GENE CLONING AND AMINO ACID SEQUENCE ANALYSIS OF TRIOSE-PHOSPHATE ISOMERASE OF *SCHISTOSOMA JAPONICUM* CHINESE STRAIN

Zhu Yinchang, Yu Chuanxin, Yin Xuren and Si Jin

Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu 214064, PR China

Abstract. A pair of primers was synthesized according to the DNA sequence of *Schistosoma japonicum* Philippine strain, and the mRNA of adult worms of *S. japonicum* Chinese strain was prepared. The gene of triose-phosphate isomerase of *S. japonicum* Chinese strain (SjC TPI) was successfully cloned from the mRNA through reverse transcription polymerase chain reaction (RT-PCR) technic with the primers. The DNA sequence of the gene showed that the open reading frame of encoding SjC TPI DNA includes 759bp, which has 84% homology to Sm TPI, 99.7% homology to SjP TPI. The analysis of deduced amino acid sequence of SjC TPI indicates that SjC TPI is 84.9% (214/252) identical with Sm TPI and 99.2% (250/252) with SjP TPI. The peptide-structure analysis presents 7 extra-surface, hydrophilic regions in the molecule of SjC TPI, the molecular weight of SjC TPI is 27,619. The isoelectric point is 7.38.

INTRODUCTION

Schistosomiasis is still a serious endemic disease in the world. In order to control the disease effectively, development of schistomiasis vaccine will be an important strategy. China is an endemic area of *Schistosoma Japonicum* Chinese strain, so that the vaccine candidates of Chinese strain in China should be developed.

Triose-phosphate isomerase (TPI) is an enzyme antigen (Harn *et al,* 1985). The full lenght cDNA and genomic DNA of *Schistosoma mansoni* TPI were cloned (Shoemaker *et al,* 1993; dos Reis *et al,* 1993). The schistosome enzyme antigen TPI was a 28 kDa antigen, and recognized immunologically by McAb M1 which was able to passively transfer partial resistance (41-49%) (Harn *et al,* 1992). It has been reported that the purified TPI of *S.mansoni* with CFA could induced a good partial protection (38%) (Harn *et al,* 1987). It is suggested that the STPI is a good candidate antigen for vaccine development and may be the target for pharmacologic attack. In this paper the gene of TPI of *S. japonicum* Chinese strain was cloned, and the deduced amino acid sequence of the gene products was characterized.

MATERIALS AND METHODS

Preparation of mRNA of S. japonicum Chinese strain

Collection of adult worms. The adult worms of *S. Japonicum* Chinese strain were collected from infected rabbits with 1,500 cercariae per rabbit at 45 days post infection.

Extraction of mRNA. The fresh adult worms were homogenized on ice, then mRNA was extracted and purified with Quick Prep mRNA Purification Kit (Pharmacia Company) according to the manufacturer's instruction.

Primers

A pair of primers P1 and P2 was designed according to the TPI DNA sequence of *S. japonicum* Philippine strain:

P1: 5′ - GCGGATCCATGTCTGGTTCTCGGA- 3′

P2: 5′ - GCGTCGACTTATTGTCTAGCTTTAC- 3′

The restriction site of *Bam*H1 was at the 5′ - end of primer P1, the *Sal*1 site was added at the 5′ - end of primer P2. The primers were synthesized by Shanghai Sangon Biological Engineering Co.

Synthesis of first strand of cDNA

Deionized water, 6.5 μl; 5 x buffer of first strand reverse transcription, 4 μl; 4 x dNTP(5mM), 2.0 μl; Oligo(dT) 12-18 (500 μg/ml) (Pharmacia), 0.5 μl; RNasin (Promega) 1.0 μl(30U); mRNA, 5 μl; murine reverse transcriptase (M-MLV, Pro-

mega), 1 μl (200U) were placed in a 0.5ml microcentrifuge tube, mixed, briefly centrifuged, then incubated at 37 °C for 30 minutes, denatured at 95 °C for 5 minutes.

Polymerase chain reaction(PCR)

P1 (12.5 μM), 1μl; P2(12.5 μM) 1 μl; 10 x buffer, 8 μl; Tag DNA polymerase (Promega), 1.5 μl (8U); deionized water, 68.5 μl were placed into the denatured first strand reaction mixture (total volume 100 μl), and centrifuged, 50 μl of paraffin oil were placed on the surface of the mixture, then the PCR was performed, at 94 °C 1 minute, 42 °C 2 minutes, 72 °C 3 minutes, for 35 cycles, following at 72 °C for 5 minutes. After PCR, 10 μl of the products were taken and analyzed by agarose electrophoresis. 1 μl of T4 DNA polymerase (3U, Promega) was added in 80 μl of PCR products, incubated at 37 °C, for 15 minutes.

Purification of the target cDNA fragment

The PCR product was a single DNA band on agarose gel electrophoresis, the molecular weight was similar to the predicted TPI gene fragment. The target DNA fragment was purified by direct purification method with Wizard PCR Preps Purification System (Promega) according to the manufacturer's instruction.

Cloning of the target DNA fragment

Digestion. The purified target DNA fragment and plasmid vector pUC18/19 (Sino-American Biotechnology Company) were digested with restriction enzymes *Bam*H1 and *Sal*1. The products were purified with Wizard DNA Clean Up System (Promega) according to the manufacturer's instruction.

Ligation. 12 μl (200ng) of the purified and digested target cDNA, 4 μl (80ng) of plasmid vector pUC18/19 and 2 μl of 10 x ligation buffer, 2 μl (6U) of T4DNA ligase (Promega) were placed in a 0.5ml sterile microcentrifuge tube, mixed, incubated at 14°C for 12-14 hours.

Transformation. The competent *E. coli* JM109 cells were prepared according to Sambrook *et al,* (1989), and placed at 4 °C for 24 hours. 200 μl of competent JM109 cells, and 20 μl of target fragment-plasmid ligated products were mixed in a 0.5 ml of sterile tube, placed on ice for 30 minutes, heat shocked at 42 °C for 2 minutes. 1 ml of pre-warmed LB medium at 37 °C was added in the tube, incubated with shaking at 37 °C for 45 minutes. 200 μl of incubated product was inoculated on a LB culture plate with 50 μg/ml of ampicillin, and incubated at 37 °C overnight.

Identification of the positive clones

The plasmid DNA containing the recombinants in the single colony was extracted by fast plasmid size identification method (Sambrook *et al,* 1989). The size of the recombinant DNA was identified by agarose electrophoresis. Analysis of the recombinant by digestion with the restrictive emzymes. A single colony containing the recombinant was placed into 3ml of LB medium, and incubated at 37 °C overnight. The recombinant cDNA was purified with Wizard Minipreps DNA Purification System (Promega). 20 μl of purified recombinant cDNA, 5 μl of x buffer (D), 2 μl (20U) of *Bam*H1, 2 μl (20U) of *Sal*1 and 21 μl of sterile deionized water were mixed in a 0.5 ml tube, incubated at 37°C for 3-4 hours. The digested product was analyzed by agarose electrophoresis.

Analysis of DNA sequence

The purified target cDNA fragment was subcloned into M13mp 18/19, and transformed into *E. coli* JM 109 cells (as above), inoculated on the LB plate, incubated at 37°C overnight. The white plaque (inserted TPI) was taken and the double strand M13-TPI recombinants were prepared according to Sambrook *et al* (1989). The M13-TPI recombinants were sent to Central Laboratory, Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine for DNA sequencing. The DNA sequence was analyzed in Nanjing Medical University.

Analysis of the deduced amino acid sequence

The amino acid sequence was deduced according to the cDNA sequence of TPI of *S. japonicum* Chinese strain and analyzed with SWISS-PROT by Dr Hawdow (Yale University School of Medicine, USA).

RESULTS

Target DNA fragment

A 700-800 bp single, specific fragment was amplified from mRNA of the adult worm of *S. japonicum* Chinese stain with primers P1, P2 reverse transcription PCR (Fig 1).

Cloning of target DNA and analysis of digested products with restrictive enzymes

The purified, end-repaired target DNA fragment was digested with restrictive enzymes *Bam*H1 and *Sal*I, then the digested fragment was ligated to digested pUC 18/19. The recombinant containing target DNA and pUC 18/19 was produced (Fig 2). The recombinant DNA (rDNA) was purified again, and digested with *Bam*H1 and *Sal*I, after that a single DNA fragment with 700-800 bp was the same as the target DNA fragment produced by RT-PCR.

DNA sequence of the target DNA fragment

The purified and digested target DNA fragment was inserted into vector M13 mp 18/19, the double strand M13-TPI recombinants were sequenced. The result showed the the size of the open reading frame of target DNA fragment was 759bp, similar to TPI of *S. japonicum* Philippine strain and *S. mansoni*. The encoding DNA sequence had 99.7% homology

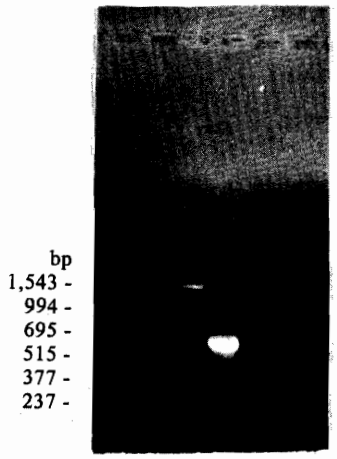

Fig 1–The target fragment amplified from mRNA by RT-PCR
lane 1– PCR DNA marker
lane 2– TPI DNA fragment

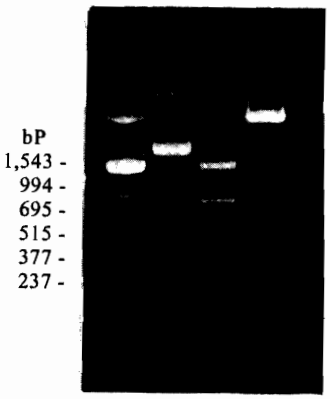

Fig 2–The recombinant (TPI + pUC18/19) and digested products
lane 1– Plasmid pUC18/19
lane 2– Recombinant (TPI+pUC18/19)
lane 3– DNA marker
lane 4– The digested products

to *S. japonicum* Philippine Strain TPI (SjP TPI) and 84% homology to *S.mansoni* TPI. This indicated that the target DNA fragment should be TPI of *S. japonicam* Chinese strain (SjC TPI). The base composition of SjC TPI: A:246, T:233, G:174, C:106, A+T/G+C: 1.7:1. GC:36.9% (Fig 3).

Analysis of amino acid sequence

The deduced amino acid sequence according to the open reading frame of encoding SjC TPI DNA sequence included 252 amino acids (Fig 3). The expected molecular weight of the molecule was 27,619. It's isoelectric point (pI) was 7.38. The amino acid composition is shown in Fig 4.

The deduced SjC TPI protein sequence was compared with the sequence of Sm TPI and SjP TPI. It had 99.2% (250/252) in homology with SjP TPI and 84.9% (214/252) homology with Sm TPI. There are 7 main hydrophilic domains which would be the surface-exposed determinants found in the peptide structure, these are amino acids 10-23, 50-58, 95-116, 130-143, 154-163, 180-208, 219-232.

DISCUSSION

It has been reported that mice immunized with natural TPI of *S. mansoni* could induce 38% resist-

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      M  S  S   S  R  K  F   F  V  G   G  N  W   K  M  N  G
1     ATGTCTAGTT CTCGGAAATT TTTTGTTGGG GGTAATTGGA AGATGAATGG
       S  R  A   D  N  K  V   L  I  H   S  L  S   E  A  H
51    CAGTAGAGCC GACAACAAAG TTTTGATTCA TTCTTTGTCT GAGGCTCATT
      F  C  G   D  T  E  I   L  I  A   A  P  F   V  Y  L  N
101   TTTGTGGAGA CACTGAAATT TTAATTGCTG CACCTTTCGT TTATCTAAAT
       E  V  R   Q  S  L  A   K  E  I   H  V  A   A  Q  N  C
151   GAAGTTCGGC AAAGCTTAGC TAAAGAGATA CACGTGGCTG CTCAAAATTG
       Y  K  V   P  K  G  A   F  T  G   E  I  S   P  S  M
201   CTATAAGGTA CCAAAGGGTG CATTTACTGG GGAAATCAGT CCTTCAATGA
      I  K  D   V  G  C  D   W  V  I   L  G  H   S  E  R  R
251   TAAAAGATGT TGGTTGTGAT TGGGTTATAC TTGGTCATTC TGAACGAAGA
       S  I  F   N  E  S  D   E  L  V   A  E  K   V  Q  H  A
301   AGTATATTTA ATGAATCCGA TGAGCTTGTA GCTGAAAAAG TTCAACATGC
       L  A  G   G  L  S  V   V  A  C   I  G  E   T  L  S
351   ACTTGCAGGA GGTCTAAGCG TTGTAGCATG TATTGGGGAA ACACTATCAG
      E  R  E   S  G  K  T   E  E  V   C  V  R  Q   L  N  A
401   AGCGTGAATC TGGTAAAACA GAAGAAGTGT GTGTAAGACA ATTAAATGCT
       I  A  N   K  I  K  S   I  D  E   W  K  R   V  V  V  A
451   ATTGCTAATA AGATTAAATC AATTGATGAA TGGAAACGAG TTGTTGTAGC
       Y  E  P   V  W  A  I   G  T  G   K  V  A   S  P  G
501   ATATGAACCT GTATGGGCTA TTGGAACAGG TAAAGTTGCT TCACCAGGTC
      Q  A  Q   E  V  H  N   F  L  R   K  W  F   K  T  N  T
551   AAGCTCAAGA AGTTCATAAT TTTCTTCGTA AATGGTTTAA AACAAATACA
       P  S  G   V  D  Q  Q   I  R  I   I  Y  G   G  S  V  T
601   CCATCTGGAG TTGACCAACA AATACGTATT ATCTATGGTG GATCAGTAAC
       A  A  N   C  K  E  L   A  Q  Q   H  D  V   D  G  F
651   CGCAGCAAAT TGTAAAGAAT TAGCTCAACA ACATGATGTG GATGGATTTT
      L  V  G   G  A  S  L   K  P  E   F  I  D   I  C  K  A
701   TAGTTGGTGG TGCTTCATTA AAACCGGAAT TTATTGATAT ATGTAAAGCT
       R  Q
751   AGACAATTAA
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Fig 3–Nucleotide sequence with amino acid translations of SjC TPI. Predicted initiation and termination codons are underlined.

ance to *S. mansoni* (Harn *et al,* 1987), indicating that Sm TPI was a good immunogen and served as a vaccine candidate. This paper reports that the TPI cDNA of *S. japonicum* Chinese strain was successfully cloned from mRNA of adult worm of *S. japonicum* Chinese strain with the primers P1, P2 synthesized according to SjP TPI DNA sequence by reverse transcription PCR technic. The analysis of cDNA sequence of SjC TPI showed that the encoding SjC TPI cDNA included 759 bp nucleo- tides, and has 99.7% homology to SjP TPI, 84% homology to Sm TPI.

TPI gene is a single-copy or low copy number enzyme gene in SjP and SjC (Hooker *et al,* 1996). The content of mRNA is also low in the organism. The TPI gene amplified from a cDNA library of SjC is not successful, so we used fresh adult worms to prepare mRNA, then to amplify immediately from the mRNA by reverse transcription PCR technic, and obtained the SjC TPI gene sequence.

Residue	Number	Mole Percent
A=Ala	23	9.127
B=Asx	0	0.000
C=Cys	7	2.778
D=Asp	10	3.968
E=Glu	19	7.540
F=Phe	10	3.968
G=Gly	21	8.333
H=His	7	2.778
I=Ile	18	7.143
K=Lys	18	7.143
L=Leu	15	5.952
M=Met	3	1.190
N=Asn	11	4.365
P=Pro	7	2.778
Q=Gln	11	4.365
R=Arg	11	4.365
S=Ser	20	7.937
T=Thr	8	3.175
V=Val	24	9.524
W=Trp	5	1.984
T=Tyr	4	1.578
Z=Glx	0	0.000

Fig 4–The amino acid composition in the SjC TPI protein sequence.

TPI is presents in all stages of the life cycle of schistosomes (Harn *et al,* 1985), the sequence of Sm TPI had strong homology to mammalian, microbial, and human TPI (Shoemaker *et al,* 1992). In this study we have showed that SjC TPI has high homology to Sm TPI (84.9%). If the full length of TPI would be used as the vaccine, the auto-immunity reactions could not be avoided (Shoemaker *et al,* 1992; . Thus, the parasite special region of TPI with the weakest homology to human counterpart should be identified and used as the candidates of vaccine of SjC.

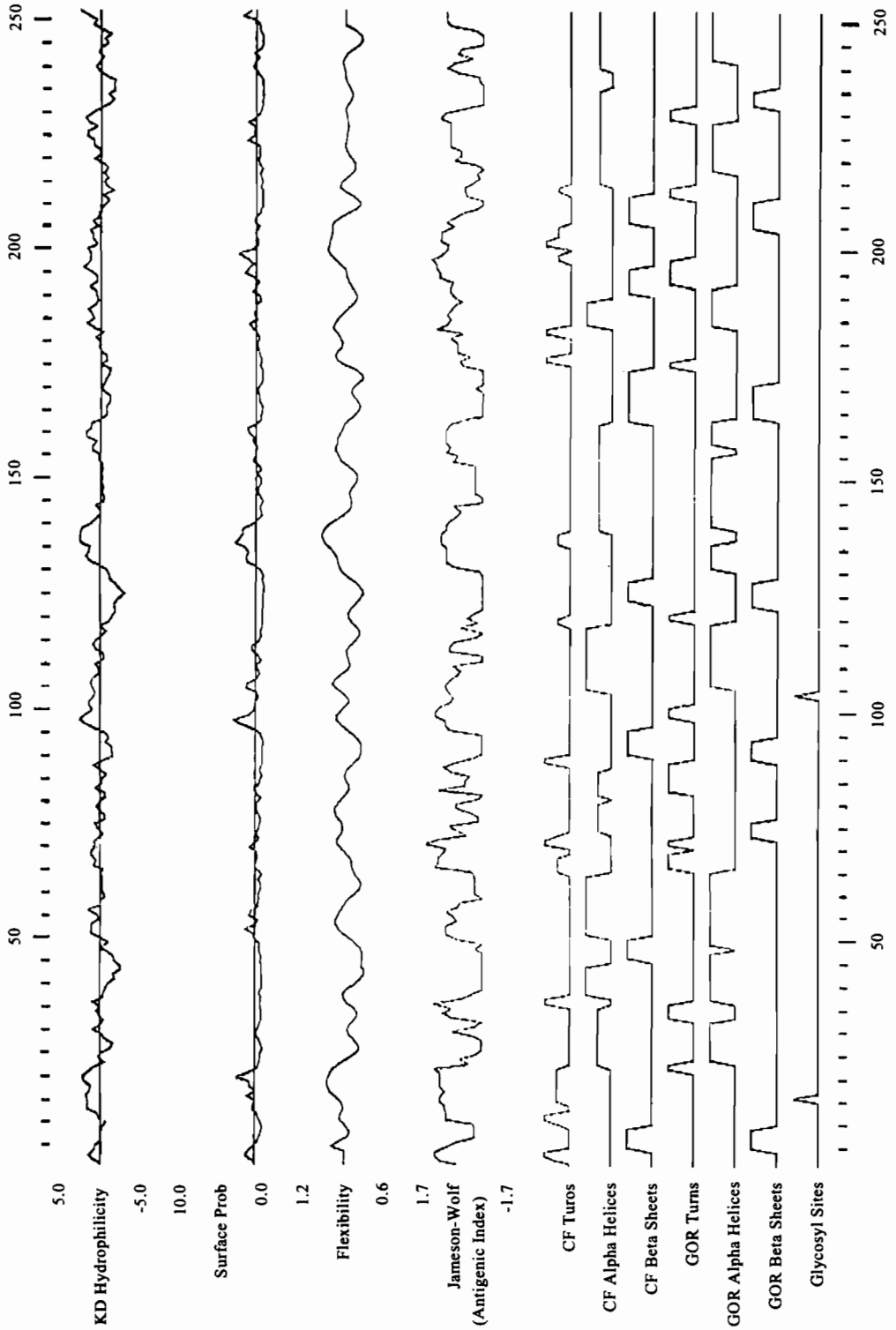

Fig 5–Peptide structure analysis of the deduced amino acid sequence from SjC TPI DNA sequence.

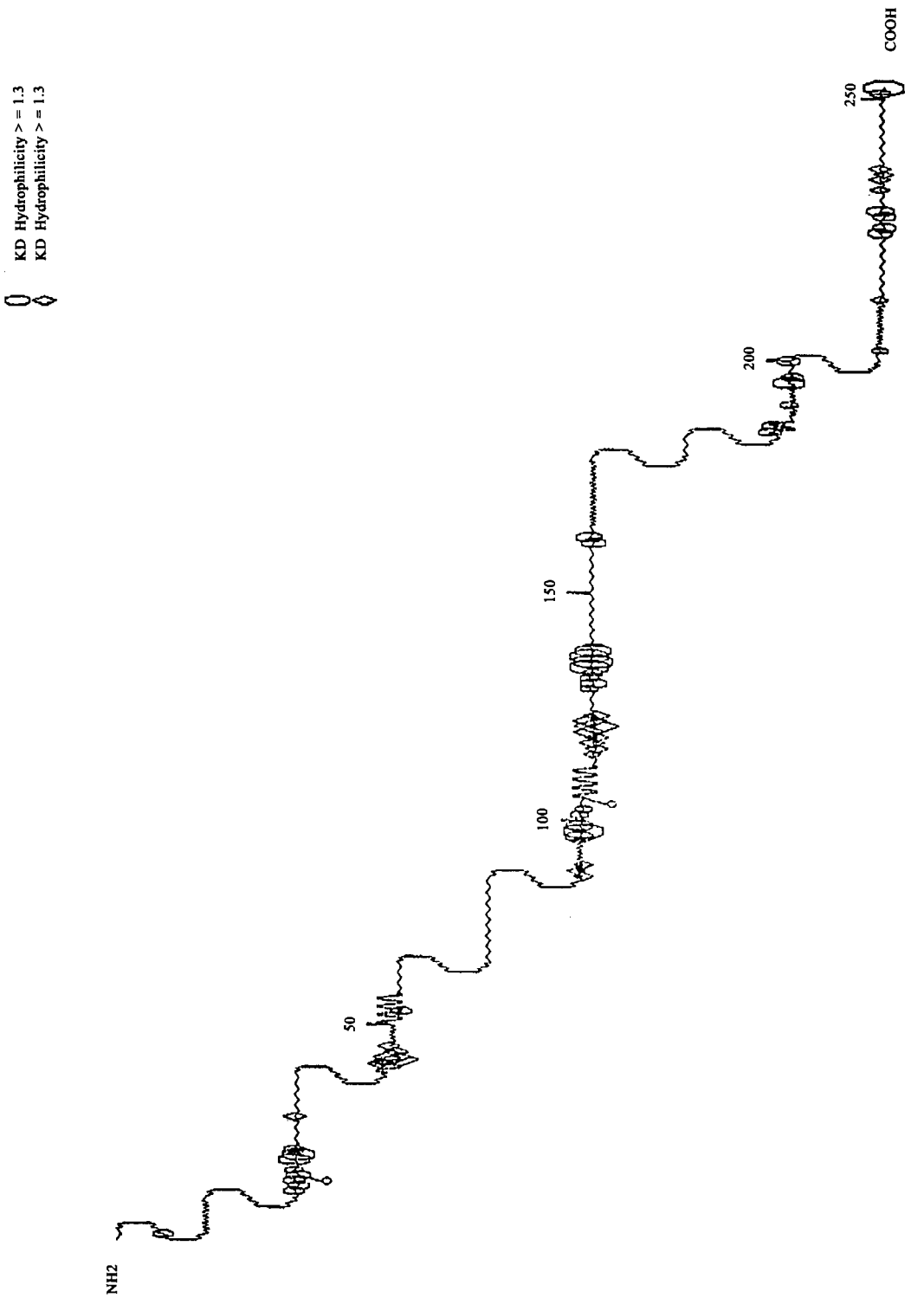

Fig 6–Plot structure of amino acid translations of SjC TPI DNA sequence.

The peptide-structure analysis showed that the SjC TPI included 7 extra-surface, hydrophilic domains, so that the parasite special, extrasurface hydrophilic domains would be a high potential vaccine candidates. Next step we shall select and define the special peptide regions with T and/or B epitopes (Reynolds *et al,* 1994).

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