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Detection and Characterization of Abrin toxin using Biochemical and Immunological Techniques

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Abstract

Naturally, several proteins occur which are poisonous and possess varied range of toxic effects. These proteins can be bacterial or plant and affect mammalian cells very strongly. Abrin is one of such toxic proteins. It is exceptionally toxic that even a single seed can be fatal to an adult human. It is used in chemotherapy for curing myeloma cells. Abrin is not yet known to be weaponized but is an easily available source for causing biohazard. They can be cultivated easily as it is a wild plant that grows best in almost dry regions at low elevations. Minute quantities of this toxin are sufficient for massive effects on large number of people. In the present study, abrin toxin from white Abrus precatorius seeds is characterized. After dialysis, crude Abrin was purified by ion exchange chromatography on DEAE-cellulose column. It was further purified by size exclusion chromatography. Estimation of protein was done by Folin-Lowry and Biuret assays. Its purity was checked by polyacrylamide gel electrophoresis (PAGE). The Abrin toxin gives a single band under non-reduced conditions and two bands under reduced conditions. The purity of toxin was also confirmed by Western blot. Hemagglutination activity was also studied. The molecular weight of abrin was found to be 65,000 approximately which when treated with 1% b-mercaptoethanol for 3 minutes converted into two peptides of molecular weight 35,000 and 30,000.

Introduction

Abrin is a glycoprotein heterodimer of 60kDa molecular weight consisting of cytotoxic A chain and surface binding B chain [1]. A and B chains are linked by a disulfide bond between cys 247 of A chain and cys 8 of B chain [2]. The A chain consists of 251 amino acids and B chain consists of 268 amino acids [3]. The B chain is a galactose specific lectin having two potential galactose binding sites which binds to the galactose recognizing receptors present on the cell surface [4]. The A chain confers cellular toxicity to abrin by removing two adenine residues from 60s ribosome, disrupting the binding site for elongation factor and finally inhibits protein synthesis leading to cell death [5,6]. Abrus intoxication gives symptoms like dehydration and shock leading to severe gastroenteritis. Liver, spleen, kidney and lymphatic system are also affected [7]. The early symptoms of toxicity include burning sensation in mouth and oesophagus, vomiting, abdominal pain, drowsiness, weakness and convulsions. Diarrhoea, haemorrhages, haematemesis are few of the later symptoms. Eye contact with the toxin cal lead to blindness and conjunctivitis [8,9].

Abrus precatorius, the source of abrin toxin can be easily cultivated in dry, low elevated regions and the preparation of pure toxin is not complicated. Therefore, abrin can be used for terrorism purposes. Toxin warfare offers horrific appeal as they are cheap, easy to make and simple to conceal. Even small traces of this toxin could cause massive destruction in very less time. This is the need of the hour to characterize this toxin to develop effective detection system and to develop antidotes, vaccines to counter its effect.

Methods

Estimation of protein: The purified toxin was estimated by two ways, Folin-Lowry assay and Biuret assay. The absorbance of the reaction mixtures and standard were recorded at 670nm and 540nm respectively. Total protein content was calculated from the absorbance of the sample.

Gel electrophoresis and molecular weight determination: The purified samples were assessed for their purity using SDS-PAGE under non-reduced and reduced conditions according to Laemmli [10]. The molecular weight was determined according to Weber and Osborn [11].

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Western blot analysis: Western blot analysis was performed as described by Caponi and Migliorini [12]. After protein transfer, nitrocellulose sheet was blocked at room temperature for 60 min with 5% SMP in PBS and incubated for 90 min with anti-abrin antibody at 1:1000 dilutions. Treated membrane was incubated with goat anti-rabbit IgG-HRP conjugate (1:2500) for 90 min at room temperature. The nitrocellulose sheet was washed three times for 5 min with PBST and developed with 3, 3'-diaminobenzidine.

Dot Elisa : The nitrocellulose combs were coated with 1µg/1µl of abrin in carbonate-bicarbonate buffer. After drying, strips were blocked with 3% BSA in PBS for overnight at 4ºC. Strip was incubated in 200µl polyclonal antibody (1:100) in PBS, later developed by using chromogenic substrate solution containing DAB. Results were recorded visually by the presence of brown colour (the dilution at which the brown spot is visible is taken as the titre of the conjugated).

Heamagglutination assay: Heamagglutination assay was performed in microtitre plates. Each well contained 100µl of diluent (0.05 M Tris-HCl/ 0.1 M NaCl buffer, pH 7.6), 100µl lectin (0.1 mg/ml) in the same buffer and serially diluted. 50µl of 1% suspension of sheep red blood cells in 0.15M NaCl was added to each well and gently mixed. The plates were incubated at room temperature for 3 hrs and the last well showing heamagglutination was taken as the end point. The heamagglutination titre was expressed as the reciprocal of dilution factor.

Results and Discussion

The purified samples contain considerable amount of protein which is easily detected by the intensity of bands in SDS-PAGE (figure 1). The purity of protein was checked by polyacrylamide gel electrophoresis. The abrin toxin gives a single band in 60kDa region under non-reduced conditions and two bands under reduced conditions (figure 2). The molecular weight of abrin molecule was approximately 65,000 but when it was subjected to reducing conditions, two peptides were found having 35,000 and 30,000 molecular weight respectively. Figure 3 shows the transblot of abrin toxin in various concentrations. The blot was prominent even at 1mg per well. The blot does not show any other band even at high concentration of protein (5mg/well) and confirms the purity of toxin. From blot analysis it is also confirmed that toxin can be detected below 1mg by SDS-PAGE and blotting. Figure 4 shows Indirect DOT ELISA that determined the purified polyclonal antiabrin rabbit antibodies titer. The titer of antiabrin rabbit polyclonal IgG was 1:12800. Agglutinins are known for high agglutination activity and low toxicity while abrin is highly toxic with low agglutination activity. The heamagglutination titer for pure abrin was found to be 1:16 whereas for crude abrin, it was found to be 1:64. The less levels of HA activity in the purified abrin confirms the purity of toxin as compared to crude abrin.

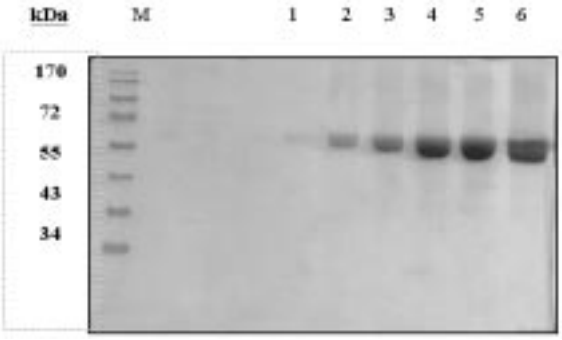

Figure 1: Sodium **D**odecyl **S**ulphate- **P**olyarylamide **G**el electrophoresis of purified proteins. M; Standard Markers; Fractions 27-32 in Lanes (1, 2, 3, 4, 5, 6 respectively).

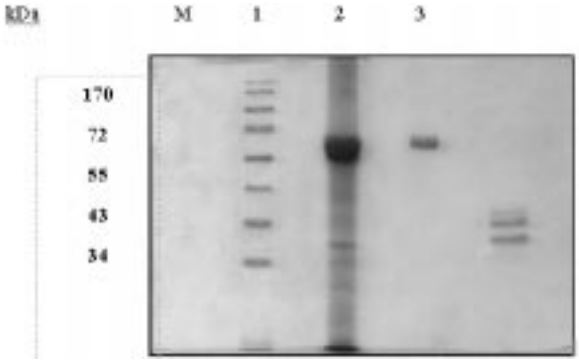

Figure 2: Sodium dodecyl sulphate- polyacrylamide gel electrophoresis of abrin. M: Molecular weight markers; Lanes: (1) Crude abrin (2) Lactamyl Sepharose affinity purified abrin (non reduced)

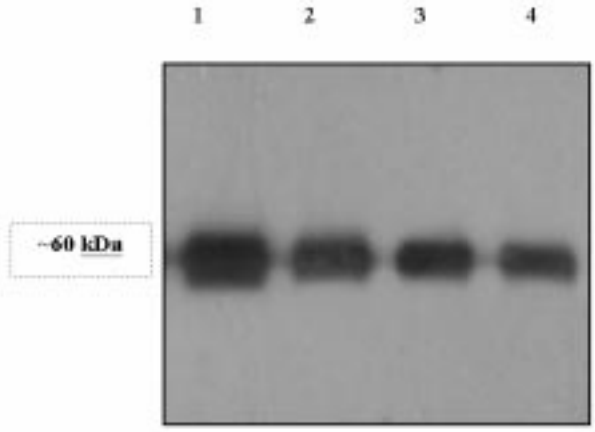

Figure 3: Transblotting of Lactamyl- Sepharose affinity purified abrin; in different concentrations of 3mg, 2mg, 1mg, and 0.5mg in Lanes 1, 2, 3, 4 respectively.

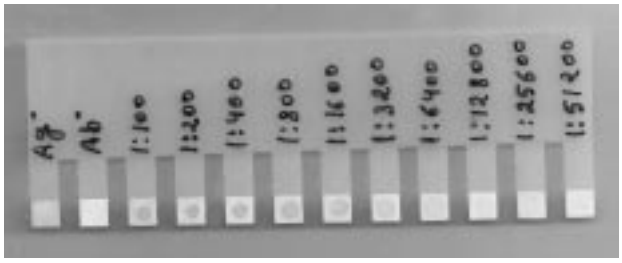

Figure 4: DOT-ELISA of abrin (anti abrin rabbit IgG)

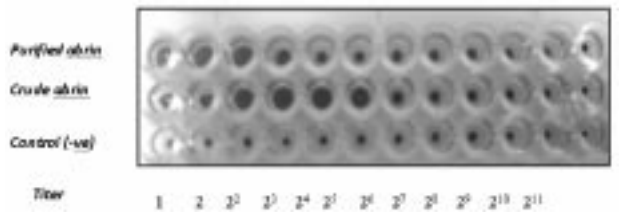

Figure 5: Hemagglutination assay of abrin

References

1. **Olsnes S. and Pihl Eur. A.**1973, Isolation and Properties of Abrin : a Toxic Protein Inhibiting Protein Synthesis Biochem., 35:179-185 .

2. **Lin J.Y., Lee T.C. and Tung T.C.** 1978, Isolation of antitumor proteins abrin-A and arbin-B from Abrus precatorius Inter..J. Pep. Protein Res., 12:311-317 .

3. **Hung C.H., Lee M.C., Lee T.C. and Lin J.Y.** 1993, Primary structure of three distinct isoabriuns determined by cDNA sequencing. Conservastion and significance J. Mol. Boi., 229:263-267 .

4. **Olsnes S., Refsnes K. and Pihl A.** 1974, Mechanism of action of the toxic lectins abrin and ricin Nature, 249:627-631 .

5. **Endo Y., Mitsui K., Motizuki M. and Tsurugi K.** 1987, The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes. The site and the characteristics of the modification in 28 S ribosomal RNA caused by the toxins J. Biol. Chem., 262:5908-5912 .

6. **Endo Y. and Tsurugi K.** 1988, The RNA *N*-glycosidase activity of ricin A-chain : the characteristics of the enzymatic activity of ricin A-chain with ribosomes and rRNA. J. Biol. Chem., 263:8735-8739 .

7. **Fodstad O., Johannessen J.V., Schjerven L. and Pihl A.** 1979, Toxicity of abrin and ricin in mice and dogs. J. Toxicol.Environ. Health., 5:1073-1084 .

8. **Gunsolus J.M. & J. Amer.** 1955, Toxicity of Jequirity beans, Med. Assoc., 157:779-880 .

9. H**art M.** 1963, Jequirity-Bean Poisoning, New Engl. J. Med., 268:885-886 .

10. **Laemmli U.K.** 1970, Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4, Nature, 227:680-687 .

11. **Weber K. and Osborn M.** 1969, The Reliability of Molecular Weight Determinations by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis, J. Bio. Chem., 244:4466-4412 .

12. **Caponi L. and Migliorini P.** 1999, Antibody usage in the lab : Immunoblotting, In: *Antibody usage in the lab: Immunoblotting.* Springer-Verlag Press .

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