Arab Gulf Journal of Scientific Research, 23 (2), pp 62-68 (2005)

Original Paper Ref. No 2344

Characterization and the Properties of *L-Serine-Glutamate Transaminase* in *Alternaria Chlamydospora*

وصف وخواص إنزيم ل-سيرين-جلوتامات ترانسأمينيز في فطرة الترناريا كلاميدوسبورا

سهام عبدالمحسن القضيب ومنى صالح الطامي

Siham Abdalmohsen Al Kadeeb and Mona Salah Al-Tami

Girls College of Education in Riyadh, Botany Department
P. O. Box 27104 , Riyadh 11417 ,Saudia Arabia

Abstract: Cell-free extracts of *Alternaria chlamydospora* contained only one *L-serine transaminase activity.* The enzyme was *L-serine-glutamate transaminase* (EC 2.6.1.52) which catalyzed the formation of 3-hydroxy pyruvate and glutamate from L α-serine and α-ketoglutarate. The enzyme had optimum activity at pH 8 and the optimal temperature of the enzyme was 40°C. The transamination reaction catalyzed by *L-serine-glutamate transaminase* was found to be irreversible. The enzyme was thermolabiale. Km of *Alternaria chlamydosporaenzyme* for L-serine and α-ketoglutarate were calculated and found to be 13.33 and 6.66 mm respectively. The activity of *L-glutamate transaminase* of *Alternaria chlamydospora* was stimulated by the addition of pyridoxal phosphate, whereas hydroxylamine inhibited it. The inhibition by hydroxylamine was overcome with pyridoxal phosphate.

Keywords : Alternaria chlamydospora, L-serine-glutamate, Characterization and properties.

المستخلص: أحتوت خلاصات خلايا فطرة الترناريا كلاميدوسبورا على إنزيم نقل اميني واحد للحمض الأميني ل-سيرين هو إنزيم ل-سيرين-جلوتامات ترانسأمينيز، والذي يقوم بتحفيز تكوين 3- هيدروكسي بيروفات والجلوتامات من ل-سيرين والفا-كيتوجلوتارات. بلغ أقصى نشاط للإنزيم عند الرقم الهيدروجيني 8 ودرجة حرارة 40° م ، وقد تبين أن تفاعل النقل الأميني والمحفز بإنزيم ل-سيرين - جلوتامات ترانساميناز غير قابل للانعكاس. كما برهنت النتائج على أن الإنزيم غير ثابت حرارياً. وفيما يتعلق بقيم ثابت ميكالس فقد تم حسابها ووجد إنها تساوي 33.13 مليمولار للسيرين و 66.6 مليمولار للألفا-كيتوجلوتارات على التوالي. يتم تحفيز إنزيم -سيرين - جلوتامات ترانساميناز الخاص بفطرة الترناريا كلاميدوسبرا بواسطة البيرودوكسال فوسفات، ويثبط بواسطة الهيدروكسيل أمين ويستعيد الإنزيم نشاطه بواسطة البيرودوكسال فوسفات.

كلمات مدخلية: الترناریاكلاميدوسبورا، ل-سيرين، جلوتامات، خواص، وصف.

Introduction

L-Serine participates in a number of transamination reactions in several bacteria including Pseudomonas AMI (Large and Qualye, 1963); *Escherichia coli* (Blatt *et al.*, 1966); *Hyphomicrobium methyllovorum* CM2 (Hagishita *et al.*, 1996 a&b) and *Paracoccus seriniphilus sp.* nov. (Pukall *et al.*, 2003) and fungi including *Cunninghamella elegans* and *Fusarium oxysporum* (Ragab *et al.*, 1990). Studies on the properties of L-serine transaminase are scarce in microorganisms. Thus (Large and Qualye 1963) showed that extracts of Peudomonas AMI catalyzed a transamination between L-serine and α-ketoglutarate or pyruvate but the specific activities were low. (Blatt *et al.* 1966) demonstrated in extracts of *Escherichia coli* the formation of L-serine from hydroxypyruvate by transamination with either L-glutamate, L-alanine or aspartate, but L-glutamate was the most active amino-group donor.

(Ragab *et al.* 1990) reported that cell free extracts of *Cunninghamella elegans* contained three L-serine activities; namely L-serine-glutamate, L-serine-glycine and L-serine-alanine transaminase, while those of *Fusarium oxysporum* contained L-serine-glutamate and L-serine-glycine transaminase activities. L-serine transamination reactions of *Cunninghamella elegans* and *Fusarium oxysporum* were found to be irreversible. The temperature and pH optima of L-serine transaminases of two organisms were determined. The Km values of L-

serine transaminases of *Cunninghamella elegans* and *Fusarium oxysporum* for various substrates were also estimated. Pyridoxal phosphate, as a co-enzyme had no effect on L-serine transaminases activities in both fungi.

The present work aims to investigate the ability of extracts of *Alternaria chlamydospora* to catalyze the transamination reactions of L-serine and to study the properties of L-serine-glutamate transaminase in *Alternaria chlamydospora*. Such studies have not been reported previously with this filamentous fungi.

Material and Methods

(I) Organism

Alternaria chlamydospora was isolated from the Al-Qassem region and identified by the International Mycological Institute, England, United Kingdom.

(II) Media and culture

The organism was grown on sucrose - Czapek - Dox liquid medium with L-serine replacing $NaNO_3$ on a nitrogen equivalent basis. Five ml aliquots of spore suspension of *Alternaria chlamydospora* was used to inoculate 250 ml Erlenmeyer flasks, each containing 50 ml of sterile medium. The inoculated flasks were incubated at 28°C for 3 days, then the mycelia were harvested by filtration, washed thoroughly with distilled water, and finally blotted dry with absorbent paper. The pH of the medium was adjusted to 5.0.

(III) Preparation of cell - free extract

The harvested mycelia were ground with cold sand in a cold mortar and extracted with cold distilled water. The obtained slurry was then centrifuged at 12.000 xgm for 10 min and the supernatant was used as the crude enzyme preparation.

(IV) Separation and quantitation of L-serine and glutamate

Identified and determined the amounts of L-serine or glutamate were identified and determined by quantitative paper chromatography, using Whatman No.1 filter paper and two solvent systems. Solvent I consisted of n-butanol-aceton-acetic acid-water (70 : 70 : 20 : 40) (Erasser and Smith, 1976) and solvent II was water - saturated phenol (Kay *et al.*, 1956). The chromatogram was continuously sprayed with the solvent for 16 hours, then left to dry at room temperature for 24 hours Drying was completed in an oven at 65°C. The dried chromatogram was then sprayed with 0.2% ninhydrin in acetone and heated at 65°C for 20 min. The colored spots of the two amino acids were located (in comparsion with standard samples), cut from the chromatogam and each was placed in tube. To each tube 5 ml of 71% ethanol was added, and the tubes were shaken for 5 min. The resulting colored solution was measured spectrophotometrically at 575 nm. A blank was prepared by cutting from the chromatogam a white of paper of the same size as the colored spots from the chromatogram, this piece then treated as described before. The concentration of glutamate was determined in a reaction mixture using a standard curve, which was prepeared using the same method.

The R_f values of identified spots were identical to those of the standard samples. The keto acids, hydroxypyruvate and α-ketoglutarate were identified by paper chromatography of their 2, 4-dinitro-phenylhydrazine derivatives (Friedemann and Haugen, 1943), using whatman No.1 filter paper and two solvent systems. Solvent I consisted of n - butanol - ethanol - water (40 : 10 : 20) (Germano and Anderson, 1968) and solvent II consisted of n - butanol - ethanol - 0.5 N NH4OH (70:10:20) (Seakins *et al.*, 1976) . The identification spots had the same R_f values as standard samples.

Protein was determined according to the method of Lowry *et al.*(1951) .

(IV) Assay of transaminase activity

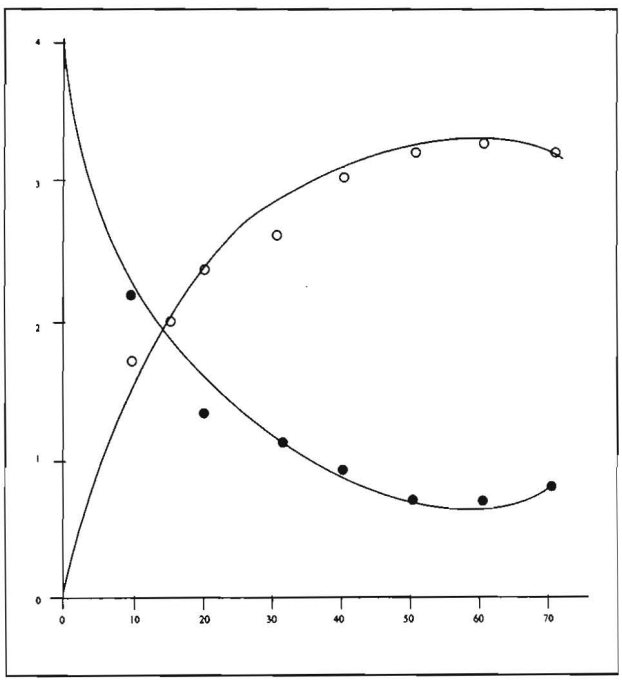
L-serine-glutamate transaminase activity was assayed by following the formation of glutamate when α-ketoglutarate and L-serine were incubated with cell-free extracts. The reverse reaction of L-serine-glutamate transaminase enzyme was assayed by following L-serine formation from hydroxy-pyruvate and glutamate. Unless otherwise specified, the standard reaction of the transaminase contained in a total volume of one ml: amino donor (L-serine 5μmoles); amino acceptor α-ketoglutarate, 5 μmoles; Tris-HCl buffer at pH 8, 80 μmoles and the appropriate amount of enzyme. All the reactions were initiated by the addition of substrates and stopped by boiling for 2 mins in a water bath. One unit of enzyme activity is defined as the amount of protein which catalyzes the formation of one μmole glutamate in 60 mins at 40°C.

All data was statistically analyzed using Person coefficient (Marija , J. and Norusis / spss Inc., 1990)

Results

(1) Rate of Glutamate Formation from α-Ketoglutarate and L-serine by Extracts of *Al. chlamydospora.*

Figure 1 demonstrates the formation of L-glutamate when extracts were incubated with L-serine and α-ketoglutarate. It shows that an increase in the amount of L-glutamate was accompanied by a decrease in the added amount of L-serine. The equilibrium of the reaction was reached after 15 mins, at which point the amount of L-serine that had disappeared was almost equivalent to that of the formed glutamate.



(Reaction mixture contained in (total volume 1ml): 3.8 μmoles L-serine , 5 μmoles α-ketoglutarate, 5 μmoles P-5-P , 80 μmoles Tris - HCl buffer at pH 8 and 2.8 mg crude extract. The reaction mixture was incubate at 40°C for time as indicated.)

○ *Glutamate* ● *Serine*		
Variables coefficient	*Pearson different*	*Significant*
Pearson coefficient	*0.916*	*0.004*
Time, Serine Conc.	*-0.844*	*0.017*

Fig. 1: Rate of glutamate formation from α-ketoglutarate and L- serine by extracts of Al chlamydospora.

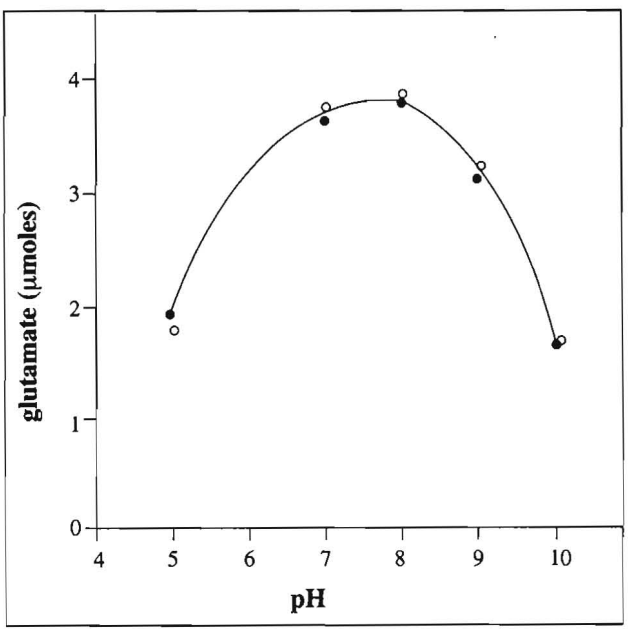
From the result cited in the accompanying table, a significant variation was noticed (0.004 and 0.017) between time, α-ketoglutarate concentration and between time, serine concentration respectively.

(2) Demonstration of the reverse reaction of L-serineglutamate transaminase of Al-chlamydospora.

The reverse reaction of L-serine-glutamate transaminase was demonstrated by determining the amount of L-serine formed when the extracts were incubated in a total volume of 1 ml, with 5 μmoles hydroxypyruvate; 5 μmoles glutamate and 80μmoles of Tris-HCl buffer pH 8 at 40°C for 1hr. Results obtained showed that no serine formation could be detected in reaction mixtures containing extracts of *Al. chlamydospora.*

(3) Effect of pH on L-Serine-Glutamate Transaminase Activity of Al. chlamydospora

Figure 2 demonstrates that the optimal pH for the formation of L-glutamate from L-serine and α-ketoglutarate catalyzed by L-serine-glutamate transaminase was pH8. At this pH optimum enzyme activity was not affected by the nature of the buffer, since identical activities were obtained in Tris-HCl and NaOH-Glycine buffer.



Reaction mixture contained in (total volume 1ml): 4 μmoles L-serine, 5 μmoles - α-ketoglutarate, 5 μmoles P-5-P, 80 μmoles buffer pH as indicated and 2.18 mg crude extracts. The reaction mixture was incubate at 40°C for 60 min.

● *Glycine – NaOH buffer* ○ *Tris - HCl buffer*		
Variables	*Pearson coefficient*	*Significant different*
Tris-HCl buffer,Glutamate	*0.023*	*0.970*
Glycine-NaOH buffer, Glutamate	*0.027*	*0.965*

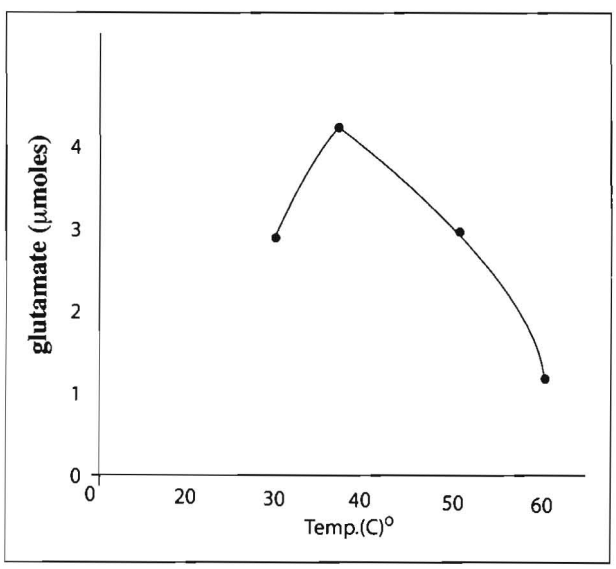
Fig. 2: Effect of pH on L-serine-glutamate transaminase activity of Al. chlamydospora.

From the result cited in accompanying table, no significant variation was noticed (0.970 and 0.965) between the Tris-HCl buffer and glutamate formation, and between the Glycine-NaOH buffer and glutamate formation respectively.

(4) Relationship Between Temperature and L-Serine-Glutamate Transaminase Activity of *Al-chlamydospora*

As shown in Figure 3, maximal enzyme activity for L-glutamate formation from L-serine and α-ketoglutarate occurs at 40°C.

Results shown in Figure 3 indicate the ratio of the activity for L-glutamate formation at 60°C to that at 40°C (optimum) is 29.5 %.



Reaction mixture contained in (total volume 1ml): 5 μmoles L-serine , 5 μmoles α-ketoglutarate ,5 μmoles P-5-P , 80 μmoles Tris - HCl buffer at pH 8, and 2.8 mg crude extract. The reaction mixture was incubate in temperature as indicated for 60 min.

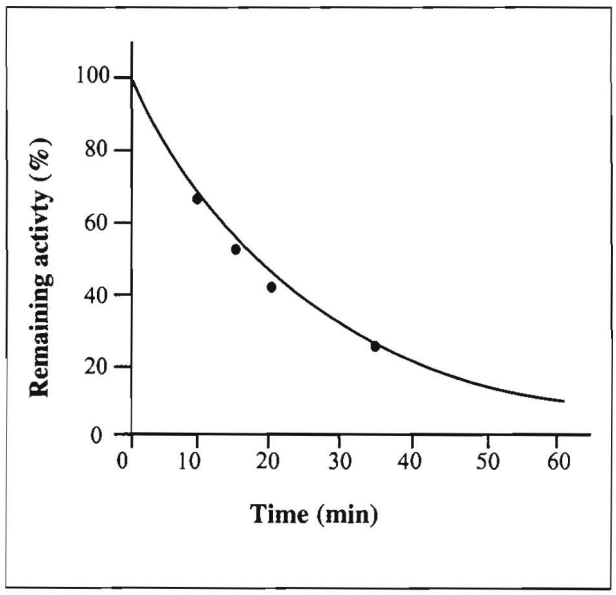
Significant different	*Pearson coefficient*	*Variables*
Temperature, Glutamate	*-0.669*	*0.331*

Fig. 3: Relationship between temperature and L-serine - glutamate transaminase activity of Al-chlamydospora.

From the result cited in the accompanying table, no significant variation was noticed (0.331) between Temperature and Glutamate formation.

(5) Thermal Stability of L-Serine-Glutamate Transaminase of Al-chlamydospora.

Activities of L-serine-glutamate transaminase was studied as a function of incubating the extracts at 45°C for different time intervals in the presence of a Tris-HCl buffer. It is clear from Figure 4 that the activity of L-serine-glutamate transaminase decreased gradually over time during incubation at 45°C. The data shows that about 15.35 % and 82.02 % of the enzyme activity was lost after 5 and 50 min incubation.



28 mg crude extract was indicated at 45° C with an equal volume of 0.2 M Tris - HCl buffer pH 8 samples were withdrawn at different time intervals and assayed for L-serine-glutamate transaminase activity.

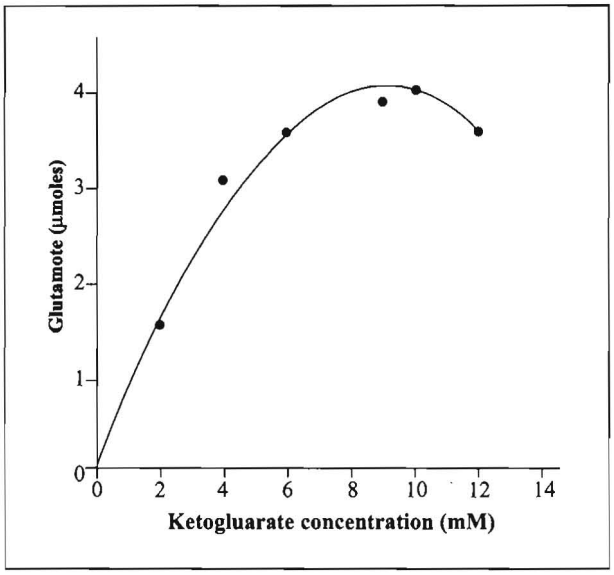
Significant different	*Pearson coefficient*	*Variables*
Time, Remaining activity %	*-0.939*	*0.00*

Fig. 4: Thermal stability of L-serine-glutamate transaminase of Al-chlamydospora.

From the result cited in accompanying table, significant variation was noticed (0.00) between Time and Remaining activity %.

(5) Michaelis constant

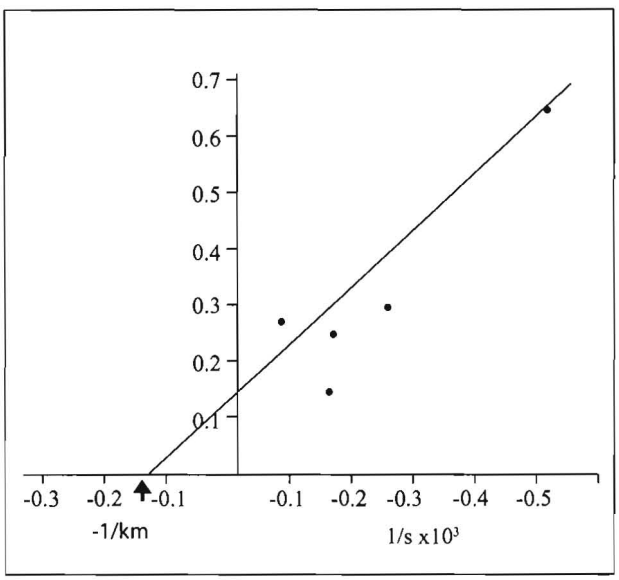
It is of interest to know the relative affinity of an enzyme for its respective substrates. This was achieved by determining the K_m values of this enzyme with each substrate. The apparent Km values of L-serine-glutamate transaminase for L-serine and α-ketoglutarate were determined from Linweaver-Burk plots of the reciprocation of initial velocities and substrate concentrations, and were found to be 13.33 and 6.66 mM respectively. These results are shown in Fig. (5a & 5b and 6a & 6b).



(Reaction mixture contained in (total volume 1ml): 8 µmoles L-serine, α- ketoglutarate as indicated, 5 µmoles P-5-P, 80 µmoles Tris - HCl buffer at pH 8 and 1.89 mg crude extract. The reaction mixture was incubate at 40°C for 60 min.
α - Ketoglutarate con., Glutamate)

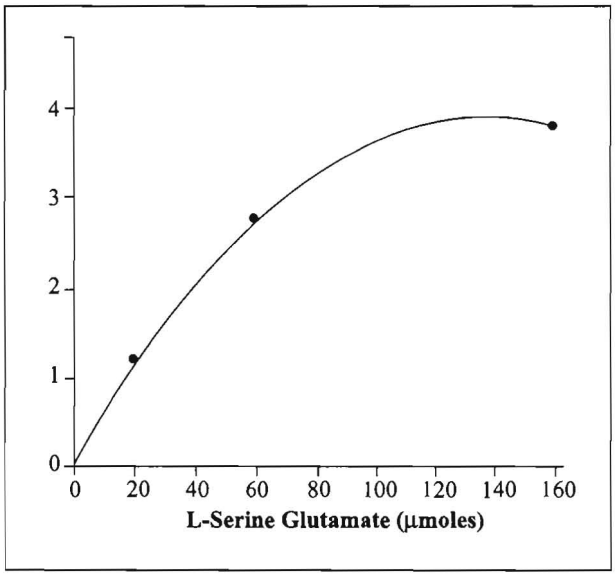
Significant different	*Pearson coefficient*	*Variables*
α - Ketoglutarate con., Glutamate	*0.753*	*0.084*

Fig. 5a: Dependence of L-serine-glutamate transaminase activity of Al-chlamydospora on α-ketoglutarate concentration



Significant different	*Pearson coefficient*	*Variables*
I/S, 1/V	*0.953*	*0.003*

Fig.5b: Lineweaver-Burk plot of the reciprocals of initial velocities and α-ketoglutarate concentrations.



Reaction mixture contained in (total volume 1ml): L-serine as indicated, 10 5 µmoles α- ketoglutarate ,5 µmoles P-5-P, 80 µmoles Tris - HCl buffer at pH 8. and 1.89mg crude extract. The reaction mixture was incubate at 40°C for 60 min.

Significant different	*Pearson coefficient*	*Variables*
L-Serine con., Glutamate	*0.940*	*0.005*

***Fig.6a:** Dependence of L-serine–glutamate transaminase activity of Al. chlamydospora on L- serine concentration*

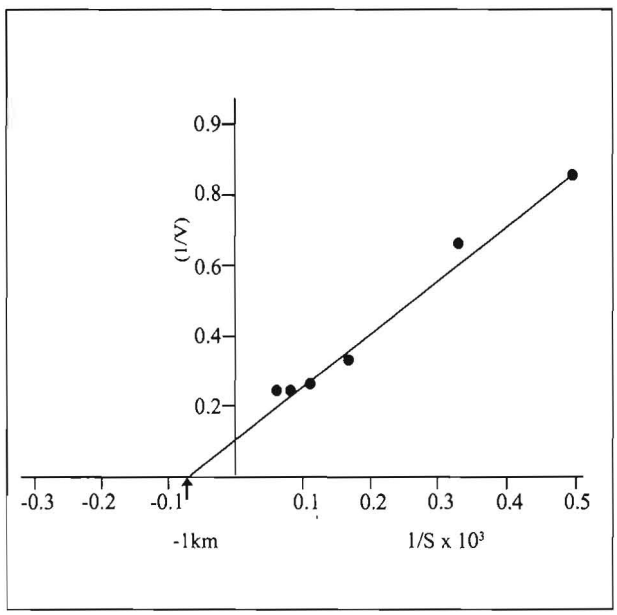


Fig.6b: Lineweaver- Burk plot of the reciprocals of initial velocities and L- serine concentration.

Significant different	*Pearson coefficient*	*Variables*
I/S, 1/V	0.953	0.003

From the results cited in the accompanying table, significant variation was noticed (0.05) between L-serine concentrations and glutamate formation, while no significant variation was noticed (0.084) between α-ketoglutarate concentrations and glutamate formation.

(6) Effect of Pyridoxal Phosphate and Hydroxylamine on L-Serine-Glutamate Transaminases Activity of *Al-chlamydospora*

Table 1 shows that the addition of pyridoxal phosphate at a concentration of 0.1 and 0.5 mM to the reaction mixture containing Lserine and α-ketoglutarate resulted in a stimulating effect. This data indicates L-serine-glutamate transaminase of Al-chlamydospora requires pyridoxal phosphate as an acoenzyme. The Addition of hydroxylamine at concentration of 0.1 and concentration of 0.5 mM

Table (1): Effect of Pyridoxal Phosphate and Hydroxylamine on L-Serine-Glatamate Transaminases Activity of *Al-chlamydospora.*

Additions	**Relative Activity (%)**
Control	100.00
Pyriodoxal phosphate (0.1mM)	114.02
Pyriodoxal phosphate (0.5mM)	100.78
Hydroxylamine(0.1 mM)	60.83
Hydroxylamine(0.5 mM)	58.73
Pyriodoxal phosphate (0.1mM)	
and Hydroxylamine (0.1 mM)	93.25

(Reaction mixture contained :(total volume 1ml) 4 μmoles L-serine, 5 μmoles, α-ketoglutarate, 80 μmoles, Tris-HCl buffer at pH 8 , 1.2 mg extract. The Reaction mixture was incubate at 40°C for 60 min.)

caused inhibition in activity of L-serine-glutamate transaminase. The inhibitory effect of hydroxylamine on the enzyme was overcome with pyridoxal phosphate. Hydroxylamine had no effect on the enzymatic activity of L-serine-glutamate transaminase.

Discussion

The present work aims to investigate the ability of extracts of *Alternaria chlamydospora* to catalyze the transamination reactions of L-serine. The obtained result revealed that *Al. chlamydospora* extracts catalyzed transamination between L-serine and α-ketoglutarate to equimolar amounts of 3-hydroxy pyruvate and glutamate, and demonstrated that the equilibrium of the reaction was reached after 15min. At this point, the amount of L-serine that had disappeared was almost equivalent to that of the formed glutamate. The transamination reaction catalyzed by L-serine-glutamate transaminase was found to be irreversible. These results were in close agreement with those reported for L-serine -glutamate transaminase of *C. elegans* and F.oxysporum (Ragab *et al.*, 1991).

On the other hand, the reaction catalyzed by L - serine-glutamate transaminase of Escherichia coli (Blatt *et al.*, 1966) was reported to be reversible. Optimum activity of L-serine-glutamate transaminase of *Al. chlamydospora* was obtained at pH 8.These results were in close agreement with those reported for L-serine-glutamate transaminase of *C. elegans* and *F.oxysporum* (Ragab *et al.*, 1991). Maximal activity of L-serine - glutamate transaminase occurred at 40°C. Similar results were demonstrated for L-serine-glutamate transaminase of *C. elegans* and F.oxysporum (Ragab *et al.*, 1991). Results have shown that the ratio of the activity for L-glutamate formation at 60 °C to that at 40°C (optimum) is 29.5 %. This data indicated the relative sensitivity of the enzyme to higher degrees C. This data also suggests that the enzyme was thermolabile. Exposure of L-serine-glutamate transaminase to 45oC for 5 and 50 min resulted in about 15.35 % and 82.02 % loss of the enzyme activity respectively. These results, along with the preceding ones, indicate that L-serine-glutamate transaminase of Al. chlamydospora was thermolabile. Km values (Michelis constant) of L-serine-glutamate transaminase for L-serine and α-ketoglutarate were calculated and found to be 13.33 and 6.66 respectively. The Km values demonstrated that L-serine-glutamate transaminase has a greater affinity for α-ketoglutarate than L-serine. It was shown that L-serine-glutamate transaminase of Al-chlamydospora requires pyridoxal phosphate as an acoenzyme. While pyridoxal phosphate did not stimulate the activity of L-serine-glutamate transaminase of C. elegans and F.oxysporum (Ragab *et al.*, 1991).

References

Blatt, L., Dorer, F.E. and Sallach, H. J. (1966). Occurrence of hydroxypytuvate-L-glutamate transaminase in Escherichia coli and its separation from hydroxypytuvate-phosphate-L-glutamate transaminase. J. Bacteriol. **92**: 668.

Erasser, R .S. and Smith , I. (1976). Amino acids and related compounds, ***In:*** **(I. Smith and J.W. T Seakins, (eds).** Chromatographic and Elctro-phoretic Techniques". Vol. I, William Medical Books, London. p.75

Friedemann, T.E. and Haugen, G.E. (1943). The determination of keto acids in blood and b urine. J. Biol. Chem., **147**: 415-442.

Hagishita, T.; Yoshida, T.; Izumi, Y. and Mitsunaga (1996a): Cloning and expression of the gene for serine-glyoxlate aminotransferase from an obligate methylotrophic Hyphomicr-obium methyllovorum CM2. Eur. Biochm. **241** (1): 1-5.

Hagishita, T.; Yoshida, T.; Izumi, Y. and Mitsunaga (1996b): Immunological characterization of serine-glyoxlate aminotransferase from a methylotrophic Hyphomicrobium methyllovorum CM2. Fems, Microbiol., Lett. **142**(1): 49-52.

Kay, R.E . ; Harris , D.C. and Entenmann , C. (1956). Quantification of aninhydrin color reaction as applied to paper chromatography. Arch. Biochem. Biophys., **63** 14 -25.

Large, P.J. and Quayly, J. R. (1963) Microbiol on C1 compounds. 5. Enzyme activities in extracts of Psedomonas AMI. Biochem. J. **87**: 386.

Lowery, O.H.; Rosebrough, N.J.; Furr, A.L. and Randell; R.J.(1951): Protein measurment with folin phenol reagent. J. Biol. Chem., **193**: 265-275.

Marija, J. and Norusis/spss Inc. (1990); Spss/pc+ statisticsTM 4.0 for the IBMPC/X T T/ AT and ps/2.444 N. Linois, Chicago,USA..

Pukall, R.; Laroche, M. Kroppenstedt, R. M.; Schummann, P.; Stackebrandt, E. and Ulber, R. (2003): Paracoccus seriniphilus sp. Nov. an L- serine dehydratase producing ccocus isolated from the marine bryozoan Bugula plumos. Int., J., Syst. Evol. Microbiol. **53** (2):443-447.

Ragab, A. M.; Ghonamy, E. A.; El-Melligy, M. A. and Ahmed, S.A. (1991). L-Serine transaminases in Cunninghamella elegans and *Fusarium oxysporum* extracts. Egypt. J. Microbiol., **26** (2): 239-256.

Seakins, J.W. T.: Smith, I. and Smith, M.J. (1976). Keto acids, ***In: I. Smithand J. W.T. Seakins, (Eds).*** chromatographic and electrophoretic techniques. Vol. 1, William Heinemann Medical Books, London. p 244.

Ref: 2344
Received: 30/07/2004
In revised form: 12/05/2005