Journal of Scientific Research
Banaras Hindu University, Varanasi

Vol. 60, 2016 : 53-63
ISSN : 0447-9483

EFFECT OF VARIOUS PHYSICAL AND CHEMICAL PARAMETERS ON THE ACTIVITY OF DEXTRANASE

Priyanka Jaiswal and Santosh Kumar
Biochemistry Division, National Sugar Institute,
Kanpur-208017, India

Abstract

Production of dextranase by *Penicillium aculeatum* has been studied. Effect of various physical and chemical parameters such as incubation period, temperature, pH, enzyme concentration etc. were studied on the activity of dextranase. The dextranase activity increased with the increase in the time of incubation up to 120 minutes and a linear relationship was obtained. Enzyme was found to be stable up to a temperature of 50^{o}C and thereafter stability and activity decreased.

***Keywords*:** Activity of enzyme dextranase

Introduction

Dextranases [(1-6)-α-D-glucan 6-glucanohydrolases or glucanases (E.C. 3.2.1.11)] are the enzymes that specifically hydrolyze the α-(1-6) linkages in dextran (Priyanka et al. 2011, Mohamed et al. 2007). Since many dextrans contain a relatively high concentration of secondary linkages, other than α-(1-6), enzyme which can break (1-2), (1-3) and (1-4) linkages in dextran, are also included together with true dextranase. The hydrolysis products of dextran by dextranase are glucose (with exodextranase), isomaltose and isomalto-oligosaccahrides. Therefore, these are called by common name as glucanases (E.C. 3.2.1.11). The prefixes show the nature of linkages attacked.

The dextranase can be utilized efficiently for hydrolyzing dextran already forme mainly in sugar mills since enzymatic method does not interfere with the sugar manufacturing process (Tilbury 1974). This is quick, easy and can be practised on commercial scale (Tilbury (1971). The enzyme can be used without changing the pH of juice and at a temperature up to 50°C. It also helps in treating deteriorated juices. The use of dextranase in sugar mills not only improves the factory performance but also improves the sugar quality (Fulcher, 1974). The excessive elongation of the crystal and viscosity of syrups and molasses may be reduced by the enzymatic decomposition of dextran (Hidi, 1975).

Materials and methods

Assay of Dextranase Activity

Mycelium free culture filtrate was used as enzyme (extracellular) source after suitable dilution (1:50) with 0.1M acetate buffer of pH 5.6. Mycelium was sonicated in a Vibronics ultrasonicator at 20 khz/sec. for 10 minutes at 4°C and centrifuged at 3000 rpm. Supernatant was used as (intracellular) enzyme.

Dextranase activity was assayed by the method of Koseric et al. (1973). Reaction mixture containing 2 ml of 2.5 % dextran in acetate buffer (0.1M, pH 5.6) and 1ml of enzyme in a total volume of 3 ml was incubated at 40°C for 20 minutes. Reaction was stopped by adding 3ml of DNS (dinitro salicylic acid) reagent and colour was read in Shimadzu U.V visible Spectrophotometer.

Dextranase Unit

One unit of dextranase is defined as the amount of enzyme, which liberates one µ mole of glucose/ml/minute.

Results and Discussion

The enzyme dextranase (1-6-α-D-glucan 6-glucanohydrolase E.C. 3.2.1.11), synthesized by some moulds and bacteria, is of great significance in the sugar industry as it is capable of degrading the biopolymer dextran formed in the cane juice during its processing for the production of sugar (Madhu et al., 1984).

To study the optimum enzyme concentration for hydrolysis of dextran, the different quantities of dextranase (1 to 6 µg protien), with 50 mg of dextran in acetate buffer (0.1 M, pH 5.6) in 3 ml reaction mixture were incubated at 40^{o}C at 20 minutes. The units of enzyme activity at different enzyme concentrations of 1, 2, 3, 4, 5 and 6 µg were 22.5, 41.2, 59.2, 81.2, 52.3 and 25.2 (units/ml). The maximum value of 81.2 units was obtained with 4 µg enzyme concentration. It is seen from table 1 that dextranase activity was proportional to the enzyme concentration (2-4 µg) and a linear relationship between µgs of protein (2-4 µg) and enzyme activity was observed and decreased thereafter.

To study the optimum time of incubation for dextranase activity, reaction mixture was incubated for 20 to 120 minutes at 40^{o}C, and assayed dextranase activity at different periods of incubation. The units of enzyme activity at different period of incubation i.e. 20, 40, 60, 80, 100 and 120 minutes were 51.2, 85.5, 135.2, 153.8, 191.2 and 241.5 (units/ml) respetively. The data showed that the dextranase activity increased with the increase in the time of incubation period up to 120 minutes and a linear relationship was obtained (Table 2).

Stability of crude and pure enzyme was ascertained by determining dextranase activity at different periods of time up to 60 days of storage. Crude enzyme (culture filtrate at pH 4.5 to 6.5) and purified enzyme solution (in acetate buffer pH 4.5 to 5.6)

were preserved at 4°C and the residual dextranase activity of both forms was determined at regular intervals. It is seen from the table 3 that there is no loss in dextranase activity in crude form at pH 4.5 to 5.6 for 24 days but after that, 30 to 75 % loss in dextranase units was seen up to 60 days. Dextranase (purified) stored at pH 4.5 to 5.6 was found to be quite stable for about 60 days.

Enzyme reaction mixture (3 ml), containing 4µg of enzyme protein and substrate (dextran of M.W 275,000, 50 mgs in acetate buffer of pH 5.6, 0.1M), were incubated at various temperatures between 20 to 80°C for 20 minutes to find out the temperature optima of dextranase. Units of enzyme activity at different temperatures of 20, 30, 40, 50, 60, 70 & 80 °C were (unit/ml) 15.4, 42.5, 80.9, 110.6, 98.5, 10.5 and 10.5 (unit/ml). The maximum value of 110.6 units was obtained at 50°C and thereafter it declined, due to denaturation of enzyme protein. The data revealed that enzyme is quite stable up to a temperature of 50°C (Table 4).

Enzyme reaction at 50°C was carried out at different pH values ranging from 3.0 to 8.0 to find out the optimum pH of dextranase activity. The highest activity of 81.2 was obtained at pH value 3.0 which was constant up to a pH value of 6.0 and declined thereafter. (Table 5).

Rate of hydrolysis of dextran at different enzyme concentrations was studied by incubating the enzyme reaction mixture containing 2 to 10 µg of enzyme (dextranase) protein with 50 mg of substrate at 50°C for 20 to 120 minutes. From the estimation of reducing sugars, percent dextran residue was calculated. It is seen from the table 6 that 16% dextran was hydrolysed in 20 minute while 28% was hydrolysed in 40 minutes at 2µg concentration of protein. However 34% dextran was hydrolysed in 60 minutes and after 60 minutes % dextran hydrolysed was constant (34%) up to 120 minutes.

At protein content 4µg, 30% dextran was hydrolysed in 20 minutes while 50% was hydrolysed in 40 minutes. In 60 minutes % dextran hydrolysis was 57 % and after which it was constant (57%) up to 120 minutes. At a protein concentration of 6 µg and above i.e. up to 10 µg, the pattern of hydrolysis was similar to that obtained with 2 & 4 µg of protein. At 6 µg of protein, 76% dextran was hydrolysed in 60 minutes while at 8 µg concentration of protein 78 % dextran was hydrolysed in 60 minutes. The maximum dextran hydrolysis % (87%) was obtained at 10 µg concentration of protein in 60 minutes. It is seen that there was an increase in dextran hydrolysis as concentration of enzyme protein was increased from 2 to 10 µg.

Various sugars such as glucose, fructose, galactose, mannose, xylose, salicin, sucrose and maltose were added (5 to 50 mM) to the enzyme reaction mixture to study their effect on dextranase activity. It is seen from table 7 that glucose, fructose, galactose, mannose, xylose, maltose and cellobiose had no effect at 5 mM concentration while at 50 mM concentration, all sugars acted as strong inhibitors of dextranase activity. Raffinose and lactose at 5 mM concentration showed slight

inhibitory effect (10%) on dextranase activity. Salicin & sucrose at 5 mM & 50 mM concentrations had no effect on dextranase activity.

6 mM concentration of different amino acids such as arginine, lysine, histidine, cysteine, threonine, methionine, aspartic acid and glutamic acid were added to the reaction mixture and incubated for 20 minutes at 50°C with substrate dextran to study their effect on dextranase activity. It is seen from table 8 that the dextranase activity was 95.0 units/ml which was taken as 100 % in the control. The units of dextranase ranged between 74.8 to 95 units/ml and remaining activity ranged between 78.8 to 100%. Arginine (92.6 units), lysine (92.7 unit) and threonine (90.8 units) showed no effect on dextranase activity and results were approximately similar to that of the control. Cysteine (74.8 units), methionine (80.56 units), aspartic acid (80.56 units) and histidine (87.2 units) had a marked effect on enzyme activity and inhibition was about 10 to 21%. However glutamic acid produced no effect on dextranase activity.

Effect of metallic salts and chelating agents was also seen for which $CoCl_2$, calcium acetate, $HgCl_2$, $FeCl_3$, $FeSO_4.7H_2O$, $AgNO_3$, $MnSO_4.4H_2O$, $MgSO_4.7H_2O$, Lead acetate, $CuSO_4.5H_2O$, EDTA, $ZnSO_4$, Barium acetate, SeO_2, $Sr(OH)_2$, $NiSO_4$, ferrous ammonium sulphate, potassium ferricyanide, sodium ascorbate, iodoacetate, PCMB, mercaptoethanol, $Na_2S_2O_4$ and NaN_3 were added to enzyme reaction mixtures at 6mM concentration and incubated at 50°C for 20 minutes. Hg^{2+}, Pb^{2+}, Cu^{2+} and PCMB completely inhibited the enzyme activity. EDTA, $K_4Fe(CN)_6$, sodium ascorbate, iodoacetate, mercaptoethanol and NaN_3 showed no inhibition. $FeSO_4.7H_2O$, $MgSO_4.7H_2O$, barium acetate, $Sr(OH)_2$ and ferrous ammonium sulphate showed slight inhibition (7%) of dextranase activity. Rest of salts showed marked inhibition from 20 to 80% on dextranase activity (Table 9).

It can thus be concluded that the dextranase activity was proportional to the enzyme concentration (2-4 µg) and a linear relationship between µgs of protein (2-4 µg) and enzyme activity was observed and decreased thereafter. The dextranase activity increased with the increase in the time of incubation period up to 120 minutes. Stability of crude and pure enzyme was checked by determining dextranase activity at different periods of time up to 60 days of storage. There is no loss in dextranase activity in crude form at pH 4.5 to 5.6 for 24 days but after that, 30 to 75 % loss in dextranase units was seen up to 60 days. Dextranase (purified) was found to be quite stable for about 60 days. The maximum value of 110.6 units was obtained at 50°C and after then it declined and the highest activity of 81.2 was obtained at pH value 3.0 which was constant up to a pH value of 6.0 and declined thereafter.

Dextran hydrolysis increased when concentration of enzyme protein was increased from 2 to 10 µg. Cystene, methionine, aspartic acid and histidine had a marked effect on enzyme activity and inhibition was about 10 to 21%. However, glutamic acid produced no effect on dextranase activity. Some metallic salts and chelating agents viz. Hg^{2+}, Pb^{2+}, Cu^{2+} and PCMB completely inhibited the enzyme

activity and EDTA, $K_4Fe(CN)_6$, sodium ascorbate, iodoacetate, mercaptoethanol and NaN_3 showed no inhibition.

Acknowledgements

Authors are thankful to Director National Sugar Institute Kanpur for providing necessary facilities for the present investigation.

References

Hidi, Staker (1975). QSSCT, 331-43.

Madhu and K.A. Prabhu, (1984). Enzyme Microb. Technol, 6, 217-220.

N.Kosaric, K.Yu, J.E. Zajic (1973). Biotech. Bioeng., 15, 729-41.

Priyanka Jaiswal and Santosh Kumar (2011). Journal of Scientific Research., 55, 71-76.

R.P. Fulcher, P.A. Inkerman (1974). Proc. Queensland, Sug. Cane tech.,43: 295.

Tilbury, French (1971). ISSCT, 1444

Tilbury, French (1974). ISSCT, 1277-87.

TABLES

Table- 1 Role of enzyme concentration on dextranase activity

S.No.	Enzyme concentration (µgs)	Enzyme activity
1	1	22.5
2	2	41.2
3	3	59.2
4	4	81.2
5	5	52.3
6	6	25.2

Table- 2 Role of time of incubation on dextranase activity

S.No.	Time of incubation (in minute)	Enzyme activity
1	0	----
2	20	51.2
3	40	85.5
4	60	135.2
5	80	153.8
6	100	191.2
7	120	241.5

Table- 3 Enzyme stability (60 days) in crude (culture filtrate) & purified stages

Time in days	Remaining activity (%)	
	Enzyme stability in crude	Stability in purified stages
	pH-4.5-5.6	pH- 4.5-5.6
0	--	100
6	100	100
12	100	100
18	100	100
24	100	100
30	75	100
36	50	100
60	25	100

Table-4 Role of temperature on the activity of dextranase

S.No.	Temperature	Enzyme activity
1	20	15.4
2	30	42.5
3	40	80.0
4	50	110.6
5	60	98.5
6	70	10.5
7	80	10.5

Table-5 Role of pH on the activity of dextranase

S.No.	pH	Enzyme activity
1	3.0	81.2
2	3.5	81.2
3	4.0	81.2
4	4.5	81.2
5	5.0	81.2
6	5.5	81.2
7	6.0	81.2
8	6.5	40.3
9	7.0	2.3
10	7.5	2.3
11	8.0	2.3

Table - 6 Dextran hydrolysis at different concentrations of dextranase

Sample No.	Enzyme dextranase protein (µg/unit value of reaction mixture)	% Residual of dextran					
		20 minute	40 minute	60 minute	80 minute	100 minute	120 minute
1	2	84	72	66	66	66	66
2	4	70	50	43	43	43	43
3	6	62	35	24	24	24	24
4	8	50	30	22	22	22	22
5	10	41	20	13	13	13	13

Table-7 Role of sugars on the activity of dextranase

S.No.	Sugar	Concentration mM	Remaining activity %
	Control	-----	100
1	Glucose	50	0
		5	100
2	Fructose	50	0
		5	100
3	Galactose	50	0
		5	100
4	Mannose	50	0
		5	100
5	Arabinose	50	0
		5	100
6	Xylose	50	0
		5	100
7	Salicin	50	100
		5	100
8	Sucrose	50	100
		5	95
9	Maltose	50	0
		5	100
10	Lactose	50	0
		5	90
11	Cellobiose	50	0
		5	100
12	Raffinose	50	0
		5	98

Table- 8 Role of amino acids on dextranase activity on Fukumota medium

S.No.	Amino acid	Dextranase activity units/ml	Remaining activity
	Control	95.0	100
1	Basic amino acids		
	Arginine		
	Lysine		
	Histidine	92.6	97.5
		92.7	97.6
3-	Neutral amino acids	87.2	91.8
	Cystine		
	Threonine		
	Methionine		
4-	Acidic amino acids	74.8	78.8
	Aspartic acid	90.8	95.6
5	Glutamic acid	80.56	84.8
6		80.56	84.8
7		95	100

Table-9 Role of inorganic salts, metal chelators and sulphydryl reagents on enzyme activity

S.No.	Chemicals	Dextranase activity % remaining
	Control	100
1	Co Cl_2	62.0
2	Calcium acetate	80.5
3	$HgCl_2$	0.0
4	$FeCl_3$	55.5
5	$FeSO_4.7H_2o$	93.5
6	$AgNO_3$	61.1
7	$MnSO_4.4H_2o$	33.3
8	$MgSO_4.7H_2o$	93.5
9	Lead acetate	0.0
10	$CuSO_4.5H_20$	0.0
11	EDTA	100
12	$ZnSO_4$	19.4
13	Ba-acetate	93.5
14	SeO_2	---
15	$Sr(OH)_2$	93.5
16	$NiS0_4$	77.8
17	Fer. ammonium sulphate	93.5
18	Pot. Ferricyanide	100
19	Sodium ascorbate	100
20	Iodo acetate	100
21	PCMB	0.0
22	Merceptoethanol	100
23	Na_2SO_4	---
24	NaN_3	100