ANALYSIS OF POLYPEPTIDE PROFILES OF STRONGYLID NEMATODES USING SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS

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ABSTRACT

Polypeptide profile of soluble extract antigens (SEA) of referral nematodes viz., *Haemonchus contortus, Bunostomum trigonocephalum and Oesophagostomum columbianum* were analysed by sodium dodecyl sulphate polyacarylamide gel electrophoresis (SDS-PAGE). The SEA of *H. contortus* showed bands at 24.0, 29.0, 35.0, 43.0, 60.0, 94.0 and 106 kDa molecular weight whereas the SEA of *B. trigonocephalum* showed bands at 21.0, 29.0, 30.0, 47.0, 60.0, 94.0 and 101 kDa size. The SEA of *O. columbianum* revealed polypeptide bands at 29.0, 39.0, 54.0, 60.0, 94.0 and 112 kDa. It was also revealed that a common polypeptide bands at 29.0, 60.0 and 94.0 kDa were present in all three antigens.

KEYWORDS : Antigenic profiles, gastro intestinal nematodes, SDS-PAGE

INTRODUCTION

Nematode antigens are biochemically complex in nature and are cross-reactive with one another (Meeusen, 1996 and Knox, 2000). Many nematode antigen in particular surface and excreted components are stage specific and provide ample scope for stage specific diagnostics and protections (Raleigh et al., 1996 and Knox, 1998). The surface bound antigenic molecules play a critical role in immune response and host parasite relationship. Serodiagnosis of helminthic infections continues to be a difficult task on account of cross-reactivity and sharing of antigenic molecules amongst various helminths. The most important impediment in the way appears to be that the specificity of antibody detection that greatly restricted by the degree of interspecific cross reactive epitopes, shared between helminths (Cuquerella et al., 1994 and Molina et al., 1999). Therefore, a study of nematode antigen in relation to immune response of host is a prerequisite for understanding immunological mechanisms between parasite and host. Hence, the present study was undertaken to examine the polypeptide profiles of economically important gastro-intestinal nematodes viz., H. contortus, B. trigonocephalum and *O. columbianum* using SDS-PAGE.

MATERIALS AND METHODS

Three species of parasitic nematodes viz., *H. contortus, B.trigonocephalum* and *O. columbianum* were collected from a local abattoir of sheep and goats. The parasites were recovered from their respective sites of predilection at necropsy following standard technique (Sahu and Misra, 1988). The parasites were washed repeatedly with distilled water followed by physiological saline and phosphate buffered saline (pH 7.4). Finally, the collected worms were suspended in 0.1 M phosphate buffered saline (PBS pH 7.4). The parasites were identified upto species level using standard keys (Soulsby, 1982).

PREPARATION OF ANTIGENS

Soluble Extract Antigen (SEA)

Soluble extract antigen for each species of the referral nematodes was obtained by processing adult parasites of *H. contortus, O. columbianum* and *B. trigonocephalum* separately using standard technique (Klesius et al., 1986).

One gram sample of freshly collected adult nematodes was suspended into homogenizing buffer (0.1 M PBS, pH 7.4 supplemented with 1mM PMSF and 10% Trition X-100). The mixture was subjected to repeated freeze-thawing cycle (approximately 8-10 times). Finally, the worms were homogenized using ground glass homogenizer and the suspension was subjected to high speed centrifugation at 10,000 Xg for 1 hr at 4^{o} C. The supernatant was designated as soluble extract antigen (SEA). It was stored at -20^{o} C until use.

Protein Estimation

The protein concentration of the referral antigens (SEA) was estimated by the method of Lowry et al. (1951) using bovine serum albumin fraction V as a standard.

Characterization of soluble extract antigen

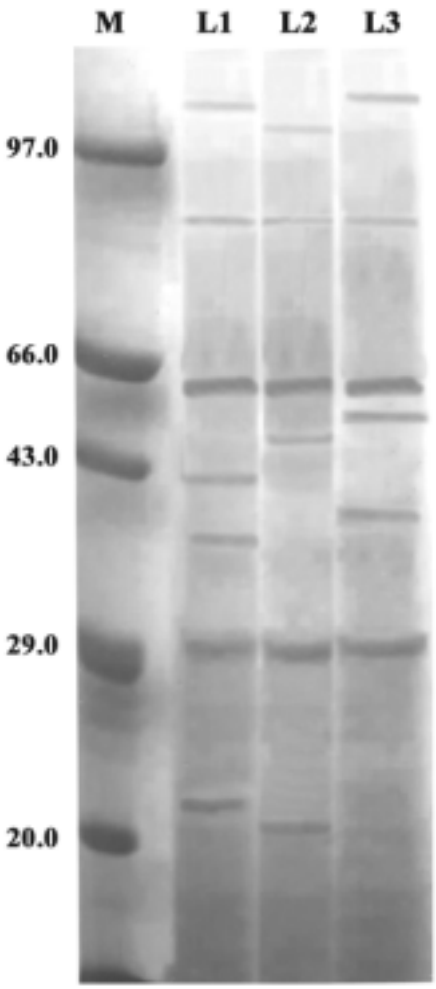
SDS-PAGE was carried out to observe the polypeptide patterns of SEA of three referral nematodes according to their molecular weights under reducing gel conditions as per the method of Laemmli (1970). The vertical slab gel electrophoresis was performed using 1mm thickness of gel in a discontinuous system (Minigel, 12% resolving gel, 5% stacking gel). The antigen samples were diluted in sample buffer (1:2) and denatured by boiling at 100 ^{o}C for 5 minutes . The molecular weight marker was also kept in boiling water bath for 5 minutes and allowed to cool. Thirty microlitre of denatured protein samples were loaded into each well with protein marker (10µl/well) in a separate well. Electrophoresis was carried out at 50 volts for 30 minutes until the tracking dye reaches the resolving gel, followed by a constant voltage of 120 V for 2 hours till the tracking dye reached the bottom of the gel. Then the gel was stained overnight with 0.1% coomassie brilliant blue R-250 and destained until the clear demarcation of bands appeared. Molecular weights of referral antigens were calculated as each antigen preparation was run simultaneously with known standards. The respective relative mobilities were calculated and plotted against the log molecular weights of the protein markers.

RESULTS AND DISCUSSION

The SDS-PAGE profile of soluble extract antigen of *H. contortus* under study revealed 7 polypeptides. *B.* trigonocephalum showed 7 polypeptides whereas, *O.* columbianum showed 6 polypeptides. The SEA of H. *contortus* showed bands at 24.0, 29.0, 35.0, 43.0, 60.0, 94.0 and 106 kDa molecular weight whereas the SEA of *B. trigonocephalum* showed bands at 21.0, 29.0, 30.0, 47.0, 60.0, 94.0 and 101 kDa size. The SEA of *O. columbianum* revealed polypeptide bands at 29.0, 39.0, 54.0, 60.0, 94.0 and 112 kDa molecular weights (Plate-1). Further, the distribution patterns of polypeptides was also demonstrated in SEA of referral nematodes. It was revealed that a common polypeptide bands at 29.0, 60.0 and 94.0 kDa was observed in all three antigens derived from the referral nematodes.

The present study revealed that the conservation of common polypeptides may be responsible for the antigenic cross reactivity among three referral nematodes. Similar polypeptide pattern was observed in soluble extract antigen of H. contortus by Knox et al., 1999, 2005. But there was no previous reports available on the polypeptide pattern of SEA of *B. trigonocephalum* and *O. columbianum* for comparison.

Some workers identified phosphoryl choline (PC) as a major immunodominant molecule present on helminths surface membrane. These PC epitopes have been identified as common antigenic determinant by polyclonal and monoclonal antibodies by ELISA and western blotting. These molecules have been identified not only from the parasite cell surface but also from intestinal structures such as intestinal membranes. This ubiquitous parasite moiety has been implicated to play



M - Molecular weight marker
L1 - SEA of *H.contortus*
L2 - SEA of *B.trigonocephalum*
L3 - SEA of *O.columbianum*

an important role in host parasite relationship. Therefore, it is essential that the degree of cross reactivity amongst strongylid nematodes is elucidated for understanding the evolutionary conservation of antigen and for designing effective immuno diagnostic/immuno prophylactic method.

Therefore, further studies are warranted for identifying species specific antigenic components of these referral nematodes. Alternatively, selection of a single defined antigen using specific purification procedure may improve the serological relevance of the referral nematode antigen. Identification of species specific antigenic components of referral nematodes is of interest to eliminate false positive reactions in infected host due to cross reactivity. Such antigens could be further used for development of specific monoclonal antibodies against the referral nematodes.

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