. KMnO_4 for 10 c.c. of sugar solution	Difference in c.c. of KMnO_4	Percentage of hydrolysis
2.5 per cent. lactose	0	13.2 } 13.3	0	0
+		13.4 }		
2.5 per cent. galactose	36 hours	14.5 } 14.4	1.1	39
		14.4 }		
	4 days	15.2 } 15.1	1.8	71
		15.1 }		

Experiment II.

ANIMAL LACTASE

2.5 per cent. lactose	0	5.9 } 5.9	0	0
		5.9 }		
	26 hours	7.7 } 7.7	1.8	62
		7.7 }		
	96 hours	8.9 } 8.9	3.0	84
		8.9 }		
2.5 per cent. lactose	0	14.2 } 14.4	0	0
+		14.6 }		
2.5 per cent. glucose	26 hours	15.2 } 15.05	0.65	22
		14.9 }		
	96 hours	16.9 } 16.85	2.45	84
		16.8 }		
2.5 per cent. lactose	0	10.3 } 10.35	0	0
+		10.4 }		
1.25 per cent. glucose	26 hours	11.5 } 11.5	1.15	40
		11.45 }		
	96 hours	12.6 } 12.55	2.2	76
		12.5 }		
2.5 per cent. lactose	0	13.8 } 13.75	0	0
+		13.7 }		
2.5 per cent. galactose	26 hours	15.7 } 15.65	1.9	65
		15.6 }		
	96 hours	16.2 } 16.2	2.45	84
		16.2 }		

Experiment III.

ANIMAL LACTASE

2.5 per cent. lactose	0	6.1 } 6.05	0	0
		6.0 }		
	89 hours	8.5 } 8.5	2.45	84
		8.5 }		
	168 hours	8.7 } 8.6	2.55	88
		8.5 }		
2.5 per cent. lactose	0	15.0 } 15.1	0	0
+		15.2 }		
2.5 per cent. glucose	89 hours	16.0 } 15.8	0.7	24
		15.6 }		
	168 hours	16.8 } 16.8	1.7	58
		16.8 }		

Concentration of carbohydrate solution	Time of action of enzyme	No. of c.c. KMnO ₄ for 10 c.c. of sugar solution	Difference in c.c. of KMnO ₄	Percentage of hydrolysis
2.5 per cent. lactose	0	10.5 } 10.45	0	0
+ 1.25 per cent. glucose	89 hours	10.4 } 11.6 } 11.7	1.25	43
2.5 per cent. lactose	0	14.1 } 13.9	0	0
+ 2.5 per cent. galactose	89 hours	13.7 } 15.8 } 15.8	1.9	65
	168 hours	15.8 } 16.4 } 16.3	2.4	83
		16.2 }		
<i>Experiment IV.</i>				
EMULSIN				
2.5 per cent. lactose	0	5.8 } 5.7	0	0
	1 day	5.6 } 6.6 } 6.6	0.9	32
	3 days	6.7 } 7.3 } 7.3	1.6	57
	11 days	7.3 } 8.3 } 8.2	2.5	89
		8.2 }		
2.5 per cent. lactose	0	14.8 } 14.8	0	0
+ 2.5 per cent. glucose	1 day	14.8 } 15.0 } 14.9	0.1	0
	3 days	16.2 } 15.8 } 16.0	1.2	42
	11 days	18.0 } 17.4 } 17.7	2.9	100
2.5 per cent. lactose	0	14.0 } 14.0	0	0
+ 2.5 per cent. galactose	1 day	14.0 } 15.0 } 14.8	0.8	28
	3 days	14.6 } 15.8 } 15.9	1.9	68
	11 days	16.0 } 17.0 } 16.9	2.9	100
		16.8 }		

In all the experiments the inhibition by glucose is very marked, whereas galactose has little or no influence. Animal lactase appears to be extraordinarily sensitive to the presence of glucose: in the first experiment 2.5 per cent. glucose entirely inhibited the action, in the second and third experiments this quantity of glucose reduced the hydrolysis from 62 and 84 per cent. to 22 and 24 per cent. Even smaller amounts of glucose produced inhibition. In the experiment with

emulsin, glucose caused total inhibition at first, but after forty hours hydrolysis occurred. The curve represents the hydrolysis of Experiment 3.

It may be concluded that animal lactase is a gluco-lactase like the lactase in emulsin.

I wish to thank Dr. Plimmer for suggesting these experiments and for assistance in the methods employed.

- A. 2.5 per cent. lactose
- B. 2.5 per cent. lactose + 2.5 per cent. glucose.
- C. 2.5 per cent. lactose + 1.25 per cent. glucose.
- D. 2.5 per cent. lactose + 2.5 per cent. galactose.

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