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Prediction, Synthesis and Antigenicity of the Antigenic Peptides of 26 kDa Glutathione S-Transferase of *Schistosoma Japonicum (*Sj26)

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Abstract: Six antigenic peptides of 26 kDa glutathione S-transferase of *Schistosoma japonicum* (Sj26) have been predicted according to their hydrophilicity, flexibility, accessibility, charge distribution and β -turn in the secondary structure by the determination of its primary structure and synthesized by solid phase method. All of them showed antigenicity with anti-*schistosoma japonicum* immunoglobulin polyclonal antibody, anti-Sj-IgG PcAb by Dot-ELISA. Three of them showed good antigenicity. They would serve as candidates of synthetic anti-schistosomal vaccine.

Keywords: *Schistosoma japonicum*, glutathione S-transferase, antigenic peptide, peptide vaccine.

Schistosomiasis is a worldwide parasitic disease that affects more than 200 million persons in 70 countries all over the world. It is very important to develop an effective vaccine including significant levels of protection against the invasive stage of the parasite for the prevention of rapid reinfection and repeated drug application over a long period.[1-4]

Up to now, many protective native antigens, such as 26 and 28 kDa glutathione S-transferases, 97 kDa paramyosins and so on, have been found. Among these candidates for an anti-schistosomal vaccine, the glutathione S-transferases have been as potential components of a vaccine against schistosomiasis[1, 4-6]. Previously, we reported the studies on the antigenic peptides of Sm26/1 and Sm26/2[7, 8]. In this paper, antigenic peptides of another GST isoenzyme Sj26 were synthesized and tested on immunoreactivity.

The epitopes of 26 kDa glutathione S-transferase of *Schistosoma japonicum* (Sj26) have been predicted using Goldkey and PC-Gene programs based on their hydrophilicity according to Hopp and Woods method, and their flexibility, accessibility, charge distribution and ϐ-turn in the secondary structure by the determination of the primary structure of Sj26[7]. The sequences of P23-46, P80-97, P86-97, P136-153, P187-202 and P206-218 are listed below.

J1, P23-46, YLEEKYEEHLYERDEGDKWRNKKF
J2, P80-97, NMLGGCPKERAEISMLEG
J3, P86-97, PKERAEISMLEG
J4, P136-153, RLCHKTYLNGDHVTHPDF
J5, P187-202, PQIDKYLKSSKYIAWP
J6, P206-218, WQATFGGGDHPPK

These predicted peptides have been synthesized by using solid phase method with the acid-labile tert-butyloxycarbonyl (Boc) group for temporary protection and acid-stable groups for the protection of side chains[7]. All protected peptide-resins were cleaved by anhydrous hydrogen fluoride with anisole, 1, 2-ethandithiol and thioanisole as scavengers and washed with cooled ethyl ether. Peptides were extracted with 30% acetic acid and purified by gel filtration on Sephadex G15 or G25. They were checked for homogeneity by reverse-phase HPLC. Impure peptides were further purified by preparative HPLC. The compositions of the peptides were confirmed by amino acid analysis and FAB-MS[9].

All synthetic antigenic peptides indicated activity in dot blot immunobinding assay[7]. Peptide J4, J5 and J6 showed good antigenicity with anti-*schistosoma japonicum* immunoglobulin polyclonal antibody (anti-Sj-IgG PcAb). Peptide J5 also showed good antigenicity with the anti-schistosoma surface membrane monoclonal antibody (A6McAb). No peptide showed antigenicity to anti-schistosoma 31/32 kDa antigen monoclonal antibody (31/32McAb). The results shown in **Table 1** indicated that these three peptides with high antigenicity located at the C-terminus and middle parts in the sequence of Sj26. They would serve as candidates of synthetic peptide vaccine against schistosomiasis.

Table 1. Antigenicities of antigenic peptides of Sj26

Peptide	J1	J2	J3	J4	J5	J6
Anti-Sj-IgG	+	+	+	++++	++	+++
A6McAb	-	-	-	+	++	-
31/32McAb	-	-	-	-	-	-

- and + — ++++ present negative and degree of positive antigenicity.

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9. Amino acid analysis results of all peptides are Satisfactory. MS analysis are listed below. $(M+Na)^+$: J1, 3225; $(M+1)^+$: J2, 1934; J3, 1359; J4, 2152; J5, 1936; J6, 1397.

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