THE TEMPERATURE - DEPENDENT EXPRESSION OF GST OF *SCHISTOSOMA JAPONICUM* (PHILIPPINE STRAIN)

Cai Zhi-hong, Song Guang-chen, Xu Yu-xin and Liu Shu-xian

Department of Immunology, Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, WHO Collaborating Center of Malaria, Schistosomiasis and Filaliaris, Shanghai 200025, People's Republic of China

Abstract. Obtained from pSj5, the cDNA gene encoding GST antigen of *Schistosoma japonicum* (Philippine strain) was ligated with efficient temperature-dependent PBV220 vector which was constructed in CAPM, and then introduced into host bacterium-DH5α *(E. coli)* by transformation. Transformants were selected by ampicillin and recombinant clones were identified by restriction mapping. The result showed that recombinant clone 43 was the one carrying recombinant plasmid PBV 220 with the correct insertion of the gene fragment. The GST expression ability of clone 43 was investigated by GST enzymic activity assay and SDS-PAGE. A relatively high level of GST enzymic activity was expressed by this clone under the temperature-dependent condition, that is, cultured at 30°C and expressed at 42°C. A more strongly stained 26 kDa protein band was identified by SDS-PAGE. The result indicated that GST of *S. japonicum* (Philippine strain) could be expressed not only by IPTG induction, but also by the temperature-dependent method.

INTRODUCTION

Encoding the candidate antigen for vaccine against schistosomiasis, the fragment cDNA of *Schistosoma japonicum* (Philippine strain) has been cloned and expressed (Smith *et al,* 1986, 1988). The native 26-28 kDa protein extracted and purified from the adult worm of the Chinese strain of *S. japonicum* by GSH agarose affinity chromatography has GST activity and obvious antigenicity to hosts such as mouse and rabbit. More than 70% homology in amino acid sequence could be found between the 26-28 kDa antigen (Chinese strain) and recombinant Sj26 (rSj26) of the Philippine strain, and there were cross reactions between 26-28 kDa and rSj26 in antigenicity and immunogenicity (Liu *et al,* 1992). It seems important to modify the expression way of rSj26 in order to find a feasible, simple method to express the GST antigen gene of the Chinese strain. PBV220 is a plasmid vector with a high copy number and efficient expression ability of the fusion protein (Zhong, 1992). In our experiment, the cDNA encoding GST of the Philippine strain from pSj5 has been recombined with the PBV220 vector, and the possibility of expression of GST by the temperature-dependent method was investigated.

MATERIALS AND METHODS

Plasmids and hosts: Plasmid PBV220 and its host bacterium DH5α *(E. coli)* were kindly provided by the Department of Genetic Engineering, Institute of Virology, Chinese Academy of Preventive Medicine. Bacterial pSj5 plasmid that directs synthesis of native Sj26 in *E. coli* was a gift from Dr Mitchell (WEHI, Melbourne) and Dr Tiu (University of Philippines, Manila).

Recombinant PBV220: Briefly, cleaved from pSj5 with restriction enzyme *(E. coli),* the gene fragment encoding GST was obtained by electrophoretic elution, and routinely ligated with the PBV220 vector treated with CIP, then transformed into fresh competent DH5α prepared using calcium chloride. Transformants were selected by ampicillin and recombinant colonies were screened and identified by restriction mapping with SalI, ScaI and EcoRI.

Expression methods: (a) expression induced by IPTG: One colony of each bacterium (DH5α containing recombinant PBV220 and JM103 containing pSj5) was cultured in LB medium containing ampicillin, shaken at 37°C overnight. The culture

was diluted until the OD value at 660 was about 1.0, then 10mM IPTG was pulsed. Two ml of each bacterium were collected before induction and 1, 2, 3, 4, 24 hours after induction, then lysed by sonication and centrifuged at 12,000 rpm for 10 minutes at 4°C. The sonicate supernatant was collected for GST activity assay and SDS-PAGE. (b) Temperature-dependent expression: Both bacteria were cultured at 30°C overnight as above. The average OD value at 660nm was 1.4-1.7. Culture was continued at 42°C. Two ml of each bacterium were collected before and 1, 2, 3, 5, 7, 24 hours after the temperature was increased at 42°C, and treated in the same way as in (a).

Expression ability assay: (a) GST activity assay: This was slightly altered from that described by Habig *et al* (1974). GST enzymic activity was investigated spectrophotometrically at 340 nm. One mM 2, 4—dinitrochlorobenzol and 1 mM reduced GSH served as substrates in 0.1 M potassium phosphate buffer (pH 6.5). 0.1 ml of each sonicated supernatant was added to each reaction mixture for 30 minutes at 37°C. The reaction was ended by addition of 0.5 N HCl. The OD value was obtained at 340 nm. (b) SDS-PAGE: 12.5% SDS-PAGE was performed routinely. Proteins were detected by staining with Coomassie blue.

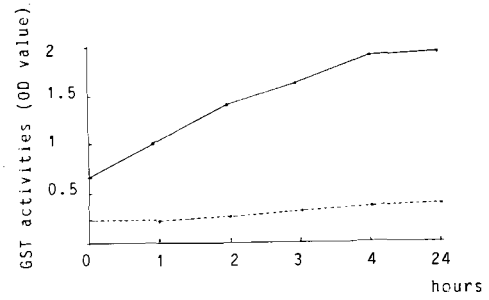

Fig 1—The GST activities of expression of PBV220 (-----) or pSj5 (——) induced by IPTG, determined spectrophotometrically.

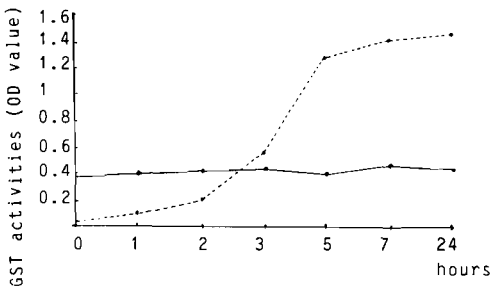

Fig 2—The enzymic activity of GST expressed by PBV220 (-----) or pSj5 (——) at 42°C, determined spectrophotometrically.

RESULTS AND DISCUSSION

The result of restriction mapping indicated that clone 43 contained the recombinant plasmid PBV220.

The expression of GST in JM103 (transformed with pSj5) induced with IPTG performed by Smith *et al* (1988) indicated that the GST expressed by pSj5 increased in a certain number of hours after IPTG induction, as shown by 13% SDS-PAGE. rSj26 purified from GSH-agarose affinity chromatography gave a yield of 3-10 mg/l of culture and had GST activity similar to that for native GST purified from adult worms. In our experiment, the GST activities of the sonicated supernatant of JM103 bacteria at different times with IPTG increased with the hours of incubation (Fig 1). Comparatively, a more strongly stained 26 kDa protein band was also shown by SDS-PAGE (Fig 3a). Both results indicated that a high expression of GST in JM103 (containing pSj5) was induced with IPTG. Without IPTG, only a low level of GST was expressed by pSj5, and did not increase with the hours at 42°C (Fig 1). No obvious 26 kDa protein band was shown by SDS-PAGE (Fig 3b).

Before and 1 hour after the temperature was increased to 42°C, only a low level of GST activity was expressed by recombinant PBV220. In 7 hours, the longest time at 42°C, a higher level of GST activity was expressed. The GST activity at 7 hours was almost the same as that at 24 hours. The curve of GST activity with different times at 42°C was sigmoidal (Fig 2), and a gradually stronger stained 26 kDa protein band was shown by SDS-PAGE (Fig 4b). The ability to express GST by recombinant PBV220 could not be induced by IPTG, even though there was a low degree of GST activity at 37°C. The curve of GST activity was almost horizontal. No strongly stained 26 kDa protein band was shown by SDS-PAGE (Fig 4a).

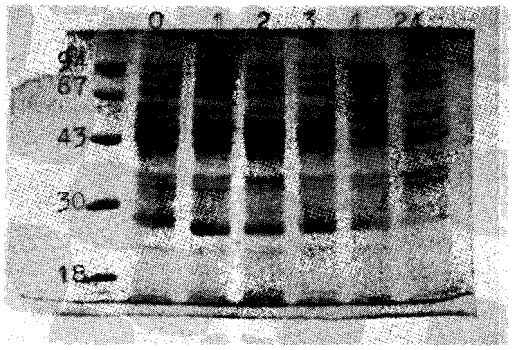

Fig 3—a) 12.5% SDS-PAGE for the sonicate supernatants of JM103 (containing pSj5) induced by TPTG at different hours. Proteins were detected by staining with Coomassie blue.

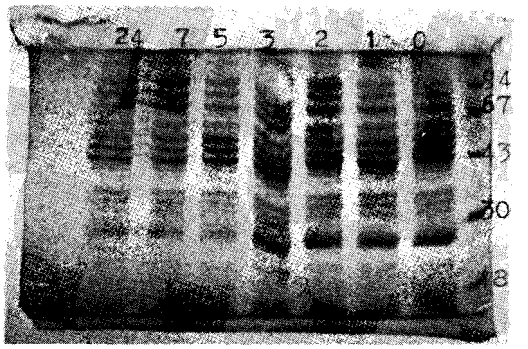

Fig 3—b) 12.5% SDS-PAGE for the sonicate supernatants of JM103 (containing pSj5) at different hours at 42°C. Proteins were detected by staining with Coomassie blue.

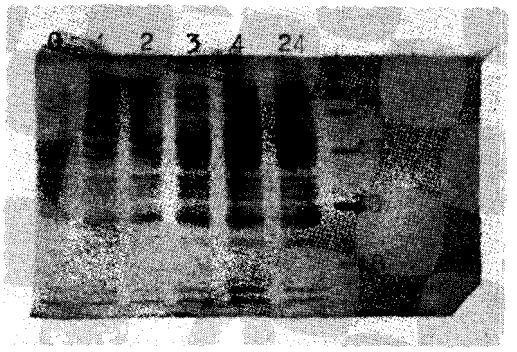

Fig 4—a) 12.5% SDS-PAGE for the sonicate supernatants of DH5α (containing PBV220) with IPTG at different hours. Proteins were detected by staining with Coomassie blue.

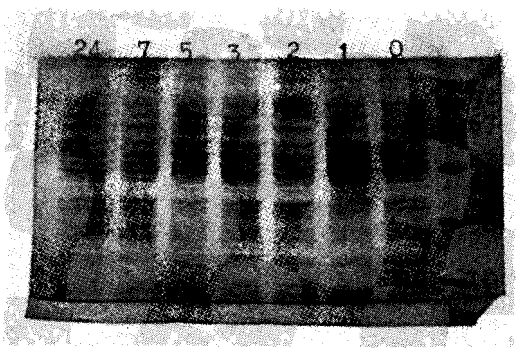

Fig 4—b) 12.5% SDS-PAGE for the sonicate supernatants of DH5α (containing PBV220) at different hours at 42°C. Proteins were detected by staining with Coomassie blue.

The results indicated that GST of *S. japonicum* (Philippine strain) could be expressed not only by IPTG induction, but also by the temperature-dependent method. Our experiment provided a method for the use of PBV220 as an efficient temperature-dependent vector for the expression of GST antigen of the Chinese strain.

ACKNOWLEDGEMENTS

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