# CLONNING AND SEQUENCE ANALYSIS OF THE 26 kDa GLUTATHIONES-TRANSFERASE GENE OF *SCHISTOSOMA MEKONGI*

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**Abstract.** The number of genomic DNA or cDNA sequences of *Schistosoma mekongi* accessible in Genbank or EMBL is very limited up to now. Recently, two reports have appeared on the molecular phylogeny of *Schistosoma* species inferred from partial sequence data of rRNA genes; no further sequence data of *S. mekongi* is available yet. Knowledge of the molecular structure of protein coding genes of *S. mekongi* will provide a better understanding of gene function in the genus *Schistosoma*. A cDNA library of *S. mekongi* adult male was constructed and a cDNA encoding the 26 kDa glutathione S-transferase protein of this species was cloned. Sequence analysis of this cDNA confirmed the close phylogenetic relationship of *S. mekongi* to *S. japonicum*.

## INTRODUCTION

The genus *Schistosoma* contains about 19 described species; all infect mammals and some cause disease in humans. *Schistosoma* species occur in Africa, several parts of Asia, and South America (Després *et al*, 1993). The *Schistosoma japonicum* group (*S. japonicum* and *S. mekongi*) that uses prosobranch snails as intermediate host is the dominant group in east and southeast Asia. Schistosomiasis caused by *S. japonicum* is a more pathogenic form of schistosomiasis resulting in more severe lesions. *S. mekongi* is found in endemic foci in Lao PDR and Cambodia. It resembles *S. japonicum* in adult and larval structures, but it has a different snail intermediate host and seems to produce a milder disease in man. Differences between *S. japonicum* and *S. mekongi* on the molecular level have been demonstrated by isoenzyme electrophoresis (Viyanant and Upatham, 1985), species specific monoclonal antibodies (Viyanant *et al*, 1994) and recently, by rRNA sequence analyses (Bowles *et al*, 1995; Barker and Blair, 1996).

Glutathione S-transferases (GSTs) are a family of multifunctional proteins found in plant and animal species including schistosomes (Mozer *et al*, 1983; Stenersen *et al*, 1987; Clarke *et al*, 1985; Smith *et al*, 1986; O'Leary and Tracy, 1988; Taylor *et al*, 1988). One important function of GSTs is their ability to detoxify a broad range of xenobiotics (Mannervik, 1985). Glutathione S-transferase isoenzymes of schistosomes have been proposed as anti-schistosome vaccine candidates (Mitchell, 1989). Most studies have been carried out on the development of *S. mansoni* (Sm28GST) and *S. japoni-cum* (Sj26GST) where the recombinant GST has proved to be protective in several experimental models (Capron *et al*, 1992). There is evidence that vaccination with Sm28GST affects worm fecundity and the hatchability of its eggs; consequently, Sm28GST is now being investigated as a diagnostic reagent for use in antigen detection. In this present study we cloned and characterized a cDNA encoding the 26 kDa GST (Sme26GST) of *S. mekongi* to provide further evidence that *S. mekongi* and *S. japonicum* are close but different species.

## MATERIALS AND METHODS

### Parasites

Adult worms of *S. mekongi* were collected by portal perfusion of infected Swiss albino mice. Freshly obtained adult worms were carefully washed in phosphate buffered saline (PBS), pH 7.2 to remove any contamination of host blood. Male and female worms were separately kept frozen at -80°C.

### Extraction of RNA

Adult male worms were disrupted in TRIZOL (GIBCO BRL) by an IKA Ultra-Turrax S25-8G at

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13,000 rpm for 30 seconds and total RNA was extracted according to the TRIZOL manual (GIBCO BRL). From 1.4 g wet weight 2 mg total RNA was extracted. Poly (A)$^+$RNA was extracted by oligo(dT) cellulose columns (GIBCO BRL). To avoid inhibition of reverse transcriptase (RT) in further experiments, no SDS was used in binding or elution buffer. From 2 mg total RNA 12 μg poly(A)$^+$RNA was isolated.

**Reverse transcriptase polymerase chain reaction (RT-PCR) cloning of glutathione S-transferase coding sequences**

A 495 bp cDNA fragment of the GST-26 gene of *S. mekongi* was amplified by RT-PCR with primers designed from the sequence of *S. japonicum* (Smith *et al*, 1986). The PCR primers used and their positions in *S. japonicum* are: GST-5: TTG GAC TTT CCC AAT CTT CC (5'-primer, position 164-183), GST-3: TCG CCA CCA CCA AAC GTG GC (3'-primer, position 638-657). The following RT-reaction was set up with poly(A)$^+$RNA extracted from *S. mekongi* adult male: 4 μl RNA (0.5 μg RNA), 1 μl NTPs @ 25 mM, 1 μl RNase Block ribonuclease inhibitor (40 u), 1 ul GST-3 (58 ng), 2 μl 10 × PCR buffer, 1 μl MMLV-RT (200 u), 10 μl water. GST-26 cDNA was generated for 10 minutes at 22°C, 1 hour at 42°C, 10 minutes at 95°C and kept on ice. PCR amplification of GST-26 cDNA was done in the following procedure: 20 μl RT-reaction, 1 μl GST-5 (95 ng), 8 μl 10 × PCR buffer, 70.5 μl water, 0.5 μl Taq polymerase (2.5 u). The thermal cycler (Biorad) was set up to 2 minutes at 94°C following 39 cycles of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C.

**cDNA library construction and screening with GST1**

A cDNA library was constructed from 5 μg of poly(A)$^+$RNA isolated from adult male *S. mekongi* using the ZAP express cDNA Synthesis Kit (Stratagene). The amplified library was plated and screened according to the instruction manual. Plaque lifts were hybridized with a $^{32}$P-labeled GST1 DNA probe. Positive plaques were picked and after *in vivo* excision (instruction manual, Stratagene) and DNA purification the size of the cDNA inserts was determined by cleavage with restriction enzymes EcoRI and XhoI and agarose gel electrophoresis.

**DNA sequence analysis**

The PCR amplified cDNA fragment (GST1) was subcloned into the EcoRV site of the Bluescript SK(-) vector (Stratagene). GST1 DNA and cDNAs isolated from the cDNA library were sequenced by plasmid dideoxy sequencing using the Sequenase 2.0 kit (USB, Amersham LIFE SCIENCE Inc). The obtained sequences were analysed using the sequence analysis programs at EMBL and the MacMolly Tetra program (Soft Gene GmbH, 1995).

## RESULTS

**RT-PCR amplificatioin of a *S. mekongi* glutathione S-transferase cDNA fragment**

We used a RT-PCR procedure to generate a specific probe for cloning of the 26 kDa glutathione S-transferase gene (GST-26) of *S. mekongi*. Comparison of the available sequences of *S. japonicum*

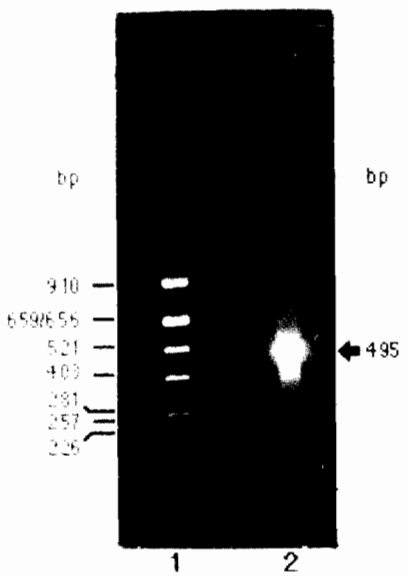


Fig 1–Agarose gel electrophoresis (1.5%) of RT-PCR product. Lane 1, DNA size marker pBR322/AluI. Lane 2, RT-PCR product of *Schistosoma mekongi* adult male mRNA with primers GST-5 and GST-3. Arrow indicates the amplified 495 bp GST-26 cDNA fragment.

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S. mekongi      acgatattcgttggacaagtctcgaataaggttactttagtcATGGCTCCGATACTCGGC   60
S. japonicum    ------------------------.tta.g..ac..g.....T.C..T.....A..T   34
S. mansoni      ---------------------------------------a.....A..T.AGT....T   19

S. mekongi      TATTGGAAAATTAAGGGCCTTGTACAACCCACTAGACTTCTTTTGGAATATCTTGGAGAA  120
S. japonicum    .....................G.........C.....................A....   94
S. mansoni      .........G.C..A.............A...C.............C.C....A....   79

S. mekongi      GAATATGAAGAGCGTTTGTACGAACGCAATGAAGGTGATGCATGGCGAAACGAAAAATTT  180
S. japonicum    A............A......T..G...G...........AA..........A....G...  154
S. mansoni      ACT.....G..A...GC...T..T.........ATC.....C...A.C.....T......  139

S. mekongi      AAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGACGGTGATGTTAAATTGACA  240
S. japonicum    G.......GST-5.....T.............A...                         214
S. mansoni      .....A..CC.......C..A.....................T......T.......A...  199

S. mekongi      CAATCCATGGCCATCATACGTTACATAGCTGACAAGCACAACATGTTGGGAGGTTCGGGA  300
S. japonicum    ..G..T.................T.........................T....GTCC.  274
S. mansoni      .....T.....T...........T...........A.............G.C..GTCC.  259

S. mekongi      AAAGAGCGTGCAGAGATTACAATGCTTGAAGGAGCAGTTTCGGATATTAGATCTGGTGTT  360
S. japonicum    .................T.................G....T...........AC......  334
S. mansoni      .....A.....G..A...T.G.............G....T.........GATG......  319

S. mekongi      TCAAGAATTGCATATAATAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAACAAGCTA  420
S. japonicum    ..G.............G.....................................G.......  394
S. mansoni      .T......C.....C.....G..A.A......C.................C.....A..T  379

S. mekongi      CCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGCCATAAAACATATTTAAATGGTGAT  480
S. japonicum    .....................................T.....................  454
S. mansoni      ....GG.G.......................G.CTA.C.....T.....G..C...A..  439

S. mekongi      CGTGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGAC  540
S. japonicum    .A.....                                                    514
S. mansoni      T.......T...........T.....A..C..T..C........G..............  498

S. mekongi      CGAAAGTGCTTGGATGCGTTTCCAAAACTAGTTTGTTTTAAAAAACGTATTGAAAATATA  600
S. japonicum    .C..T....C..........C......T.........................GC...C  574
S. mansoni      TC.C........A.C.A..........T......C...C.....GT........G..T..  559

S. mekongi      CCACAAATCAATGAGTACTTGAGATCTAGTAAGTATATAGAATGGCCTCTGCAGGGCTGG  660
S. japonicum    ........TG..A.........A...C..C..........C.......T...........  634
S. mansoni      ...........GA.C.....A.AT.....C.G...C...A.............A..T...  619

S. mekongi      CAAGCCACGTTTGGTGGTGGCGACCATCCTCCGAAATAAattattaattgttgttttggt  720
S. japonicum    ...........GST-3.....................A...........ag...ga.......a..  694
S. mansoni      G.T.................A..TAC......A.....G....ac.g.aaa.c.gg.aaa  679

S. mekongi      aaacattatttatcacctatgattgtgctaaataaaaatgtcgataaaaaaaaaaaaaaa  780
S. japonicum    ................t..ca...aaa.......t.........c                739
S. mansoni      t.....a.aaagc.tgt...atg.t.a...........ataa                   721

S. mekongi      aaaa                                                         784
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Fig 2–Aligment of the cDNA sequence of *Schistosoma mekongi* GST-26 with those from *Schistosoma japonicum* (M14654) and *Schistosoma mansoni* (M31106). The alignment was obtained with the COMPLIGN program in the MacMolly Tetra suite (Soft Gene GmbH). Similarity of the aligned part of *S. mekongi vs S. japonicum* is 89.9%, the number of mismatches is 75, *S.mekongi vs S. mansoni* shows 78.6% similarity with 154 mismatches within the aligned part. Underlined uncleotides from 164-183 and 638-657 within the sequence of *S. japonicum* indicate the position of the primers GST-5 and GST-3 used for RT-PCR cloning of the *S. mekongi* GST-26 cDNA fragment GST1. Amino acids identical to those of the top line are indicated by ".", double underlined sequences in *S. mekongi* are ATG translational start site, TAA stop codon and AATAAA poly (A) signal. Capital letters indicate the open reading frame,' including start and stop codon. Small letters indicate the 5'- and 3'-untranslated regions, respectively. "-" indicates nucleotides not available in M14654 and M31106.

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S. mekongi      MAPILGYWKIKGLVQPTRLLLEYLGEEYEERLYERNEGDA   40
S. japonicum    .S.....................E.K...H....D...K   40
S. mansoni      ...KF....V............H.E.T....A.D...I..   40

S. mekongi      WRNEKFKLGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHN   80
S. japonicum    ...K..E.................................   80
S. mansoni      .S.D..................F.................   80

S. mekongi      MLGGSGKERAEITMLEGAVSDIRSGVSRIAYNKDFETLKV  120
S. japonicum    ....CP......S......L...Y.......S........  120
S. mansoni      ...ACP......S......L...M..L.......EY.....  120

S. mekongi      DFLNKLPEMLKMFEDRLCHKTYLNGDRVTHPDFMLYDALD  160
S. japonicum    ...S.....................H.............  160
S. mansoni      .......GR........SN......NC.............  160

S. mekongi      VVLYMDRKCLDAFPKLVCFKKRIENIPQINEYLRSSKYIE  200
S. japonicum    .......M................A....DK..K.....A  200
S. mansoni      ......SQ..NE.....S...C..D....KN..N..R..K  200

S. mekongi      WPLQGWQATFGGGDHPPK                        218
S. japonicum    ..................                        218
S. mansoni      ......D.......T...                        218
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Fig 3–Alignment of the deduced amino acid sequence of *Schistosoma mekongi* GST-26 with those from *Schistosoma japonicum* and *Schistosoma mansoni*. Amino acids identical to those of the top line are indicated by ".". The number of different amino acids between *S. mekongi* and *S. japonicum* is 22 (89.9% similarity) whereas 41 amino acids are different betwen *S. mekongi* and *S. mansoni* (81.2% similarity).

(M14654) and *S. mansoni* (M31106) led to the construction of primers GST-5 and GST-3. Agarose gel electrophoresis of the cDNA products amplified from poly(A)$^+$RNA of adult male worm showed a predominant DNA fragment which had the expected size of ~495 bp (Fig 1). We subcloned this cDNA fragment (GST1) into the EcoRV site of the Bluescript SK(-) vector, and subsequent sequence analysis confirmed that GST1 is homologous to the corresponding DNA fragment of GST-26 of *S. japonicum* and *S. mansoni* (Fig 2).

### Screening of the cDNA library of *S. mekongi* with GST1

50,000 plaques were plated and ~800 plaques reacted positively with GST1. Six plaques were picked for analysis, processed and the size of the cDNA inserts determined by agarose gel electrophoresis. Two cDNA clones carried additional sequences and were not analysed further. The remaining four cDNA clones contained single inserts ranging from 480-780 bp length. The longest cDNA clone cGST-3 was sequenced by dideoxy sequencing using the Sequenase 2.0 kit (USB).

### Comparison of the sequence of *S. mekongi* with those of *S. japonicum* and *S. mansoni*

The obtained cDNA sequence confirmed that we have cloned GST-26 of *S. mekongi*. The 784 bp cDNA consists of an open reading frame (ORF) of 657 bp length encoding the 26 kDa GST protein and ending up with the stop-codon TAA. The ORF is preceded by 52 bp of a 5'-untranslated region and 85 bp of a 3'-untranslated region containing the poly(A) signal (AATAAA, position 751-756) and a poly(A) tail of 19 nucleotides length. The DNA sequence of *S. mekongi* shows a higher similarity towards the one of *S. japonicum* (89.9%) than to that of *S. mansoni* (78.6%) . The deduced amino acid sequence of *S. mekongi* is also more similar to *S. japonicum* than to *S. mansoni* (89.9% versus 81.2%).

## DISCUSSION

Glutathione S-transferase isoenzymes have been shown to be protective antigens in various animals (Boulanger *et al*, 1991; Capron *et al*, 1992). The 26 kDa GST of *S. japonicum* (Smith *et al*, 1986) and the 28 kDa GST of *S. mansoni* (Henkle *et al*, 1990)

confer partial protection in several experimental animals including the baboon (Boulanger *et al*, 1991; Henkel *et al*, 1990). When using these enzymes to vaccinate animals, there is a risk of stimulating autoimmune reactions as with other genetically conserved antigens. These compounds remain attractive as they can be targeted by designer drugs (Bergquist, 1995). In this study, we gave further evidence that *S. mekongi* and *S. japonicum* were different species by cloning, sequencing and comparing the cDNA sequence of the 26 kDa GST of *S. mekongi* with the published sequence of *S. japonicum* (Smith *et al*, 1986) and *S. mansoni* (Trottein *et al*, 1990 unpublished). Our data showed that the DNA sequence of *S. mekongi* is 89.9% and 78.6% similar to *S. japonicum* and *S. mansoni*, respectively. The deduced amino acid sequence is 89.9% and 81.2% similar to *S. japonicum* and *S. mansoni*, respectively. These data support the results obtained from isoenzyme electrophoresis, species specific monoclonal antibodies (Viyanant and Upatham, 1985; Viyanant *et al*, 1994) and rRNA sequence analyses (Bowles *et al*, 1995; Barker and Blair, 1996), and confirms that *S. mekongi* is a different oriental *Schistosoma* species from *S. japonicum*.

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The cDNA sequence of *S. mekongi* 26 kDa glutathione S-transferase has been submitted to EMBL. The accession number is Y07663.

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