Sci. J. Fac. Sci. Menoufia Univ. Vol. V (1991). 83 - 103

PURIFICATION AND CHARACTERIZATION OF D - FRUCTOSE 1.6 DIPHOSPHATE ALDOLASE FROM STREPTOMYCES SPOROCINEREUS

Magda A. El. Meleigy; A. M. Ragab; Eglal A. Ghonamy and Ebtessam N. B.

Faculty of Science for Girls, Al - Azahar University Nasr City, Cairo, Egypt.

SUMMARY

Fructose diphosphate aldolase was purified 5.6 - fold from cell free extracts of cultured cells of Streptomyces sporocinereus. Maximal activity was obtained at 45 °C and pH 8.5. Km value was 2 x 10^{-3} M. The effect of some salts and chemical reagents on enzyme activity was studied. Enzyme activity was stimulated by KCL, cysteine and 2 - mercaptoethanol and inhibited EDTA, O - phenanthroline, iodoacetate and heavy metals. Exposure of the enzyme to PH7.0, 8.5 and 9.2 for 2 hrs caused moderate loss in enzyme activity but exposure to pH 5.0 for few min. completely inactivated the enzyme. Exposure of the enzyme to 45 °C and 55 °C resulted in appreciable inactivation while exposure to 60 °C resulted in rapid inactivation of the enzyme. Addition of 2 - mercaptoethanol or FDP (enzyme substrate) and bovine serum albumin (BSA) stabilized the exposed enzyme to 45 °C and 55 °C. The present studies revealed that **S. sporocinereus** *aldolase resembles class II aldolases found in bacteria and fungi.*

Key words. FDP - aldolase, Streptomyces sporocinereus.

INTRODUCTION

FDP Aldolase, like most of the other enzymes of the glycolytic pathway is widely distributed in living organisms. The

properties of this enzyme were extensively studied in several bacteria (Kowal *et al.*, 1966; Amelunxen and Lins, 1968; James and Richard 1971; Bang and Banmann, 1978), and fungi (Ingram and Hochster, 1967; Siddiqui and Banerjes, 1978).

Yet to our Knowledge these properties were not demonstrated before in thermophilic actinomycetes except *Streptomyces thermocinereus* (Ragab *et al.*, 1982); In case of mesophilic actinomycetes the only work been done by Cochrane *et al.*, (1953), Wang *et al.*, (1958), Maitra and Roy (1959) and Cochrane (1965). Those authors detected aldolase enzyme in a survay including other enzymes in Embden Meyerhof - parnas (EMP) pathway of glucose catabolism.

Our preceding report aims to purify, study and compare some of the properties of aldolase enzyme produced by Streptomyces sporocinereus with those produced by other microorganisms.

MATERIALS AND METHODS

Organism.

Streptomyces sporocinereus (Hussein *et al.*, 1979) was used in the present study.

Medium

Sucrose - nitrate medium was used, it has the following composition (g/L); sucrose 20.0; KNO_3, 2,0; K_2HPO_4, 1. 0; $MgSO_4$, 0.5; NaCl, 0.5; $FeSO_4$, 0.001; $CaCO_3$, 3.0; yeast extract, 2.0. Distilled water $_1$L.

Cultural condition and harvest of mycelium.

Preparation of cell free extract, enzyme assay and protein determination are presented in a previous communication (El-Melegy *et al.*, 1991).

Acrylamide gel electrophoresis:

Polyacrylamide gel electrophoresis was carried out according to the method described by Davis (1964).

RESULTS AND DISCUSSION

Enzyme precipitation :

Some procedures have been used for the precipitation of FDP aldolase enzyme including ammonium sulphate, cooled organic solvents and also calcium phosphate gel fractionation but all these trials failed to precipitate the enzyme.

Another trial was made by the addition of bentonite using different concentrations for every 5 ml extract°ε. The most active enzyme protein was retained in the supernatant while the precipitate

contains trains of activity. As shown in table (1) the highest yield of specific activity was obtained at a concentration of 0.25 g bentonite per 5 ml cell free extract, with a specific activity 7. 4 and 89 % recovery.

Table (1) : precipitation of aldolase from cell - free extract by bentonite.

Bentonite g / 5 ml	Activity units	Protein mgm	Specific act. (units / mgm)	Purification (fold)	Recovery (%)
-	8.4	2.4	3.5	1	100
0.25	7.5	1.01	7.42	2.12	89
0.50	5.19	1.10	4.71	1.34	61.7
0.75	4.3	1.10	3.9	1.11	51.1
1.00	3.4	0.90	3.8	1.07	40.4

DEAE Cellulose column chromatography :

The supernatant containing the enzyme after the addition of bentonite was then applied to a DEAE cellulose column chromatography 1.2 x 45.5 cm packed and equilibrated with 60.0 ml 0.05 M Tris - HCI buffer, pH 8.5. Batch - wise elution of the enzyme was then begun with 25 ml portions of increasing molarities of NaCl.

The results obtained (Fig.1) showed the most active fractions of the enzezme in Nacl Concentrations, 0.5 - 0.5 M. Table (2) demonstrates a 5.64- fold purification and 87.8 % recovery

Table (2) : Purifiation of *S. sporocinereus* aldolase by DEAE - Cellulose chromatography.

Purification Steps	Activity (units)	protein mgm	specific activity	purification (flod) (units/mgm)	Recovery (%)
Crude ext.	88	33	2.60	1	100
Bentonite	80	14.4	5.50	2.13	90.9
DEAE-cellu-lose column	77.25	5.26	14.68	5.64	87.7

It is worth to add the fractions 1 - 29 and fractions 35 - 42, which contain the inactive proteins (Fig.1). They may be contaminating proteins associated with the enzyme molecule that separated by the column. On the other hand, it is worth - mentioning that acrylamid gel electrophoresis of the fractions of the column revealed that enzyme protein was highly purified.

Properties of S. speorocinereus aldolose :

Effect of temperature and pH on enzyme activity :

Fig. 2, demonstrates the maxim alactivity of FDP aldolase occurred at 45°C. Increasing the temperature resulted in a decrease in the activity of the enzyme. At 70°C the activity was 40 % of that at optimum temperature. This result was in accordance with that reported by Edward and Seed (1970) who reported that aldolase of *I. brucei; I. gambiens; I. equioerdum* and *I. lewisi* had optimum temperature ranfus from 41 - to 45 °C james and Richard (1971)

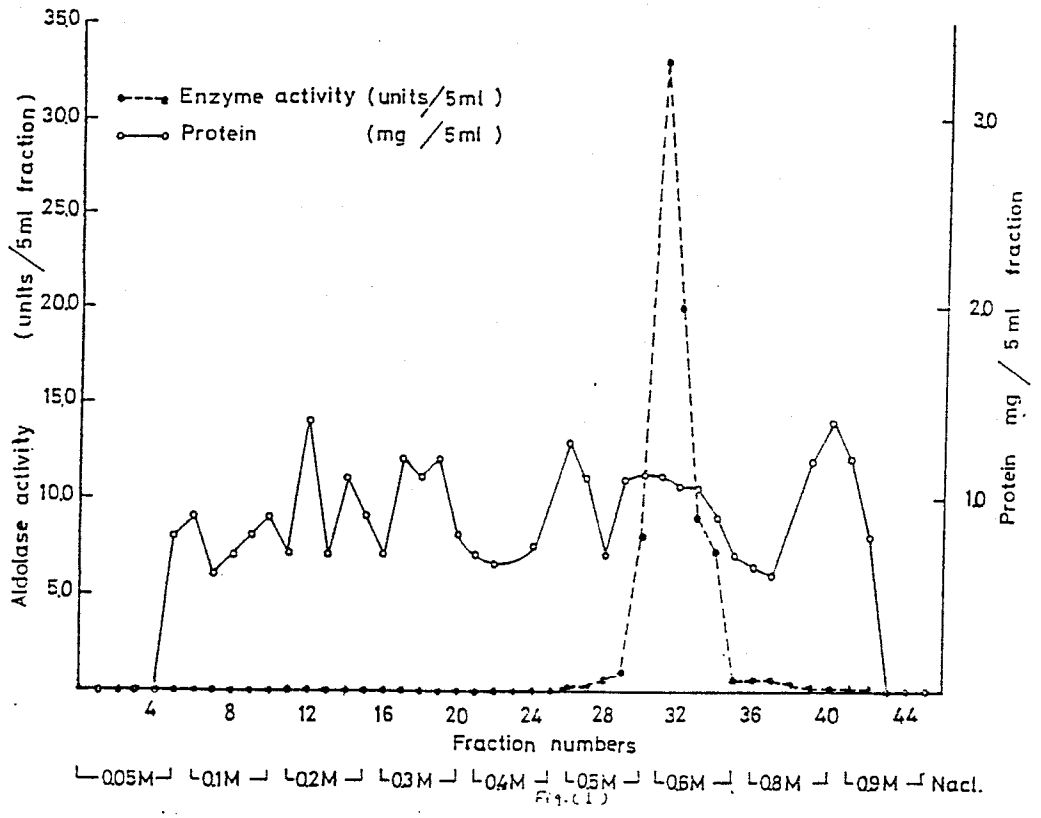


Fig. (1) : Purification of *S. sporocinereus* aldolase by DEAE Cellulose column chromatography.

found that *C. pasteurianum* aldolase exhibited maximum activity at 42°C.

On the other hand, maximum enzyme activity was obtained at pH 8.5 (Fig. 3). Similar findings were reported by Shinichi and Yoshiaki (1971), were found that the optimum activity of *B. stearothermophilus* aldolase occurred at pH 8.5 - 8.6 in Tris or borate buffer. Siddique and Banerjee (1975) found that the aldolase enzyme in *Rhizobium* sp. had an optimum activity at pH 8.4 in veronal buffer and pH 8.8 in Tris - HCL. Thus the nature of the buffer was affect the enzyme activity to some extent. Phosphate buffer exhibited 68 % of the activity of Tris - HCL at pH 8.0 and carbonate buffer at pH 8.0 vstnpmsyr niggrt exhibited 35% of the activity of Tris - HCL at the same pH value.

The ionic strength of the reaction mixture affected the enzyme activity. FDP aldolase activity increased with increasing buffer molarity from 0.05 - 0.4; Maximal activity occurred at 0.25M. Further increasing in molarity did not affect the enzeyme activities.

Rate of glyceraldehyde phosphate formation from fructose 1, 6 diphosphate

Fig. 4 reveals the formation of glyceraldehyde phosphate from fructose 1, 6 diphosphate by the partially purified enzyme. It is clear that the extent of catalytic action is a true response of the concentration of enzyme.

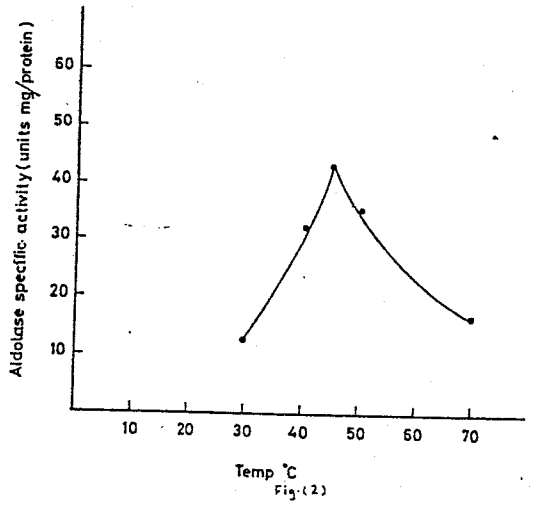


Fig. (2) : Effect of temperature on the activity of *S. sporocinereus* aldolase. Reaction mixture contained : FDP, 10.0 µ moles ; Tris - HCI buffer pH 8.5, 80 µ moles; enzyme protein, 25.0 µg; total volume, 2.0 ml; temp asindicated; reaction time, 20 min.

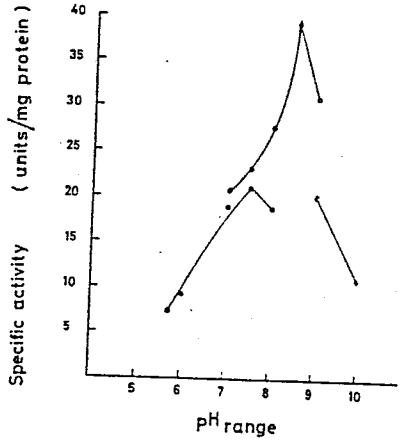


Fig. (3) : Effect of pH on the activity of *S. sporocinereus* aldolase.
Reaction mixture contained, FDP, 10.0 µ moles, hydrazine sulphate; 56.0 µ moles; enzyme protein, 40.0 ug; buffer, Ph, as indicated, 80 moles; total volume, 2.0 ml; temp. 45°C; reaction time, 20 min.
(o_o) Tris - HCI buffer. (._.) Sod phosphate buffer.
(x - x) Carbonate - bicarbonate buffer.

Substrate specificity:

Investigation of substrate specificity revealed that the aldolase of *S. sporocinereus* showed highly specificity for fructose 1,6 diphosphate since the activities determined by using other substrates such as fructose -6- phosphate and glucose 6- phosphate were 49.3 % and 20.7 % respectively (Table 3).

Table (3) : Substrate specificity of alolase of *S. sporocinereus.*

Different substrates	Aldolase sp. activ. Unit/mg protein	Sepecificity
F - 6 - diphosphate	40.5	100
F - 6 - phosphate	20.0	94.3
Glucose - 6 - phosphate	8.4	20.7

Aldolase activity of *S.sporocinereus* as a function of substrate concentration.

The relationship between FDP aldolase activity and concenurtions of FDP have been shown in Fig.(5) KM was calculated from the Line Weaver - Burk plot and found to be 2.0 $x10^{-3}$ M. This is in comparable with that reported by sadoff *et al* (1969) who found that Km value of Bacillus cereus was 2.0 x 10^{-3} M. Ragab *et al.*, (1982) reported that the Km value of *S. thermocinereus* was 2.0 x 10^{-3} M.

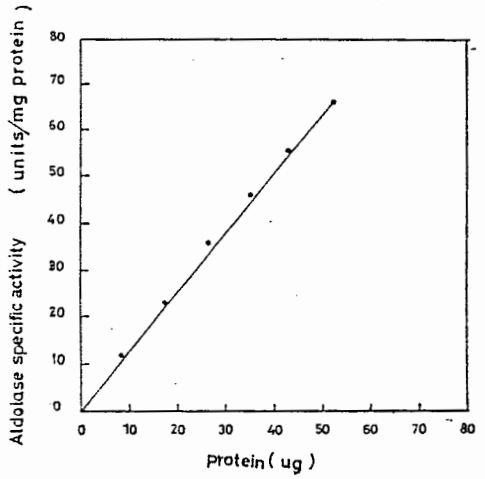


Fig. (4) : Rate of glyceraldehyde phosphate formation from fructose 1, 6 diphosphate
Reaction mixture contained : FOP, 100μ Moles;hydrazine sulphate, 56.0 μ moles; Tris - HCI buffer, pH 8.5, 80 u moles; enzyme protein, as indicated, total colume, 2. 0 ml, temp, 45°C reaction time 20 min.

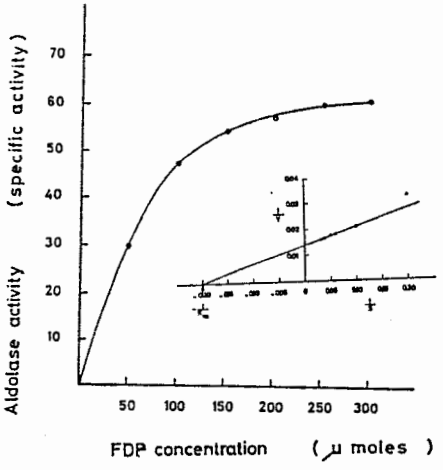


Fig. (5) : Aldolase activity of *S. sporocinereus* as a function of substrate concentration.
Reaction mixture contaned : FDP, as indicated; hydrazin sulphate, 56.0 μ moles; Tris - HCI buffer, pH 8.5,80 μ moles; enzyme protein, 35. 0 ug; total volume 2. 0 ml, temp., 45°C; reaction time, 20 min.

Effect of some metal salts and chemical reagent on aldolase activity of S. sporocinereus.

The effect of the addition of the tested salts on the enzyme activity was offectted by the additein of the welalo oolts results showed that potassium cloride and L - cysteine activated the effect of aldolase enzyme of *S. sporocinereus* at the two concentrations used (10^{-2} and 10^{-3}M). Willard and Gibbs (1968) and Husain and Rao 91973) reported that aldolase enzyme was stimulated by K^{+} and cysteine. Calcium chloridehad little inhibit ary effect at the two concentrations used. Zinc sulphate, copper sulphate, ferrous sulphate, nickel chloride, manganese sulphate, cobalt chloride and magnesium sulphate had high inhibitory effect. These results are in accordanc with that reported by Husain and Rao (1973).

Th addition of some chmical ragnts such as O- phnanthroline, EDTA,KCN and 4- chloromrcury benzoate resulted in a noticeable inactivation of aldolase enzyme, while 8-Hydroxyquinoline, iodine sodium azide and iodoacetic acid have a slight inactivation on enzyme activity.

Kowal *et al.*, (1966) reported that aldolase enzyme is strongly inhibited by chelating agents such as, EDTA, O-phenanthroline and pyrophosphate. Verma (1964) mentioned that EDTA O- phenanthroline produced marked inhibition. Raymond and kenneth (1971) reported that aldolase was inactivated by metal chelating agents.

Table (4) : Effect of the addition of some metal salts and some compounds on the activity of S. Sprocinereus.

Componds added	Concentration (N)	Aldolase up activity (units / mgm)	Inhibiton (%)	Activation (%)
Control	-	36.6	-	100%
k cl	10^{-2}	41.3	-	12.7
	10^{-3}	38	-	3.5
ca cl_2	10^{-2}	30.6	16.3	-
	10^{-3}	32.6	10.9	-
L- Cystein	10^{-2}	43.0	-	18.2
	10^{-3}	39.3	-	7.5
Mn So_4	10^{-2}	13.3	63.6	-
	10^{-3}	14.6	60.0	-
Mg So_4	10^{-2}	23.3	36.4	-
	10^{-3}	25.3	30.9	-
Ni Cl_4	10^{-2}	12.6	65.4	-
	10^{-3}	16.0	56.4	-
Cu So_4	10^{-2}	6.8	87.1	-
	10^{-3}	8.6	76.3	-
Fe So_4	10^{-2}	12.0	67.2	-
	10^{-3}	30.6	16.3	-
Co cl_2	10^{-2}	18.0	50.9	-
	10^{-3}	22.0	40.0	-
Zn So_4	10^{-2}	6.5	82.1	-
	10^{-3}	16.0	56.3	-
Iodoaxetic acid.	10^{-2}	34.6	-	-
	10^{-3}	36.0	-	-

Table (4) : cont.

Componds added	Concentration (N)	Aldolase up activity (units / mgm)	Inhibiton (%)	Activation (%)
sodium azide	10^{-2}	32.6	-	-
	10^{-3}	35.3	-	-
EDTA	10^{-2}	5.6	85	-
	10^{-3}	7.0	82.1	-
K CN	10^{-2}	11.6	86.7	-
	10^{-3}	23.3	37.5	-
O-phenathroline	10^{-2}	5.1	86.2	-
	10-3	12.1	66.9	-
Choromercury	10^{-2}	19.6	47.3	-
benzoate	10^{-3}	30.6	17.8	-
Hydroxyquinoline	10^{-2}	22.0	41.0	-
	10^{-3}	33.6	10.8	-
Iodine	10^{-2}	32.6	12.5	-
	10^{-3}	37.3	-	-
2-mercaptoethanol	10^{-2}	38.7	-	6.8
	10^{-3}	40.6	-	12.0
EDTA +2-mercapto	10^{-2}	6.4	83.2	-
(equimolat amounts)	10^{-3}	8.12	77.0	-
Iodoacetic	10^{-2}	36.0	-	-
acid + 2 mercaptoe.	10^{-3}	41.8	-	15.5

Reaction mixture contained :
FDP, 10 moles; Hydrazine sulphate, 55u moles; Tris - HCI buffer PH 85, 80u moles : enzyme protein, 35u g; concentrations of compunmds added, as indicated; total coume, 2.0 ml; temp, 45 & reaction time, 20 min.

The inhibitory effect of iodocetic acid suggests the requirements of sulfhydryi group in the enzyme. 2-mercaptoethanol was added in equimolar amouns of iodoacetic acid to counteract this inhibition. As shown in table 4, the containing iodoacetic acid reversed the inhibition at the first concentration (10^{-2} M), whereas, the, activation of the reaction that this inhibition is then reversible while mixture at the low concentration (10^{-3}M) which revealed that occured by EDTA is irreversible since it was not affected by the addition of 2- mercaptoethanol.

pH stability of *S. sporocinereus* aldolase :

Four test tubes were prepared each containing 1 ml enzyme (160 ug protein) and 2 ml 0.1 M buffer at different pH value. The first tube receives actetate buffer pH 5.0 and 9.2 were added separately to the other tubes, and left for 2 hrs. At specific time intervals 0.6 ml aliquot were taken each and assayed (Fig.6) revealed that exposure of the enzyme to PH5 resulted in complete inactivaton of enzyme activity within few minutes of incubation. Reactivation of the inactivated enzyme by transferring an aliquot to borate buffer at pH 9.2 and incubating for two hours was unsuccessful. These results indicate that exposure of the enzyme to acidic conditions resulted in an irreversible denaturation of the enzyme protein. On the other hand, exposure of the enzyme to pH 7.5, 8.5 or 9.2 were similar in their behaviours and resulted in a gradual decrease in enzyme activity at the first hour of incubation.

The remaining activity was stable during the second of incubation which was higher in Tris than phosphate or borate buffers (50.2 %, 34.4 % and 29.7 % respectively).

Heat inactivation kinitics of S. Sporocinereus aldolase.

It is clear from Fig 7 that when enzyme extracts were incubated at 60°C a rapid loss of activity occured through the first hour (the remaining activity was 6.2 % of the activity at 45°C) and complete loss of activity was after 30 min later.

Exposure of the enzyme to 45°C and 55°C resuled in about 52.4& and 69.9% loss in enzyme activity respectively during the first hour of the incubation period. The remaining activity was stable during the second and the third hour at 45°C whereas at 55°C there was a gradual loss in enzyme activity and the remaining activity was about 17.6 %. These results indicated that aldolase of *S. sporocinereus* is thermolabile. It is evident also, that although the optimum temperature for the activity of the enzyme was 45°C yet there was appreciable inactivation when incubated in a buffer solution at the same temperature and in absence of the substrate. This indicates that the presence of the substrate in the reaction mixture protects the catalytic site from heat inactivation. Siddiqui and Banerjee (1978) found that on treating the crude extract of aldolase of *Rhizobium leguminosarum* at 50°C for 1 hr, aldolase activity decreased by 33%, and the remaining actvity remained stable on further incubation at 50°C for up to 5 hrs.

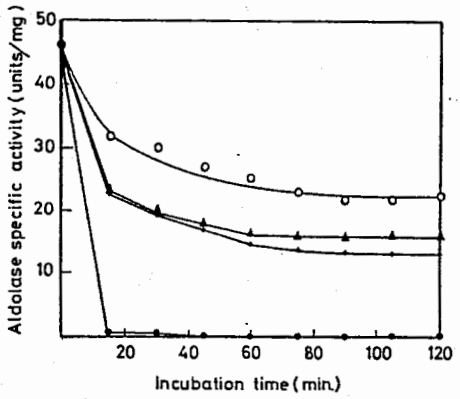


Fig. (6) : pH Stability of *S. sporocinereus* aldolase. Reaction mixture contained. Aldolase (160ug protein / ml) was incubated in 0.2 M borate buffer, pH 9.2; 0.2M Tris - HCI buffer pH 8.5; 0.2 M phosphate buffer, pH 7.5, and 0.6 ml sample were removed and assayed for aldolase activity at specific time intervals.

(* - *) Enzyme incubated at PH 9.2.

(o - o) Enzyme incubated at PH 8.5.

(Δ - Δ) Enzyme incubated at PH 7.5.

(ω - ω) Enzyme incubated at PH 5.0.

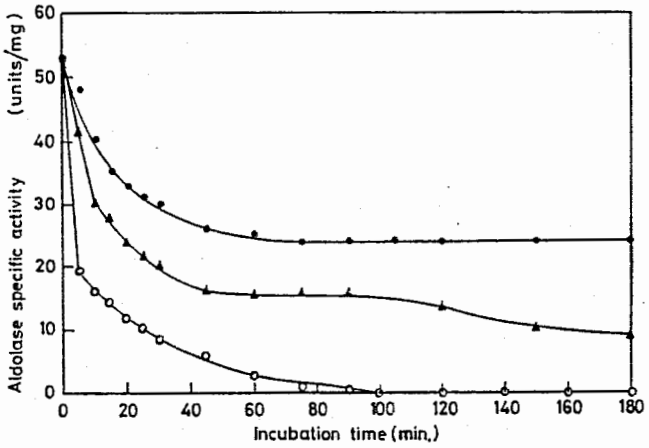
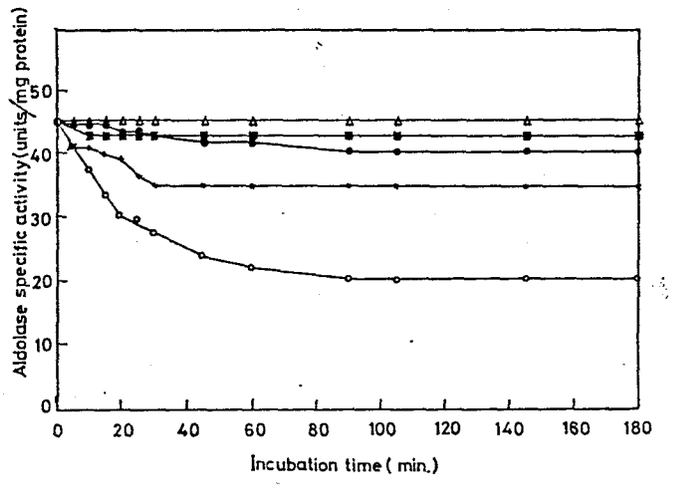
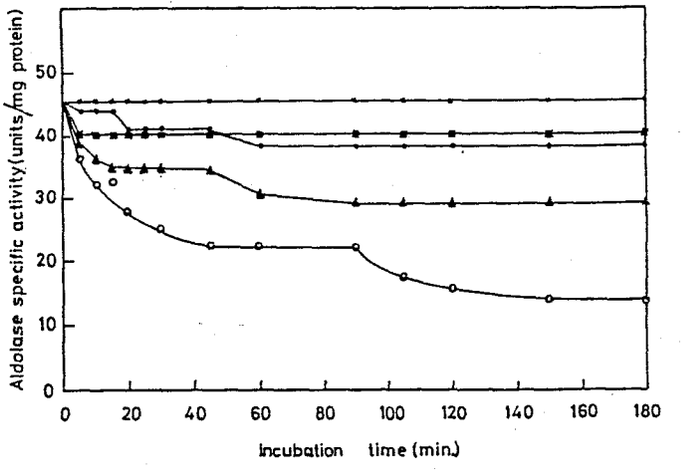


Fig. (7) : Heat inactivation Kinitics of aldolase of *S. sporocinereus.*

Reaction mixture contained : Aldolase (150 ug protein / ml), was incubated in 0.2 M Tris - HCI buffer, PH 8.5; at 45,55 and 60°C. Samples were removed at the time intervales and assayed for aldolase activity.





Figs. (8 & 9) : Effect of some compounds on the thermal stability of S. sporocinereus at 45°C and 55°C respectively.
Reaction mixture contained : as mentioned in the experiment.
(ω - ω) Control.
(x - x) FDP.
(Θ- Θ) 2 mercaptoethanol.
(Δ _ Δ) half volume of BSA.
(* - *) equal vlume of BSA.

Attemptes to protect the enzyme at 45°C and 55°C were made by the addition of fructose 1,6 diphosphate (FDP), 2-mercaptoethanol and bovine serum albumin (BSA). Figs (8,9) demonstrated that 2- mercaptoethanol and fructose 1,6 diphosphate had a complete protective effective effect on the enzyme during the whole period of incubation at 45°C. From these results we can conclude that sulfhydryl compounds like 2-mercaptoethanol can counteract the effect of high temperature on the enzyme molecule. The substrate protects the enzyme from denaturation when exposed to high temperature. On the other hand when BSA was added in half amount to the enzyme solution (on protein basis) and exposed to 45°C and 55°C for 180 minutes there was appreciable protective effect on *S. sporocinerus* aldolase. BSA caused complete protective effect of stability of the enzyme when added in equal amount to the enzyme. Then BSA as a protein protects the enzyme at these high temperatures.

REFERENCES

Amelunxen, R. E. and Lins, M. (1968). Comparative thermostability of enzymes from B. Stearothermophilus and B. Cereus. Arch. Biochem., 125 - 765.

Bang, S. S. and Baumann,P. (1978). Properties of fructose 1,6 diphosphate aldolase from pseudomonas putida. Curr. Microbiol 1,5.

Cochrane, V. W. ; peck, H. D. and Harrision, A. (1953). Metabolism of species of streptomyces VII : The hexose monophosphat shunt and associated reactions. J. Bacteriol. 66,17.

Cochrane, V. W. (1965). Reactios of Embden - Meyerhof - parnas sequence in *streptomyces coelicolar*. ibid., 69, 256.

Davis, B. (1964). Disc electrophoresis. II Method and application to human serum protein. Ann. N. Y. Aced. SCi, 121, 404.

Edward, R. L. and seed, J. R. (1970) Trypanosome aldolase. J.Biochem. 1(2), 209.

El-Meleiqy, M. A. ; Ragab, A. M. ; Gohnamy E. A. amd Badawy. E. N. (1991). Studies on the induction of aldolase and pyruvate kinase of mesophilic strptomyces sporocinereus. (in press).

Husain, M. M. and Roa, V. K. (1973). Fructose 1,6 diphosphate aldolase activity of axenically growing Hartimanella culbertson. Indian J. Microbiol., 13,37.

Hussein, A. M. ; Ragab, A. M. ; Amira A. El - Gammal; Zeinat Adeep and Nadia H. Abdel - nasser. (1979) : Taxonomy of some grey non - pigmented isolates of streptomycetes obtained from Egyptian soils. Soc. Appl. Microbiol. Ann. Meet. 217.

Ingram, J.M. and Hochster, R. M. (1967) : Purification and properties of fructose diphosphate aldolase from Fusarium oxxsporum. Can. J. Biochem., 45,929.

James, A.M. and Richard H. H. (1971) : Properties of fructose 1,6 diphosphate aldolase from 2 thermophilic and mesophilic clostridia. Biochem. Biophys. Acta. 227(1). 199.

Kowal,J; Cremona, T. and Horecker, B. L. (1966) : Fructose 1,6 diphosphate aldolase of Candida utilis, purification and properties. Arch. Biochem. Biophys. 114, 13.

Ragab, A. M.; Zeinab A. El-Awamry and Eglal A. Ghonamy (1982) : Purification and properties of Fructose diphos. Aldolase of streptomyces thermocinereus. Egypt. J. Bot., 25, No. 1 - 3,171.

Raymond, B. W. and Kenneth, W.S. (1971). Properties of aldolase from Francisella tularensis. J. Bacteiol; 105 (3), 1232.

Sadoff, H.L.; Hitchins, A. D. and Celikkol Emel. (1969): Properties of fructose 1,6 diphosphate aldolases from spores and vegetative cells of Bacillus cereus. J. Bacteriol. 98, 1208.

Shinichi, S. and yoshiaki; N. (1971). Thermal properties of fructose 1,6 diphos. aldolase from thermophilic bacteria. Biochem. Biophy. Acta, 235,(1) 210.

Siddiqui, K. A. I. And Banerjee, A. K. (1975) : Fructose 1,6 diphosphate aldolase activity of Rhizobium species. Folia Microbiol. 20, 412.

Siddiqui, K. A. I. And Banerjee, A. K. (1978) : Presence of a thermostable Fructose 1,6 diphos. aldolase in Rhizobium leguminosarum Indian J. Microbiol. 18, 158

Verma, J. P (1964). Enzyme make - up of Alternaria V. Aldolase activity. Mycologia, 56,909.

Wang, C. H.; Bialy, J. J., Klungsoyr, S. And Glimour, C. M. (1958) : Biochemistry of streptomyces III Glucose catabolism in streptomyces greseus. J. Bacteriol 75, 31.

Willard, M. James and Gibbs,M. (1968) : Purification characterization of the fructose diphosphate aldolase from Anacystis nidulans saprospira thermalis Acta. 151,438.

الملخص العربي

تنقية وصفات انزيم الالدوليز بواسطة ستربتوميسيس سبوروسينيريس

تم تنقية أنزيم الألدوليز بعد محاولات عديدة كان آخرها إضافة مادة البنتونيت والتى وجد أنها أفضل ما أستخدم حيث أدت إلى ترسيب جزء كبير من البروتينات غير النشطة الموجودة مع الأنزيم. ثم أمكن تنقيته على عمود كروماتوجرافى من السليلوز. وقد أختبرت نقاوة الانزيم على أعمدة هلامية من البولى أكريلاميد. ووجد أن أنسب الظروف لأعلى نشاط للإنزيم هو الرقم الهيدروجينى ٨ر٥ ودرجة حرارة ٤٥ درجة مئوية وثابت ميكائيل هو $X2 \cdot ^{-3}10$ مولار. وأثبتت دراسة أضافة بعض المعادن أو المواد الكيميائية على نشاط الانزيم أن بعض هذة المواد بإستثناء إيون البوتاسيوم ومادة السيستين كان لها تأثير مثبط. وعند تعريض الانزيم لدرجة حامضية وقلوية مختلفة لمدة ساعتين وجد أن للحامضية تأثير ضار على نشاط الانزيم بينما لم يكون للقلوية مثل هذا الاثر. كما وجد أنه بتعرض الانزيم لدرجتى ٤٥ ، ٥٥ درجة مئوية لمدة ساعتين لم يفقد نشاطة كلية وإنما أدى الى ضعف ملحوظ فى نشاطه ويزداد هذا الضعف بإزدياد درجات الحرارة أما بتعرضة لدرجة حرارة ٦٠ درجة مئوية فقد كان لها الاثر المباشر والسريع لفقد الانزيم نشاطه كلياً. ولقد كان لإضافة ثانى مركبتو الإيثيل ومحلول ثنائى فوسفات الفركتوز ومحلول بروتين الاليومين نتائج واضحة فى تثبيت الانزيم عند درجتى ٤٥ ، ٥٥ درجة مئوية وأمكن القول بناءً على النتائج السابقة أن انزيم الالدوليز محل الدراسة يتبع الالدوليز رقم ٢ الموجود فى الكائنات الدقيقة الأخرى .