

Characterization of Purified Glutathione S-Transferase (GSTs) from *Fasciola hepatica* and Liver Tissue by Two-Dimensional Electrophoresis (2-DE)

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Abstract

Two-dimensional electrophoresis (2-D electrophoresis) is a powerful and extensively used method for analysis of complex protein mixtures extracted from cells, tissue, or other biological samples such as helminth parasites including, *F. hepatica*. Each spot on the resulting two-dimensional collection corresponds to a single protein species in the sample. This study was carried out to detect of GSTs isoenzyme spots map for collection of highly specific proteins. For this purpose, GSTs were purified from adult parasite of *F. hepatica* and sheep liver tissue as an enzyme pool by a glutathione affinity matrix using a wash-bath method and investigated for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) pattern. For 2-DE, purified GSTs from *F. hepatica* and sheep liver tissue were resuspended in sample buffer and then run on a IPG strip in the first dimension and then on an Excel Gel SDS in the second dimension before protein spots staining with Coomassie blue. The obtaining spots in the gels were compared and GSTs protein spots were detected with similar molecular weight, 26 kDa. The protein spots which are recorded in this paper could be GSTs isoenzymes and are highly specific peptides. These findings may be considered for vaccination or chemotherapeutic targets in sheep and human fascioliasis.

Keywords: Double dimension electrophoresis (2-DE), Glutathione S- Transferases (GSTs), *Fasciola hepatica*, Iran

Introduction

Two-dimensional electrophoresis (2- D electrophoresis) method is used for analysis of complex protein mixtures (proteomic) extracted from biological samples. This technique sorts protein according to independent properties in two discrete steps: the first-dimension step, isoelectric focusing (IEF), separates protein according to their isoelectric points (pI). In the second-dimension step, SDS-poly acrylamide gel electrophoresis (SDS-PAGE) separates protein according to their molecular weight (MW). Thousands of different proteins can thus be separated, and information such as the protein pI, the apparent molecular weight, and the amount of each protein is obtained (1).

F. hepatica is the causative agent of fascioliasis. A wide variety of mammals including man may be infected with *F. hepatica* by ingestion of contaminated vegetables such as watercress. Glutathione (GSH) transferase is one of the major detoxification systems found in helminths including, *F. hepatica* (2). GSTs are found in high levels in *F. hepatica* parasite and the level of this enzyme is approximately 4% of the total soluble protein (3, 4). GSTs protein spots can be detected using two-dimensional electrophoresis. 2- DE can be complicated by a number of factors. The presence of endogenous proteases can result in loss of protein spots; this is especially so in parasitic organisms like, *F. hepatica*, that secretes potent proteases (5, 6).

The aim of present paper was detection of GSTs isoenzyme spots map from *F. hepatica*. These spots have been compared with GSTs sheep liver protein spots as a host.

Materials and Methods

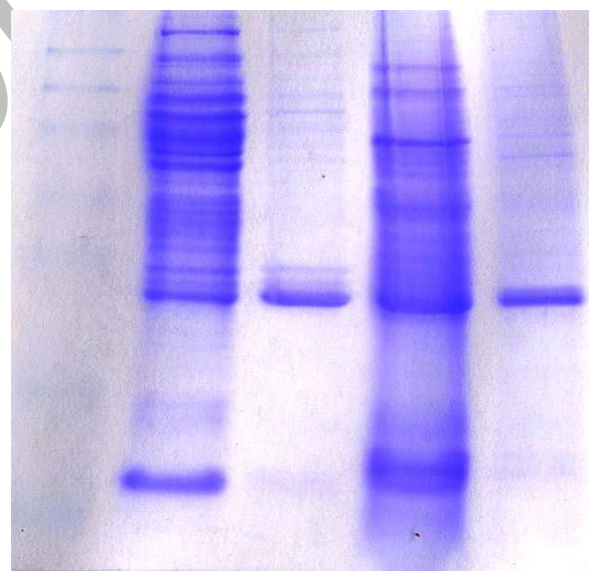
GSTs Purification by Glutathione-Agarose Gel Purified GSTs from *F. hepatica* and sheep liver tissue were obtained by bath affinity technique (7) as follow: Approximately 200 μ l wet volume of freshly prepared glutathione-agarose gel (Sigma) was washed with 1000 μ l of 20 mM potassium phosphate buffer pH 7.0 containing 50 mM sodium chloride (solution A) in a micro centrifuge tube (1500 μ l capacity) by centrifugation at low speed (9000 rpm) for 10 sec. The extract, 1000 μ l (3 mg protein for *F. hepatica* and 3 mg protein for liver tissue), was mixed with the gel for 30 min at 4°C. The supernatant was removed by centrifugation and the gel matrix washed with 1000 μ l of solution A. Polypeptides were eluted from the gel by washing with 5 \times 200 μ l of 50 mM Tris-HCL pH 9.6 buffer (4°C) contained 5 mM glutathione (reduced). Concentration of solubilized protein of *F.hepatica* and liver tissue, before and after purification was determined by Bio-Rad protein Assay, based on method of Bradford. For SDS-PAGE, whole extract of *Fasciola hepatica*, liver tissue and purified GSTs were run on a vertical thin layer gel (final gel was 12.5%).

Double Dimension electrophoresis (2-DE) of Purified GST Enzyme Purified GST Enzyme was resuspended in sample buffer and finally run on a IPG strip (7cm, pH 3-10) in the first dimension and then on an Excel Gel SDS (Gradient 8-18) in the second dimension as follows: The IPG strip was placed into the tray and poured of mineral oil on top of it and allowed IPG strip for IEF for overnight (0-100v for 2 hours; 100v-3500v 4 hours; 3500v-3500v 12 hours). After IEF the second-dimension separation was performed on Excel gel SDS. Excel gel SDS consists of four steps: 1) preparing the

second-dimension gel 2) equilibrating the IPG strips in SDS buffer 3) placing the equilibrated IPG strip on SDS gel and 4) electrophoresis by multiphor II system. Multiphor II connected the power supply and carried out electrical setting and running time for Excel gel SDS. After removing gel from multiphor II system, it was placed on of hoefer automated gel tray. Finally, the hoefer automated gel stainer was pre-programmed for coomassie staining.

Results

SDS-PAGE of homogenized and purified GSTs revealed a similar molecular weight. As shown in Fig. 1, the purified GSTs from *F. hepatica* and liver tissue have one band protein with molecular weight 26 kDa.



Lane.1 Lane. 2 Lane. 3 Lane. 4 Lane.5

Fig. 1: SDS-Page of whole and purified extract of GSTs from sheep liver tissue and *Fasciola hepatica* : Lane1. Marker sample. Lane2. Whole extract of *F.hepatica*. Lane3. Purified fraction of *F.hepatica* Lane4. Whole extract of sheep liver tissue. Lane 5. Purified fraction of sheep liver tissue.

Two-D electrophoresis revealed that purified *F.hepatica* and sheep liver tissue GSTs have an equally molecular weight (26 kDa) and comparable protein spots map (Fig.2).

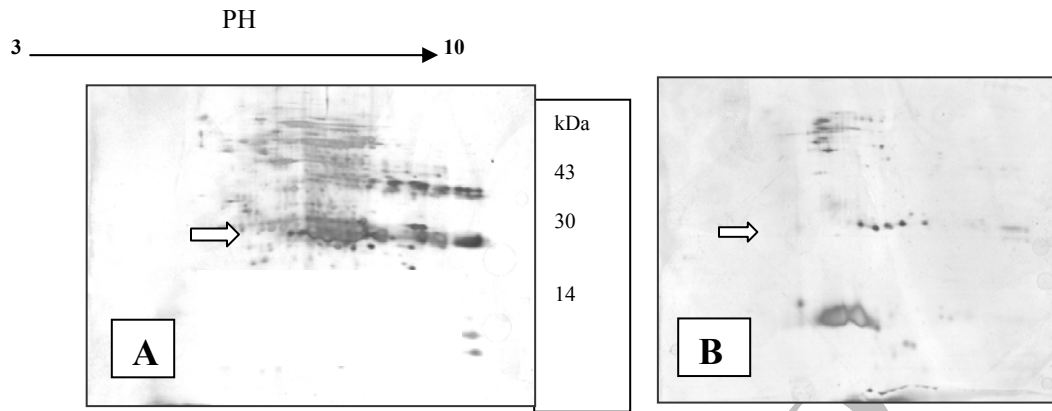


Fig. 2: Protein spots of GSTs from sheep liver tissue sample (A) and *F. hepatica* sample (B).

Discussion

Four protein spots of *F. hepatica* were detected by 2-DE technique. Moreover four cDNAs encoding GST (rGST1, rGST7, rGST47 and rGST51) of *Fasciola hepatica* have been expressed in *Escherichia coli*. All four rGST proteins from *F. hepatica* actively conjugate glutathione to the universal substrate, 1-chloro-2, 4-dinitrobenzene (8). Therefore four protein spots of *F. hepatica* which recorded in this study could be related to GSTs isoenzymes and have 26 kDa molecular weight. Furthermore the results of SDS-PAGE in this study and previous studies of author have shown that the molecular weight of whole extract and purified GSTs from *F. hepatica* and liver tissue have as same as molecular weight (9,10). Striking in the protein spots map of liver tissue may be related to high sample concentration. Recently both hot SDS and TCA precipitation treatments have been used on whole *Fasciola hepatica* sample preparation and excretory-secretory product sample preparation for resolution of this problem(6). Further identification of protein spots of gel which recorded in this paper is achievable by mass spectrometry. For this purpose protein spots of gel could be excised and digested with a specific protease (e.g., trypsin cleaves only on the carbonyl side of lysine and arginine) and the fragments measured by Matrix assisted laser desorption ionisation time-of-flight (MALDI-

TOF) mass spectrometry. This peptide mass fingerprint can then be used to search databases to identify the protein (11, 12).

Briefly, in the era of human genome map preparing, identifications of protein encoded by gene (functional genomic) is most important. This research has been revealed a small image of *F. hepatica* GSTs isoenzymes. Due to highly specific of the protein spots in the gel, these findings may be considered for vaccine development against *F. hepatica* or chemotherapeutic targets in sheep and human fascioliasis.

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References

1. Barrett J, Jefferies JR, Brophy PM (2000). Parasite proteomics. *Parasitology Today*, 16(9): 400-403.
2. Barreet J (1997). Helminth detoxification mechanisms. *Journal of Helminthology*, 100: 85-9.
3. Brophy PM, Crowley P, Barrett J (1990). Detoxification reaction of *Fasciola hepatica* cytosolic glutathione transferases. *Molecular and Biochemical Parasitology*, 39: 155-62.

4. Howell MJ, Board PG, Boray JC (1988). Glutathione S-transferases in *Fasciola hepatica*. *J Parasitol*, 47: 5-8.
5. Smith AM, Dowd AJ, McGonigle S et al. (1993). Purification of a cathepsin L-like proteinase secreted by adult *Fasciola hepatica*. *Mol Biochem Parasitol*, 62(1):1-8.
6. Jefferies JR, Brophy PM, Barrett J (2000). Investigation of *Fasciola hepatica* sample preparation for two-dimensional electrophoresis. *Electrophoresis*, 21(17): 3724-29.
7. Brophy PM, Patterson LH, Brown A, Pritchard DI (1995). Glutathione S-transferase (GST) expression in human hook worm *Necator americanus*: potential roles for excretory-secretory forms of GST. *Acta Tropica*, 59:259-63.
8. Salvatore L, et al. (1995). Biochemical analysis of recombinant glutathione S-transferase of *Fasciola hepatica* *Mol Biochem Parasitol*, 69: 281-88.
9. Farahnak A, Brophy J (2003). Study on Glutathione S-Transferases (GSTs) Inhibition Assay in the purified Extract of *Fasciola hepatica* and Sheep Liver Tissue by Hexachlorophene. *Acta Medica Iranica*, 42(3):168-71.
10. Farahnak A, Barrett J (2001). Comparative Study on Glutathione S-Transferases (GSTs) Inhibition Assay in the Whole Extract of *Fasciola hepatica* and Sheep Liver Tissue by Hexachlorophene. *Iranian J. of public health*, 30(3-4):125-28.
11. Jefferies JR, Campbell AM, Barrett J, Brophy PM (2001). A Proteomic approach to genome unverified parasitic organisms; does it work? [Abstract] *Spring meeting of British Society for parasitology, Keel, UK, April*.
12. Yates JR (2000). Mass spectrometry- from genomics to proteomics. *Trends in Genetics*, 16: 5-8.