Volume : 2 | Issue : 4 | Apr 2013 • ISSN No 2277 - 8179

Research Paper

Molecular Weight Determination of Glutaminase Enzyme Produced from Erwinia

Biotechnology

KEYWORDS : Glutaminase Enzyme, SDS-PAGE,Molecular Weight Determination

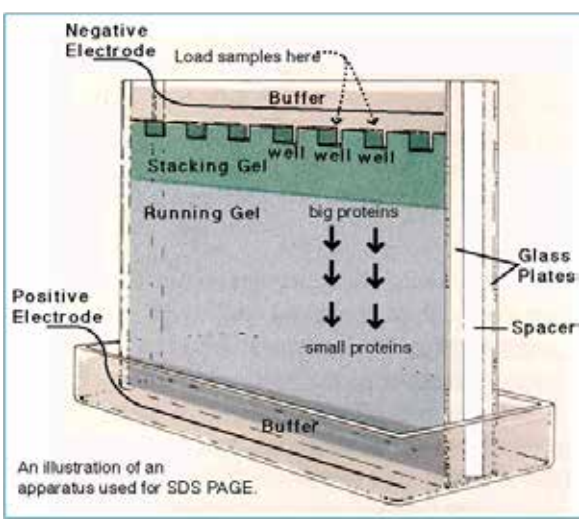
Pushpinder Paul Department of Biotechnology, M.G.C. Fatehgarh Sahib,Punjab

ABSTRACT

The purpose of this study was to determine molecular weight of glutaminase enzyme by SDS-PAGE.Glutaminase enzyme was produced from Erwinia under optimized condition. After production of glutaminase enzyme it was isolated by acetone precipitation & ammonium sulphate precipitation method. After isolation purification of enzyme was done by Gel Filtration chromatography and Ion Exchange chromatography. Then SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) was performed to determine the molecular weight and the purity of the sample. It was observed after SDS-PAGE, that the number of bands after acetone extraction were less as compared to number of bands observed for protein crude sample before acetone extraction. Also the number of bands observed after ammonium sulphate fractionation are less. So the ammonium sulphate fractions was subjected in Gel Filtration which showed further purification. The Ion exchange chromatography showed more diffusion and less recovery of protein sample as compared to Gel Filtration Chromatography. This was confirmed by the achievement of one band during SDS-PAGE which confirmed the purity of enzyme. From the results of SDS-PAGE ,it was clear that molecular weight of Glutaminase isolated from Erwinia species came to be 145 Kda.

MATERIAL & METHODS:

SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) was performed to determine the molecular weight and the purity of the sample.SDS was performed by following the procedure given by Laemmli, (1970) in 10% to check the molecular weight and purity of enzyme.The protein staining was done by using Commasive blue.



An illustration of an apparatus used for SDS PAGE.

REAGENTS:

Separating (4x) gel buffer

Tris-HCl (18.3g) was dissolved in 100 ml of distilled water and pH was adjusted 8.8 with 1M HCl.

Stacking (4x) gel buffer

Tris-HCl (6.055g) was dissolved in 100 ml of distilled water and pH was adjusted 6.8 with 1M HCl.

Bisacrylamide (30%)

29.2g acryl amide was mixed with 0.8g of bis-acrylamide and mixture was dissolved in total 100 ml of distilled water.

Sample buffer:

For the preparation of sample buffer, following components were mixed:

Tris-HCI (pH 6.8) buffer	:	0.4 ml
SDS (10%)	:	2.5%
2-mercaptoethanol	:	0.4ml
Glycerol	:	2.0 ml
Bromophenol blue	:	0.002 gm
Distilled water	:	4.7ml

Electrode buffer

Tris HCL	:	6.05 g
Glycine	:	28.8 gm
SDS	:	2.0 gm
Distilled water	:	2.0 L
pH	:	8.3

Separating gels

Distilled water	:	19.5 ml
Bisacrylamide (30%)	:	10 ml
4x separating gel buffer	:	10 ml
SDS (10%)	:	0.8 ml
Glycerol (10%)	:	0.35 ml
TEMED	:	20 µl
APS (2%)	:	0.6 ml

Immediately, whole mixture was poured in a vertical mould. Then saturated butanol was added and gel was allowed to polymerize. After half an hour, butanol was removed and upper portion of gel was washed with deionized water.

Stacking gel

Distilled water	:	6.3 ml
Bisacrylamide (30%)	:	2.0 ml
4x separating gel buffer	:	2.5 ml
SDS (10%)	:	0.2 ml
Glycerol (10%)	:	0.15 ml
TEMED	:	10 µl
APS (2%)	:	0.13 ml

This mixture was poured in vertical moulds of plates on the separating gel. Placed the comb in it and gel was allowed to settle for 30 minutes.

After the polymerization of stacking gel, the comb was removed. The samples were prepared by heating in boiling water bath for 2-3 minutes. After cooling, 40µl of sample was loaded in sample wells with the help of auto pipette. Electrophoresis was carried out at 50V up til dye front reached into the separating gel and then voltage was increased to 100V. After run completed, the gel was taken out and washed with water. Then, with commassive blue, protein staining was carried out.

Staining solution

Water	:	90 ml
Methanol	:	90 ml
Acetic acid	:	10 ml
Commassive Blue dye	:	0.25g

Destaining solution

Water	:	90 ml
Methanol	:	90ml
Acetic acid	:	10 ml

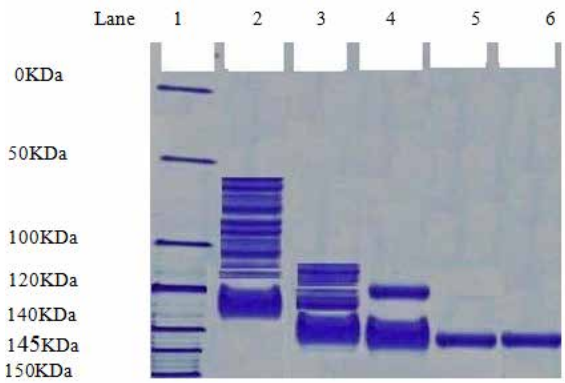
Volume : 2 | Issue : 4 | Apr 2013 • ISSN No 2277 - 8179

Procedure:
Placed the gel in 100 ml of staining solution for 4 to 5 hours. Then, placed the gel in the destaining solution overnightly.

RESULT

4.4 SDS-PAGE (Sodium dodecyl sulphate polyacryamide gel electrophoresis)

It was observed after SDS-PAGE, that the number of bands after acetone extraction were less as compared to number of bands (4) observed for protein crude sample 2, before acetone extraction. Also the number of bands(2) observed after ammonium sulphate fractionation are less. So the ammonium sulphate fractions was subjected in Gel Filtration which showed further purification. This was confirmed by the achievement of one band during SDS-PAGE which confirmed the purity of enzyme.



SDS-PAGE

Lane 1 - Protein marker of 150 KDa.
Lane 2 - Crude sample
Lane 3 - Purified sample after Acetone Extraction.
Lane 4 -. Purified sample after Ammonium ion Extraction.
Lane 5and 6 - Purified samples by Gel Filtration Chromatography.

CONCLUSION:

The Ion exchange chromatography showed more diffusion and less recovery of protein sample as compared to Gel Filtration Chromatography. So, Gel Filtration Chromatography was found to be better technique for purification(Hartman and Stochaj., 1973; Lu et al., 1996). From the results of SDS, it was clear that molecular weight of Glutaminase isolated from Erwinia species came to be 145 KDa. Various researchers have done Gel Filtration Chromatography and found the molecular weight of glutaminase enzyme(Tate and Meister, 1975).

REFERENCE

• Shimazu, Y., Ueyama, A., and Goto, K.(1991). Purification and characterization of glutaminase from Bacillus subtilis GT strain. J .Brew. SOC Jpn. Vol. 86:pp441-446. • Ramadan ,M. A., Asmar, F E, and Greenberg ,D. M.(1964). Purification and properties of glutaminase and asparaginase from a pseudomonad. J. Arch Biochem Biophys. vol. 108: pp 143-149. • Palekar, A. A.; Vasudevan, P. T.; Yan, S. (2000).Purification of lipase: a review. Biocatalysis Biotransformation, [S.l.], vol. 18: pp. 177-200. • Hartman, S. C. (1968). Glutaminase of Escherichia coli. Purification and general catalytic properties. J. Biol. Chem. vol.243. • Allen,R.C., Saravis,C.A. and Maurer,H.R.(1984). Gel Electrophoresis and isoelectric Focusing of proteins. pp255. • Andrews,P. (1965). The Gel–filtration Behavior of proteins related to their molecular weights over a wide range. Biochem.J.vol.96:pp595-606.