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Modification of carbohydrate compositions of 31/36 kDa proteins of plerocercoids (sparganum) of *Spirometra mansoni* grown in different intermediate hosts

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Abstract: We purified specific 31/36 kDa antigenic molecules from sparganum in different intermediate hosts (snakes and mice) and analyzed their monosaccharide compositions. Compositional analysis showed that glucose and mannose concentrations were 2-3 fold higher in the 31/36 kDa molecule purified from snakes than those from mice. This result implies that antigenic glycoproteins of sparganum from snakes might be modified in mammalian sparganosis with respect to their carbohydrate composition.

Key words: sparganum, glycoprotein, monosaccharide

Human sparganosis, is a disease caused by the ingestion of plerocercoid larva (sparganum) from snakes or procercoid from unfiltered water, and occurs worldwide particularly in East Asia especially in Korea (Cho et al., 1975). Serologic diagnosis of human sparganosis can be performed by ELISA (Kim et al., 1984) and by immunoblot (Choi et al., 1988). Among the many antigenic proteins involved in sparganosis. 31/36 kDa excretory-secretory proteins (Cho et al., 1992) have been shown to be highly specific and to be useful for the diagnosis of human sparganosis (Choi et al., 1988). Antigenic proteins in parasitic infections appear to be distributed on parasite surface as glycoproteins. The 31/36 kDa proteins were also localized to the syncytial tegument and tegumental cells of sparganum (Kim et al., 1992). In parasitic infections, glycoconjugates in parasites are mainly targeted against host immune response and are known to be involving in organism survival, infectivity, and host-parasite interactions (Cummings and Nyame, 1996). Therefore, we suggest that the carbohydrate compositions of antigenic molecules in sparganum may be modified to facilitate parasites survival in mammalian intermediate host infections. This study involved carbohydrate monosaccharide analysis of the purified 31/36 kDa antigenic proteins in sparganum collected from different intermediate hosts (snakes and mice).

Sparganum was collected from the subcutaneous tissue of naturally infected snakes. After washing with sterile physiologic saline, worms were stored at -70°C until required. The 31/36 kDa proteins were partially

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Table 1. Measurements of monosaccharide concentration (nmole/50 μg each sample) in purified 31/36 kDa molecules of sparganum from snakes and mice

Sugars	31 kDa (snake)	31 kDa (mouse)	36 kDa (snake)	36 kDa (mouse)
Fucose	0.1	0.1	0.1	0.2
N-acetylgalactosamine	–[a)]	–	–	–
N-acetylglucosamine	0.2	0.1	0.2	0.4
Galactose	0.3	0.1	–	–
Glucose	2.4	0.7	2.1	1.1
Mannose	3.1	1.3	2.7	1.5

[a)]–; not detected.

purified by gelatin affinity chromatography (Kong et al., 1991) and a Mono Q HR 5/5 column using AKTA FPLC system (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The purified antigenic proteins were confirmed by Western blot using human sparganosis patient sera. A total of 50 μg of both molecules was separated by 10% SDS-PAGE and transferred onto a PVDF (polyvinylidene difluoride) membrane. The membranes were then cut into strips and the strips were stored in Eppendorf tubes. For monosaccharide analysis, the 31/36 kDa molecules on PVDF strips were hydrolyzed in 6N HCl at 100°C for 4 h for amino sugar (glucosamine and galactosamine) analysis and in 2M trifluoroacetic acid at 100°C for 4 h for neutral sugars (glucose and mannose) analysis. The neutral and amino sugars were separated and quantitated on a CarboPac PA10 column (2.0 x 250mm) with a Bio-LC DX-600 (Dionex Co., Sunnyvale, CA, USA) using a cartridge (2.0 x 50mm). Eighteen mM NaOH was used as an eluant at a flow rate of 0.25 ml/min. Duplicated data was analyzed using Peaknet on-line software.

Monosaccharide compositions of the purified molecules are shown in Table 1. The glucose and mannose concentrations of the purified molecules in sparganum from snake were over 2-fold that of mouse. However, other monosaccharides showed no significant concentration differences. The 31/36 kDa proteins contain N-linked oligosaccharides which can be released by PNGase F treatment (data not shown). The monosaccharide compositional analysis indirectly indicated that the 31/ 36 kDa proteins of sparganum contain N-linked complex type oligosaccharides composed of at least N-acetylglucosamine and mannose. It is of interest that minimal amounts of some common monosaccharide components of mammalian N-linked carbohydrates, such as fucose, in N-glycans of 31/36 kDa proteins were observed. Also, it is interesting that the glucose concentration of the 31/36 kDa proteins in sparganum from mouse was lower than those of snake. Oligosaccharides in mammals are known to be deglucosylated prior to export from the cell (Kornfeld and Kornfeld, 1985). Therefore, the glucose reduction phenomenon of the 31/36 kDa proteins in sparganum from mouse may, in part, be a survival mechanism in mammalian intermediate host. The maintenance of high levels of N-glycosylation in sparganum from snake could insure proper targeting of its antigenic/pathogenic related surface proteins, and during larval migration in the host, prevent worm degradation by the host's immune cells. The oligosaccharides of pathogens are important at mediating the first contact with the host's innate immune system. In *Trichinella* sp., some specific carbohydrates may also provoke an antibody response and serve as a target for specific antibodies (Ellis et al., 1994). However, the oligosaccharides of *Leishmania* sp. gp63 do not play an important role with respect to contact with the plasma membrane, as the removal of the oligosaccharides does not significantly affect enzymatic activities (Funk et al., 1993). The immunogenicity of some parasite glycans may be due to their different biochemical structures as compared with mammalian host glycans. This suggests that differences in the glycosylation pathways between host and parasite reflect evolutionary distance (Ferguson and Homans, 1988).

However, the biological significance of carbohydrate changes in the infection of mammalian hosts is not well known, and it also remains to be determined whether this monosaccharide or all N-linked carbohydrates are involved in biological and immunological functions in mammalian sparganosis.

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