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Activity and Stability of Purified Amylase Produced by *Streptomyces aureofaciens* 77

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Abstract The effects of pH values, temeprature and some elements on the amylolytic activity and stability of the purified *S. aureofaciens* 77 amylase were studied in this investigation. The purified enzyme showed its maximum activity at pH 6 within 8 min incubation at 40 °C. None of the tested 6 metals showed on stimulatory effect on the enzymatic activity, Fe^{+++}, Cu^{++} and Hg^{++} at high dose inhibited the enzyme activity to great extent as compared with Zn^{++}, Mn^{++} and Fe^{++} which gave less effect in this respect. The enzyme liquor was found to be thermolabile, since it lost completely its activity after 4 days incubation under room temperature and showed maximum activity during this period as a result of additions of Ca^{++} and NaCl. Gradual reduction was however recorded until activity reached 30% after 60 days of incubation.

Keywords Activity and stability of amylase, *Streptomyces aureofaciens.*

It is of great interest to study the stability of *S. aureofaciens* 77 amylase at different pH values and at different degrees of temperature lower and higher than the optimum, in order to find out whether this enzyme inactivation is reversible or not. Since it is known that certain changes in the native structure of the protein molecule may take place at low temperature.

Samia[1] found that the amylase produced by *S. orientalis* was thermolabile, at 60 °C up to 15 min and the enzyme lost its activity after 30 min at 80 °C and 90 °C. While amylase stored at 30 °C at pH 7 it maintained 82% of its activity up to one week and 60.5% after 3 weeks.

The effect of temperature on α-amylase activity of *B. subtilis* was studied by many investigators.[2,3] They reported that the purified enzyme is stable at 70 °C in the presence of 0.01 M. Ca^{++} and 0.1 M NaCl over abroad pH range from 5.5-9.5[4], illustrating that at very low temperature the rate of amylase inactivation is so slow that it does not have to be considered.

Obi and odibo[5] found that the optimum activity of thermostable *Actinomycetes* β-amylase was at 60 °C at pH 7 and it was stimulated by Mn^{++} and Fe^{++} but strongly inhibited by Hg^{++}. Abramov *et al.*[6] found that the optimum temperature was 65 °C for α-amylase produced by *Thermoactinomyces vulgaris* strain 42 and the optimum pH was at pH 5.0-6.0. Fairbairn *et al.*[7] found that amylase of *S. limosus* was unstable above 45 °C.

MATERIALS AND METHODS

S. aureofaciens 77 amylases

Purified amylase was chromatographically obtained.[8]

pH values

The activity of the purified enzyme was determined at different pH values (pH 4–10). This was carried out by using a series of buffers.[9] The buffered substrate was prepared by using 65 mg soluble starch per 100 m*l* of each buffer at various pH values. Enzyme activity and relative amylolytic activity were determined.

Determination of amylase activity

The amylase activity is measured photometrically according to the method adopted by Smith & Roe[10] and Garaway.[11]

Temperature

Constant amount of amylase was added to the

Table I. Effect of different pH values on the activity of purified amylase enzyme

Activities	pH Values						
	4	5	6	7	8	9	10
Amylolytic activity (U/ml)	11.70	38.25	45	37.5	18.45	16.6	0
Relative activity (%)	26	85	100	83	41	37	0

substrate (soluble starch) prepared in test tubes. These were incubated together at varying temperatures ranging from 20-80 °C with 5 °C interval. At certain intervals of incubation periods aliquots were taken from each tube and assayed for the enzyme activity.

Microelements

Manganese, ferrous, zinc, mercuric, copper and ferric were added individually to a diluted amylase enzyme solution sample. They were added at two concentrations (0.01 M and 0.0001 M). The activity of *S. aureofaciens* 77 amylase sample was determined before and after the addition of the trace elements.

Enzyme stability against pH changes

Seven test tubes (containing one ml of purified amylase solution) were prepared. Amounts of 9 ml of acetate buffer at pH 4.0 and 5.0; 9 ml of phosphate buffer at pH 6.0, 7.0 and 8.0; 9 ml of borate buffer at pH 9.0 and 10.0 were added, respectively. At specific intervals of time, samples were taken from each tube after 30, 60, 90, 120, 150, 180, 210 and 240 minutes at 5 °C and assayed at the optimum conditions to determine the remaining enzyme activity.

Thermostability

A suitable dilution of the purified enzyme at pH 6.0 was incubated at different temperatures ranging from 30 °C to 80 °C with 10 °C intervals. The determination of the enzyme activity was carried out after 15, 30, 45, 60, 90, 120, 180 and 240 min.

RESULTS AND DISCUSSION

pH values

Results from Table I showed that the maximum activity of *S. aureofaciens* 77 amylase was at pH 6.0 (45 U/ml). Increasing or decreasing the pH value of 6.0 resulted in a progressive drop in the enzyme activity. However, at lower pH values, it could be noticed that a sharp drop in enzyme activity was demonstrated at pH 4.0 untill it reached 26% of the original activity. At the highest pH value (pH 10.0) no enzymatic activity was recorded.

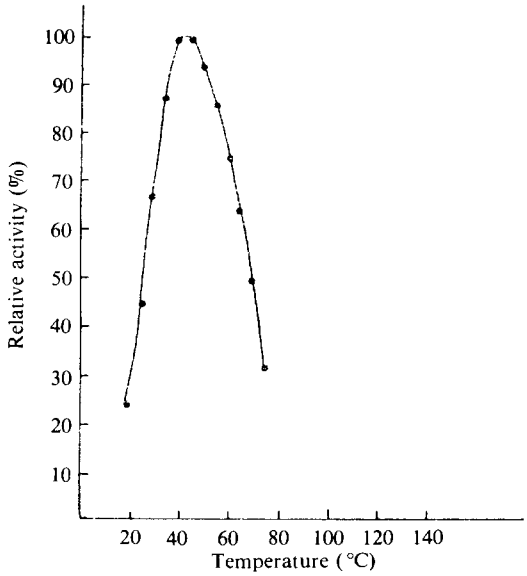

Fig. 1. Effect of temperature on the activity of the purified amylase.

The amylolytic enzyme system elaborated by *S. aureofaciens* 77 appeared to consist of only one type of amylase, as evidenced by a single activity peak, within the pH range of 4.0 to 10.0. These results were in agreement with those obtained by Okazaki *et al.*[12], who found that α-amylase of *B. subtilis* reached its maximal activity at pH 6.0 and the enzyme was active in pH range from 5.0 to 7.5 by Obi and Odibo[5] who reported that the optimum activity of thermostable *Actinomycetes* β-amylase was at pH 7.0, and by Abramov *et al.*[6] who reported that the optimum pH for enzymatic hydrolysis of amylase from *Thermoactinomycetes vulgaris* was at 5-6.

Temperature

Data from Fig. 1 cleared that 25% of the enzyme activity (12.5 U/ml) was attained at 20 °C. As temperature increased, a progressive increase in enzyme activity was observed till it reached its maximum between 40 and 45 °C (50 U/ml), which can be considered as the optimal temperature range of amylase produced by *S. aureofaciens* 77. Above 45 °C,

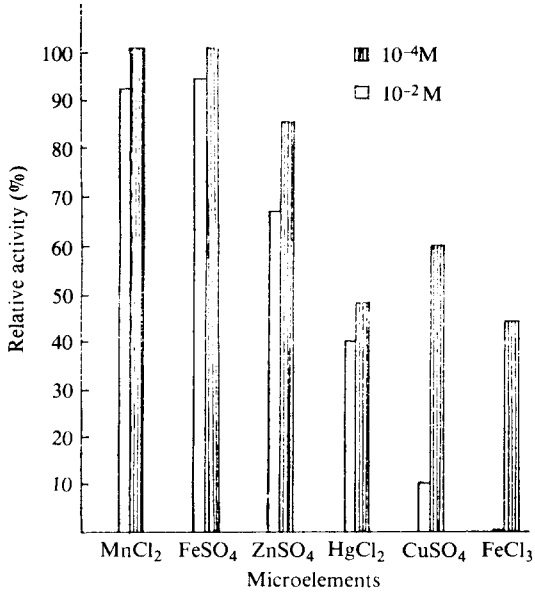

Fig. 2. Effect of some microelements on the purified amylase activity.

the enzyme activity showed a gradual decrease till it reached 50% (24.9 U/m*l*) at 70 °C, then sharply decreased till it reached 7% only at the original activity at 80 °C.

These results were in agreement with those of Fairbairn *et al.*[7] who found that amylase by *S. limosus* was unstable above 45 °C.

Microelements

Fig. 2 showed that none of the tested trace elements ions possessed any activating effect on the activity of the enzyme. Addition of 10^{-2}M manganese (Mn^{++}) and ferrous (Fe^{++}) resulted in a slight reduction of the enzyme activity amounted to 8 and 6% from the original activity, respectively.

Addition of Zinc (Zn^{++}) showed a significant reduction effect on the enzymatic activity which reached 33 and 15% at 10^{-2} and 10^{-4}M, respectively. Similary, mercuric (Hg^{++}) at the both concentrations made the enzyme inhibition reached 60 and 52% at 10^{-2} and 10^{-4}M, respectively.

The amylase enzyme activity appears to be specially sensetive to cupric and ferric ions which were proved to be strong inhibitors for the activity of amylase. The inhibition was 90% with copper (Cu^{++}) and 100% with ferric (Fe^{+++}) at the highest concentration (10^{-2}M).

Enzyme stability at various pH

Table II. Stability of *S. aureofaciens* 77 amylase at different pH values

Time (min)	Relative activity (%) at various pH						
	4	5	6	7	8	9	10
30	26	86	100	84	40	35	0
60	26	86	100	84	40	35	0
90	26	86	100	84	40	32	0
120	10	86	100	84	40	27	0
150	10	86	100	84	40	20	0
180	0	86	100	84	40	12	0
210	0	86	100	84	40	0	0
240	0	86	100	84	40	0	0

The optimum pH for the amylolytic activity was found to be pH 6.0 in the present investigation. This shows naturally the pH nature of *S. aureofacins* 77 amylase enzyme. Therefore, it was found of interest to study the stability of this enzyme at different pH values lower and higher than this value.

Table II showed that exposure of the enzyme to pH 6.0 for 4 hours did not affect the protein molecule, where the activity was still 100% of the original enzyme. However, keeping of the enzyme at pH 5.0, 7.0 and 8.0 for the same time, reduced its activity to about 86, 84 and 40% of the original activity, respectively. In addition, the activity of the used enzyme decreased with the decrease or increase in the pH value of the exposed enzyme. On the other hand, it was found that exposure of the enzyme to pH 4.0 and 9.0 apparently affected the enzyme molecules as the activity of the enzyme exposed at pH 4.0 was destroyed drastically within 180 min to get completely lost its activity. The activity of the exposed enzyme was gradually abolished within 210 min at pH 9.0. This activity showed complete unstability at pH 10.0, where the enzyme completely lost its activity after 30 min from the start of the exposure.

Thermostability

As shown before, the optimal temperature for the amylase activity was ranged between 40-45 °C, indicating the mesophilic nature of the enzyme. Therefore it was found of interest to study the stability of this enzyme when exposed to different degrees of temperature lower and higher than this value.

Data in Table III showed that the enzyme kept its activity at 30-40 °C for 240 min incubation.

Table III. Thermostability of *S. aureofaciens* 77 amylase

Time (min)	Relative activity (%) at various temperature (°C)					
	30	40	50	60	70	80
15	100	100	96	74	50	0
30	100	100	96	71	41	0
45	100	100	94	68	30	0
60	100	100	94	65	14	0
90	100	100	90	58	0	0
120	100	100	90	52	0	0
180	100	100	90	46	0	0
240	100	100	90	46	0	0

Table IV. Effect of calcium ion on the thermostability of *S. aureofaciens* 77 amylase

Treatments	Temperature (°C)	Relative activity (%) Time (min)							
		15	30	45	60	90	120	180	240
Control	60	74	71	68	65	58	52	46	46
	70	50	41	30	14	0	0	0	0
	80	0	0	0	0	0	0	0	0
10^{-4}M Ca^{++}	60	78	73	70	68	60	52	45	44
	70	53	44	32	14	0	0	0	0
	80	0	0	0	0	0	0	0	0
10^{-2}M Ca^{++}	60	92	90	87	84	79	71	62	53
	70	88	85	80	75	68	58	48	37
	80	24	8	0	0	0	0	0	0

However, incubation of the enzyme at 50 °C gradually decreased its activity with the increase of the incubation time to reach 90% of the original activity at the experimental period, namely 240 min. Increasing the temperature from 50 to 60 °C resulted in an appreciable decrease in the enzyme activity which amounted to 50% from the original activity. Sharp drop of the enzyme activity took place at 70 °C, where the activity reached 14% only after 60 min. No activity was however, detected after that. Moreover, increasing of the temperature to 80°C resulted in complete loss of the amylase activity.

Addition of calcium ion

Addition of calcium ion to the purified amylase in the form of calcium sulphate, greatly increased the thermostability of the enzyme. (Table IV) Addition of 0.01 M Ca^{++} proved to be the suitable concentration of calcium sulphate which gave the highest active stability for amylase enzyme after 4 hr incubation at 60, 70 and 80 °C. The addition of 0.01 M $CaSO_4$ helped the enzyme to attain its amylolytic activity up to 68% at 70 °C incubation for 90 min, where the enzyme lost its amylolytic activity during the same treatment without the addition of Ca^{++} ions. Moreover, the enzyme showed relative stability at 80 °C for 30 minutes (8% of the original activity).

In this respect slight activation was recorded with the addition of the lowest concentration of Ca^{++} ions namely 0.0001 M. Hence, it could be concluded that the removal of calcium from these preparation gave sensitive amylase for denaturation and loss activity after short period of incubation at 70 °C. The addition of calcium ion into the amylase preparations proved to be very important for the production of active and stable enzyme. It is well known that the addition of Ca^{++} ion to the amylase enzyme preparation is of importance for improving its quality, activity and stability.

These results are in agreement with those of Moseley and Keciy[2] who found that the amylase enzyme has remarkable heat stability in the presence of 0.01 M Ca^{++} and 0.1 M NaCl.

Storage of liquor enzyme

Since it is well known that certain changes in the native structure of the protein molecule take place at natural conditions. Therefore preservation of the amylase liquor without significant loss of its activity is well known to be of great important for subsequent industrial technological process. Hence, an experiment was designed to record the amylase stability under natural conditions using 0.01 M of $CaSO_4$ and 0.1 M NaCl as stabilization and preservation agents for amylase activity. Storage was continued at room temperature (20-35 °C) for a period extending for 60 days. Logically, cooling was avoided during storage to study the effect of the stabilization and preservation agents only and to know the stability of the purified amylase activity at room ten . rature, since refrigeration of the liquor by itself is a mean of stabilization and preservation.

Table V showed that storage of the enzyme liquor containing 0.01 M $CaSO_4$ and 0.1 M NaCl at room temperature for a period of 4 days did not affect its activity. This began to decrease gradually reaching to 60% of the original activity after 14 days of storage, then dropped sharply to reach 30% after 60 days of storage.

Table V. Effect of Ca^{++} and Na^{+} on the stability of amylase solution when stored at 5°C and room temperature

		Relative activity (%)													
		Storage time (days)													
		1	2	3	4	5	6	7	14	21	28	35	42	50	60
5 °C	Without $Ca^{++} + Na^{+}$	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	With $Ca^{++} + Na^{+}$	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Room Temp.	Without $Ca^{++} + Na^{+}$	75	40	10	0	0	0	0	0	0	0	0	0	0	0
	With $Ca^{++} + Na^{+}$	100	100	100	100	97	92	85	60	35	30	30	30	30	30

In this respect, it must be mentioned that the amylolytic activity of the enzyme liquor did not changed as a result of storage in the refrigerator (5 °C) for a period extended to 60 days. These feature is of great importance for subsequent industrial technological process. Hence it was found that storage of the enzyme which containing Ca^{++} and NaCl at room temperature was more thermostable than the enzyme which not containing Ca^{++} and NaCl, so the enzyme activity in the presence of Ca^{++} and NaCl at the fourth day of storage was 100% while it was 0% in the absence of Ca^{++} and NaCl at room temperature.

LITERATURE CITED

1. Samia, A.I.: Production of amylase by *Streptomyces*. M. Sc. Thesis, Cairo Univ. (1969).
2. Mosely, M.M. and Keciy, L.: Purification and characterization of the α-amylase of *B. subtilis* NRRL. B 34 II, *Biotech. Bioeng.* **12**, 251 (1970)
3. Dawod, M.A.: Biochemical studies on amylase and protease activity and stability during production. M.Sc. Thesis. Faculty of Agric., Cairo Univ. (1984).
4. Omer, N. and Berkhan, B.: Activity of *B. subtilis* amylase heated at different temperatures. (1978).
5. Obi, S.K.C. and Odibo, F.J.C.: Partial purification and characterization of thermostable *Actinomycete* β-amylase. *Appl. Environ. Microbiol.* **47**(3), 571 (1984).
6. Abramov, Z.T., Tsaplina, I.A., Mstepanov, V. and Gloginova, L.: Purification and properties of α-amylase from *Thermoactinomyces vulgaris strain* 42, *Biokhimya* **51**(1), 119 (1986).
7. Fairbairn, D.A., Priest, F.G. and Stark, J.R.: Extracellular amylase synthesis by *S. limosus*. *Enzyme Microb. Technol.* **8**(2), 89 (1986).
8. Arafa, M.A.I.: Microbiological studies on the production of certain enzymes by some strains of *Streptomyces*. Ph.D. Thesis, Faculty of Agriculture, Al-Azhar Univ., 1988.
9. Dawson, R.M., Elliott, D.C., Elliot, W.H. and Jones, K.M.: Data for biochemical research. Oxford at the Clarendon Press Second Edition, p. 483 (1969).
10. Smith, B. and Roe, J.: Colorimetric determination of amylase activity. *J. Biol. Chem.* **227**, 357 (1957).
11. Caraway, W.T.: A stable starch substrate for the determination of amylase in serum and other body fluids. *Am. J. Clin. Pathol.* **32**, 97 (1959).
12. Okazaki, K., Hata and Komatsu, H.: Digestive drugs. XX-Antiseptics. XXI-β-amylase 1. XXX-β-amylase 2. *Yakuzaigaku*, **20**, 23; 26; 32, (1960).