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Precepitation and Purification of Amylase Enzyme Produced by *Streptomyces aureofaciens 77*

A.N. Ibrahim, F.H. Ahmed*, M.M.K. Ibrahim* and M.A.I. Arafa*
Botany Department, Faculty of Agriculture, Al-Azhar University, Madinet Nasr
**Mycology Department, National Organization for Drug Control and Research*
***Faculty of Home Economic, Hellwan University, Cairo, Egypt*
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Abstract □ Precipitation and purification of amylase secreted by *Streptomyces aureofaciens* 77 in liquid inorganic salts-starch medium under the optimum conditions were carried out. Ammonium sulphate fractionation was used to precipitate amylase in cell free culture filtrate. $(NH_4)_2SO_4$ at a concentration of 50-70% saturation gave the highest enzyme yield. The obtained precipitates were redissolved in phosphate buffer (pH 7.0) and subjected to dialysis. The dialyzed enzyme preparation was applied to DEAE-cellulose column chromatography which resulted in an increase of purification up to 59.48 fold. A further step of purification was done by applying the obtained purified sample to Sephadex-G200 column chromatography which resulted in an increase of purification up to 73.92 fold. The results clearly indicated that the isolated amylase from *S. aureofaciens* 77 was only on type.

Keywords □ Purification of amylase, *Streptomyces aureofaciens.*

Enzymes are found in nature in complex mixture, usually in cells which perhaps contain a hundred or more different enzymes, or in cultural filtrate. In order to study any enzyme properly it must be isolated and purified. The ready availability of isolated enzymes has been considerably valuable in number of medical and industrial applications.[1]

Grootegeed *et al.*[2], obtained best results of amylase separation by using 50-70% ammonium sulphate as starting material, yielding 72% of the total amylase activity in the low molecular weight fraction. Upton and Fogarty[3] purified amylase to 313 fold by precipitation with n-propyl alcohol, dialysis against tap water, adsorption on $Ca_3(PO_4)_2$, and fractional on Sephadex G-100. Ghai and Chahal[4] used ammonium sulphate for amylase precipitation, and DEAE-cellulose column for amylase purification. Obi and Odibo[5] reported that the amylase from *Thermoactinomyces sp.* No. 2 was purified 36 fold by acetone precipitation, ionexchange chromatography and Sephadex G-200 gel filtration.

This study contributed for revealing a microbial source of amylase from local isolates of *S. aureofaciens* 77 in purified form that could have applicable and clinical importance.

MATERIALS AND METHODS

Fractional precipitation by ammomium sulphate

Fractional precipitation was carried out periodically by adding various amounts of solid ammonium sulphate to 100 m*l* of crude enzyme preparation to bring a saturation percentage from 20-100% interval. The mixture was left overnight at 0 °C and then centrifuged. Each fractional precipitate was dissolved immediately in 0.2 M phosphate buffer at pH 7. The dissolved fractional precipitates were tested four amylolytic activity and protein concentration.

Purification by DEAE-cellulose column chromatography

The crude enzyme solution obtained from 7 days old cultures filtrates was fractionated with ammonium sulphate. The fraction precipitating between 50-70% saturation was dissolved in 100 m*l* of 0.01 M phosphate buffer (pH 7). This solution was dialyzed against the same buffer for 24 hr and against sucrose until the volume became 50 m*l*. Ten m*l* of the dialyzed solution was applied at a rate of 60 m*l*/hr onto a column (40 × 1.6 cm) of diethyl aminoethyl cellulose (DEAE-cellulose) equilibrated with 0.01 M phosphate buffer (pH 7.0). After washing with 100 m*l* of the equilibration buffer, amylase was eluted with a linear gradient from 0.01 M to 0.2 M of phosphate buffer (pH 7.0). The

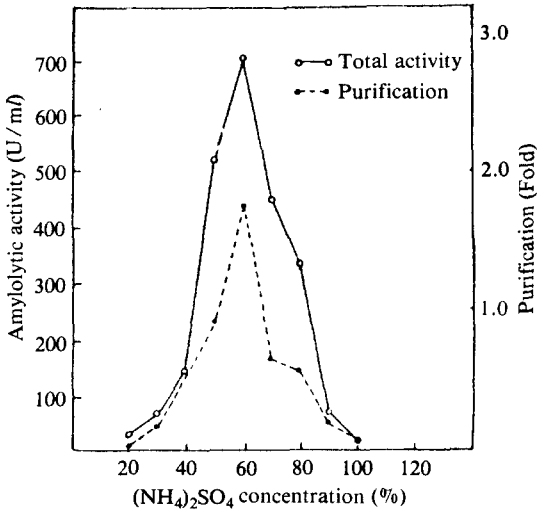

Fig. 1. Fractional precipitation by ammonium sulphate.

various fractions were tested for both amylase activity and total protein.[6]

Purification by gel filtration using Sephadex G-200

The fractions containing amylase activities, which collected from the amylase purification by chromatography and rechromatography on DEAE-cellulose column, were again fractionally precipitated with ammonium sulphate between 50-70% saturation. After dissolving the resulting precipitate in 0.01 M phosphate buffer, pH 7.0, ten m*l* of this enzyme solution was applied to a Sephadex G-200 column (40 × 1.6 cm) which was eluted at a rate of 30 m*l*/hr with 0.01 M phosphate buffer, pH 7.0. Representative fractions were examined for amylolytic activity and total protein.[7,8]

Determination of amylase activity

The amylase activity was measured photometrically according to the method adopted by Smith & Roe[9] and Caraway.[10]

Determination of total protein

Total protein was estimated according to Lowry *et al.*[11]

RESULTS AND DISCUSSION

Fractional precipitation by ammonium sulphate

Fractionation by ammonium sulphate was made to precipitate the *S. aureofaciens* 77 amylase enzyme, using different concentrations of ammonium sulphate (20-100% saturation). The results recorded in Table I illustrated by Fig. 1 showed clearly that specific activity recovery (yield %) and purification (fold) in the precipitates increased with the increase of ammonium sulfate concentration reaching a peak of specific activity of 47.3 U/mg, recovery yield 10.57% with 1.77 folds of purification at 60% $(NH_4)_2SO_4$ concentration, after which all were dropped. Therefore it could be concluded that addition of 50-70% saturation of $(NH_4)_2SO_4$ is recommended for the first step in the purification process of the enzyme.

Accordingly further studies were carried out using $(NH_4)_2SO_4$ (50-70%) saturation for precipitating the enzyme.

Purification of S. aureofaciens 77 amylase by using DEAE-cellulose column chromatography

Table I. Fractional precipitation of *S. aureofaciens* 77 amylase by different concentration of ammonium sulfate

$(NH_4)_2SO_4$ (%)	Volume (m*l*)	Activity (U/m*l*)	Total activity (U)	Protein (mg/m*l*)	Total protein (mg)	Specific activity (U/mg)	Recovery Yield (%)	Purification (Fold)
0	1200	5.6	6720	0.21	252	26.7	100	1.0
20	100	0.3	30	0.37	37	0.8	0.45	0.03
30	100	0.7	70	0.15	15	4.7	1.04	0.18
40	100	1.5	150	0.09	9	16.7	2.23	0.63
50	100	5.25	525	0.21	21	25.0	7.81	0.94
60	100	7.1	710	0.15	15	47.3	10.57	1.77
70	100	4.55	455	0.25	25	18.2	6.77	0.68
80	100	3.35	335	0.21	21	15.9	4.99	0.60
90	100	0.7	70	0.12	12	5.8	1.04	0.22
100	100	0.2	20	0.09	9	2.2	0.30	0.08

Table II. Column chromatography of *S. aureofaciens* 77 amylase on DEAE-cellulose column

Molarity of phosphate buffer pH 7.0	Number of fraction	Amylolytic activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
0.01 M	1	0.0	0.0	0.0
	2	124.2	0.240	517.5
	3	161.1	0.240	671.3
	4	180.5	0.160	1128.1
	5	154.0	0.138	1116.0
	6	91.9	0.143	642.7
	7	77.7	0.141	551.1
	8	74.1	0.192	385.9
	9	0.0	0.0	0.0
	10	0.0	0.0	0.0
0.05 M	11-20	0.0	0.0	0.0
0.10 M	21-30	0.0	0.0	0.0
0.15 M	31-40	0.0	0.0	0.0
0.20 M	41-50	0.0	0.0	0.0
Mixture of fractions	1-10	130	0.11	1181.8

Data presented in Table II illustrated by Fig. 2 showed clearly that the activity of the amylase enzyme began to appear from the second fraction up to the eighth fraction 0.01 M phosphate buffer (pH 7.0), after which the enzyme did not give clear activity. Maximum activity of the enzyme was shown at fraction No. 4. These results clearly indicated that the amylase enzyme isolated from *S. aureofaciens* 77 was only one type.

The amylolytic active fractions (No. 2-8) were collected, added together and fractionally precipitated again with $(NH_4)_2SO_4$ (50-70% saturation). After dissolving the enzyme precipitate in 0.2 M phosphate buffer, pH 7.0 and dialyzing against the same buffer, the enzyme was again chromatographed on DEAE-cellulose using 0.01 M phosphate buffer, pH 7.0 (Table III).

The above rechromatography process was significantly effective for further purification of the enzyme.

Purification of amylase enzyme by gel filtration using Sephadex G-200 column chromatography

Ten ml of the enzyme solution, which purified by using DEAE-cellulose column chromatography,

Table III. Rechromatography of *S. aureofaciens* 77 amylase on DEAE-cellulose column

No. of fraction	Amylolytic activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
1	25	0.0	0.0
2	190	0.310	612.9
3	210	0.260	807.7
4	280	0.210	1333.3
5	200	0.151	1324.5
6	145	0.190	763.2
7	129.5	0.196	660.7
8	110	0.240	458.3
9	0.0	0.0	0.0
10	0.0	0.0	0.0
11-50	0.0	0.0	0.0
Mixture of fractions (1-8)	270	0.170	1588.2

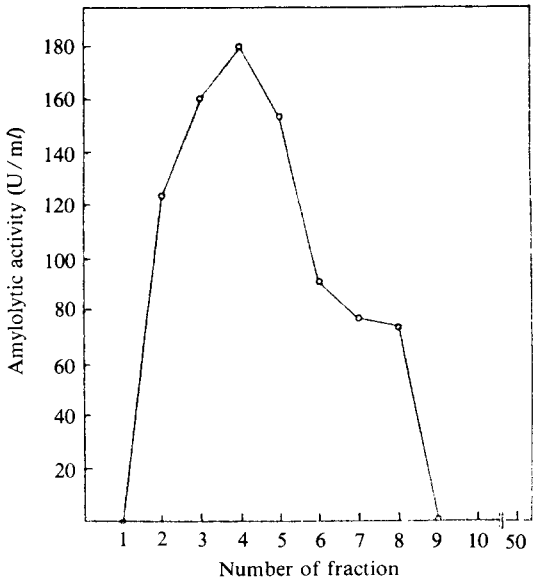

Fig. 2. Chromatography of *S. aureofaciens* 77 amylase on DEAE-cellulose column.

was applied at the top of Sephadex G-200 column. Fractions of 10 ml aliquots were collected separately. Fifty fractions were collected. A graphical representation for both the enzymatic activity (U/ml) and protein content (mg/ml) is shown in Table IV.

Table IV. Gel filtration of *S. aureofaciens* 77 amylase on Sephadex G-200 column chromatography

No. of fraction	Amylolytic activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)
1-5	0.0	0.0	0.0
6	345	0.400	862.5
7	447.5	0.400	1118.7
8	510.0	0.269	1896.0
9	427.8	0.230	1860.0
10	255	0.238	1071.4
11	216	0.235	919.1
12	206	0.320	643.7
13-50	0.0	0.0	0.0
Mixture of fractions (6-12)	375	0.190	1973.7

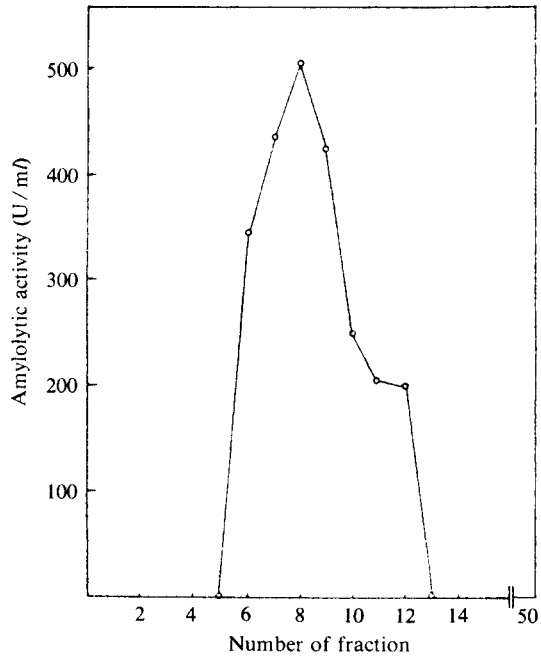

Fig. 3. Gel filtration of *S. aureofaciens* 77 amylase on Sephadex G-200 column chromatography.

The most active fraction was fraction No. 8 which represented a high activity (510 U/ml) and specific activity (1896.0 U/mg).

In view of the findings of other investigators, it has been reported that the major aim of any enzymologist is to obtain the enzyme in the form of pure state. For the purpose at purification, various methods were used according to the nature of the enzyme and its properties. Accordingly, different methods were given by many investigators.[2-5,12-16]

The subsequent steps of purification of the present amylase under investigation, *i.e.* purification by using of DEAE-cellulose and Sephadex G-200 column chromatography, resulted in a purified enzyme preparation of specific activity of 1973.7 units/mg protein and purification folds of 73.92 (Table V).

The degree of purity of the obtained purified enzyme preparation was testimonized by more than one criterion. One of these was obtained from DEAE-cellulose column chromatography which resulted in a single sharp peak of amylolytic acti-

Table V. Summary of purification procedures of *S. aureofaciens* 77 amylase

Step	Fraction	Volume (ml)	Activity (U/ml)	Total activity (U)	Protein (mg/ml)	Total protein (mg)	Specific activity (U/mg)	Recovery yield (%)	Purification (fold)
1	Broth (supernatant)	1200	5.6	6720	0.21	252	26.7	100	1
2	Fractionation by $(NH_4)_2SO_4$	100	7.1	710	0.15	15	47.3	10.57	1.77
3	Dialysis	50	16	800	0.08	4	200	11.90	7.49
4	Column chromatography on DEAE-cellulose	10	130	1300	0.11	1.1	1181.8	19.34	44.26
5	Re-chromatography on DEAE-cellulose	10	270	2700	0.17	1.7	1588.2	40.18	59.48
6	Gel filtration on Sephadex G-200	10	375	3750	0.19	1.9	1973.7	55.80	73.92

vity, the second was given by Sephadex G-200 column chromatography which resulted in a single sharp peak of activity also (Fig. 2,3).

In general, it is believed that the sharp peak obtained at the end of purification steps reflect a good support for the degree of purity of the enzyme under investigation. However, it is worthy to mention that partial denaturation of the enzyme during purification attempts would not be ignored. The results clearly indicate that 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.

LITERATURE CITED

1. Price, N.C. and Stevens, L.: Fundamentals of enzymology. Oxford Univ. Press, Oxford, New York (1982).
2. Grootegeed, J.A., Lauwers, A. and Heinen, W.: Separation and partial purification of extracellular amylase and protonase from *B. caldohyticus*. *Arch. Microbiol.*, **90**, 223 (1973).
3. Upton, Mary E. and Fogarty, W.M.: Production and purification of thermostable amylase and protease of *Thermomonspora viridis*. *Appl. Environ. Microbiol.* **33**(1), 59 (1976).
4. Ghai, S.K. and Chahal, D.S.: Alpha-amylase from *Aspergillus flavus*. Production, purification and properties. *Indian. J. Agric. Res.* **14**, 146 (1980).
5. Obi, S.K.C. and Odibo, F.J.C.: Partial purification and characterization of thermostable Actinomycete β-amylase. *Appl. Environ. Microbiol.* **47**, 571 (1984).
6. Himmelhoch, S.R.: *Methods Enzymol.* **22**, 273 (1971).
7. Fischer, L.: An introduction to gel chromatography, North-Holand, Amesterdam (1969).
8. Reiland, J.: *Methods Enzymol.* **22**, 287 (1971).
9. Smith, B. and Roe, J.: Colorimetric determination of amylase activity. *J. Biol. Chem.* **227**, 357 (1957).
10. 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).
11. Lowry, O.H., Rosebrough, N., Farr, A. and Randall, R.: Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265 (1951).
12. Refaat, A.A.: Studies on the production of microbial amylase. Ph.D. Thesis, Faculty of Agric., Ain Shams Univ., (1978).
13. Ibrahim, M.M.K.: Production of some enzymes by Streptomyces. Ph.D. Thesis, Faculty of Agric. Al-Azhar University, 1983.
14. Safwat, M.S.A., Mahmoud, S.A.Z., Abdel-Nasser, M. and Attia, R.M.: Isolation and purification of bacterial alpha-amylase. *Zentrable. Milkrobiol.* **138**(3), 247 (1983).
15. De Mot, R. and Verachtert, H.: Purification and characterization of extracellular amylolytic enzymes from the yeast *Filobasiduim capsuligenum*. *Appl. Environ. Microbiol.*, **48**, 1474 (1985).
16. Shatta, A.M., Ibrahim, A.N., El-Kousy, S.M. and Ibrahim, M.M.K.: Proteinase production by *Streptomyces griseus* 550. III-Precipitation and purification of proteinase. *Al-Azhar. J. Agric. Res.*, **3**, (1986).