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Study of *E. coli* JM109 glutamate decarboxylase: isolation and partial purification

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Abstract: Glutamate decarboxylase (GAD) was isolated from a cell-free extract of *E. coli* JM109.and partially purified by applying crude intracellular extract on Sephadex G-100. The enzyme was eluted in the first peak of the column and it was purified 27%. First peak showed highest GABA production activity on TLC plates. Absorption spectrum showed 0.33mg/ml of protein.

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INTRODUCTION

Glutamate decarboxylase (GAD), EC 4.1.1.15 is a pyridoxal enzyme. It catalyzes the removal of the carboxyl group of L-glutamic acid adjacent to the α-amino group to produce γ-aminobutyric acid and carbon dioxide:

$$HOCO-CH_2CH_2CH(NH_2)CO_2H \longrightarrow HOCO-CH_2CH_2CH_2NH_2 + CO_2$$

GAD of *E. coli* exists as a hexamer of approximately 50 kDa identical subunits, each containing one molecule of pyridoxal phosphate (PLP). *E. coli* GAD is reported to maintain cellular pH under acidic conditions in the intestines. It allows *E. coli* to survive gastric acidity and volatile fatty acids produced during fermentation in the intestines[1].

In humans and higher mammals, the enzyme is known to play important physiological roles in the nervous system by balancing excitation and inhibition of neurons. It also acts as an autoantigen in insulin-dependent diabetes[1]. GAD has been isolated from different sources like *Lactobacillus* and *Streptococcus pneumonia*[2, 3]. It has got diverse applications like detection of *E. coli* in food and water samples[4] and in determination of glutamate and aspartic acid in protein samples[5].

MATERIALS AND METHODS

All the materials and reagents used in this study were of reagent grade. Culture media was purchased from Oxoid and Co.

Bacterial culture

The bacterial isolate used in this study was *E. coli* JM109 was provided by the Department of Biotechnology, University of Karachi. Culture was maintained on Luria Bertani agar slants and also stored in glycerol at -70ºC.

Culture cultivation

Culture was grown in 1 Liter media containing Glucose (1.0%), Sodium glutamate (1.0%) Yeast extract (0.2%), Ammonium sulfate (0.25%), Di potassium hydrogen phosphate (0.5%) in de-ionized water. Medium was sterilized by autoclaving at 121ºC for 15 min. at 15psi. After sterilization medium was inoculated with 5% of starter culture and incubated for 20 hrs with agitation at 37ºC.

Harvesting of cells

After cultivation, the cells were harvested by centrifugation at 5000rpm for 15 min at 4ºC. The cell pellets was stored at -20ºC until further treatment.

Extraction of enzyme from cells

Release of intracellular enzyme from bacterial cells was achieved by bead lysis method. Glass beads were activated by immersing in concentrated solution of HCl. Beads were then thoroughly washed with distilled water before used. Cells were suspended in 10ml of buffer A (20mM sodium phosphate buffer pH 7.0 containing 0.1mM pyridoxal-5-phosphate, 0.1mM 2-mercaptoethanol, 0.2% Brij35) with 10gm of activated beads. Cells were vortexed for 3.0 min. and kept on ice bath for 3.0 min. The cycle was repeated 10 times and total procedure takes 30 min. Sample was then centrifuged at 5000rpm for 15 min. The supernatant was again centrifuged at 16,000rpm for 30 min. at 4ºC. The clear supernatant was used for further studies.

Total protein estimation

Total protein was estimated from supernatant by the method described by Bradford[6].

Purification of enzyme using gel permeation chromatography

The sample was then subjected to sephadex G-100 for partial purification. Glass column (27x 510mm, BioRad) was packed with pre-swollen Sephadex G-100 gel and equilibrated with Buffer A containing 0.15M NaCl and 5mM EDTA. 5% v/v sample was subjected to the column with flow rate of 12ml/hr. The elution of protein was monitored by taking absorbance at 280nm.

Electrophoresis

SDS-PAGE and Native gel electrophoresis was done by the method described by Laemmli[7].

Enzyme assay

Enzyme solution (0.1ml) was mixed with 0.1ml of 4M ammonium sulfate and 1.3ml of substrate solution (0.2M pyridine-HCI, pH 4.6, containing 0.4M NaCl and 20mM sodium glutamate) and incubated at 37°C for 60 min. After the incubation, the reaction was stopped in boiling water for 5 min. Then the produced GAD was analyzed by TLC (Kiesel gel F254 (Merck). The solvent system was used n-BuOH-AcOH-HzO (3: 2: I, v/v)[8].

Absorption spectrum

The active fractions from G-100 column were analysed by taking absorption spectrum in the range of 200-600nm.

RESULTS AND DISCUSSION

Maintenance and cultivation of culture

The pure culture was maintained successfully on agar plate and slant (Figure 1). The culture was cultivated at 37°C for 20hrs.with continuous shaking.

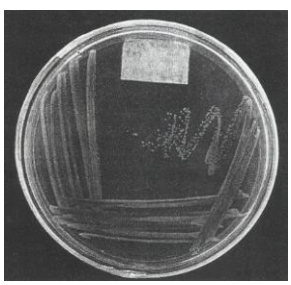

Figure 1: isolated colonies of the pure culture *E.coli* JM109 on solid agar plate.

Bead lysis

Cells were harvested by centrifugation and approximately 3.5g wet cell mass was collected from 1 liter of culture. Intracellular protein was extracted by bead lysis method and protein concentration was 1.1mg/ml in crude cell extract.

Sephadex G-100 column chromatography

The crude cell extract was applied to Sephadex G-100 column. The elution profile of G-100 at 280nm is shown in figure 2.

Enzyme assay

The enzyme activity was analyzed in crude ell extracts as well as in different fractions of G-100 column using TLC. The activity was observed in peak no. 1 of the column and peak no 2 did not show any activity. The results of enzyme assay are depicted in figure 3.

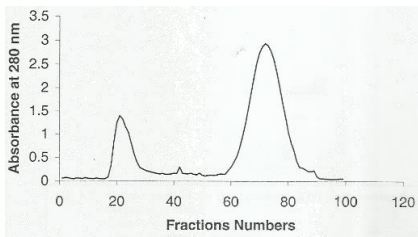

Figure 2: Elution profile of GAD on sephadex G-100, eluted with buffer A containing 0.15M NaCl and EDTA at a flow rate of 12ml/hr at 280nm.

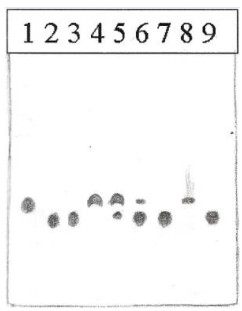

Figure 3: Enzyme assay of GAD produced. TLC plate showing the formation of GABA; Lane 1: Standard GABA; Lane 2: Standard Glutamic Acid; Lane 3: Standard GAD; Lane 4: Crude extract; Lane 5: 1st peak (top part) after sephadex chromatography; Lane 6: 1st peak (trailing end) after sephadex chromatography; Lane 7: Sample before the elution of 1st peak; Lane 8: Sample after the elution of 1st peak; Lane 9: Standard glutamic acid.

Electrophoresis

The protein profile of the crude extract from Native PAGE and SDS-PAGE is depicted in figure 4 and 5 respectively.

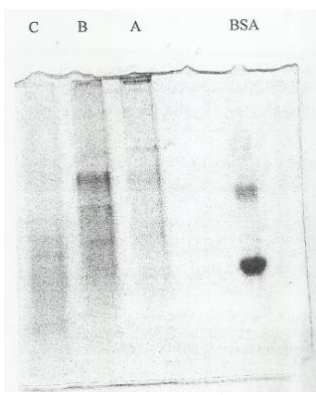

Figure 4: Native electrophoresis of pooled fractions after sephadex G-100 chromatography. BSA: bovine serum albumin (67KDa); A: peak 1 fraction no. 18-24; B: peak 1 fraction no. 25-34; C: fraction no. 35-50.

Absorption spectrum

The Absorption spectrum of Active fractions is presented in figure 5. Recently, many researchers have reported great advances in the field like immobilization of GAD has been done and

expression of enzyme has been studied for improved production of enzyme[9,10].

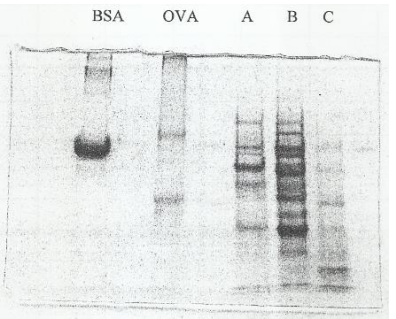

Figure 5: SDS PAGE of the pooled fractions after sephadex G-100 chromatography. BSA (64KDa); OVA (45KDa); A: fraction no. 18-24; B: fraction no. 25-34; C: fraction no. 35-50.

CONCLUSION

In this study GAD from *E. coli* JM109 was successfully isolated and preliminary studies were done. In future, further investigation is required to achieve an economically feasible production of enzyme for industrial and biotechnological applications.

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