

Construction and Expression of Recombinant Streptolysin-O and Preevaluation of Its Use in Immunoassays

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Commercially available immunoassays for assessment of anti-streptolysin-O antibodies use native streptolysin-O obtained by a complex process. We prepared a biologically active recombinant streptolysin-O with higher yield and a simpler purification process. An enzyme-linked immunosorbent assay developed with this recombinant showed good correlation with a commercial test, suggesting that it could be suitable for immunoassays.

Streptolysin-O (SLO) is a pore-forming cytolysin secreted by most strains of *Streptococcus pyogenes* (4). Patients infected with *S. pyogenes* develop antibodies to SLO, and strong antibody responses to SLO have been shown to correlate with the onset of acute rheumatic fever and acute poststreptococcal glomerulonephritis. Titration of anti-SLO antibodies in sera of patients with recurrent infection with group A streptococci is then important for risk factor assessment (16). Most commercially available immunoassays for assessment of anti-SLO antibodies in sera use native SLO purified from *S. pyogenes* cultures (2, 3). However, purification of native SLO has several drawbacks: it has very low yield and considerable batch-to-batch variation, the industrial process carries biological risks, and wasting is a problem. The use of recombinant SLO (rSLO) is an alternative that could solve most of those problems.

A DNA fragment coding for SLO amino acid sequence 78 to 571 of the whole protein (5) was amplified by PCR, using appropriate primers and purified chromosomal DNA from *S. pyogenes* as template (10). Forward primer (5'-AATGGATC CCTTGCTCCCAAAGAAATGCC-3') corresponded to nucleotides 430 to 450 of the *slo* complete gene and was designed with addition of a BamHI (underlined) site at the 5' end. Reverse primer (5'-CCGGAATTCCTACTTATAAGTAATC GAACC-3') corresponded to nucleotides 1894 to 1914 and had an EcoRI site (underlined) added at the 5' end. PCRs were performed using a polymerase with proofreading activity (Vent Polymerase; New England Biolabs Inc., Hertfordshire, United Kingdom). The amplified fragment was ligated into plasmid pGEX-2T (Amersham Pharmacia Biotech, Uppsala, Sweden) previously digested with the same enzymes and transformed into *Escherichia coli* TG2 cells. Selected transformants were grown in Luria-Bertani broth, and expression of the glutathione *S*-transferase fusion protein was induced with isopropyl- β -D-thiogalactopyranoside (IPTG; Sigma, St. Louis, Mo.). Cells were recovered by centrifugation and disrupted by soni-

cation, and the soluble GST-rSLO fusion protein was affinity purified with a glutathione-Sepharose column (Amersham Pharmacia Biotech) (13). Purified protein yield was 1.5 mg/liter. Analysis of the purified product by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed two bands of similar intensity corresponding to approximately 77 kDa and 27 kDa and a third band of minor intensity of approximately 70 kDa (data not shown). The two major bands (77 kDa and 27 kDa) were consistent with the predicted sizes for GST-rSLO and GST, respectively. Immunoblot analysis demonstrated that human anti-SLO sera recognize the bands of 77 kDa and 70 kDa, whereas a mouse anti-GST serum recognizes all bands; preincubation of anti-GST mouse serum with soluble GST resulted in a marked reduction of all bands in the immunoblot (Fig. 1). These results confirm that all three bands contain GST and that the two bigger bands were different forms of GST-rSLO fusion, while the 27-kDa band seems to correspond to GST alone. The shorter fusion may be a degradation product or a modified form of the fusion protein due to alternative translation termination sites within the *slo* gene, resulting in the expression of shorter fragments of SLO (11, 14). The appearance of a product with a molecular weight corresponding to GST that is only recognized by anti-GST serum could be the result of sensitivity of the hybrid protein to host's proteases or early stop in the transcription/translation process. Similar results have been reported with other proteins fused to GST (12, 13). Early stop in the transcription/translation process could arise from the presence in the fused gene of codons of low usage in *E. coli*, generating a pause in the ribosome traffic when these codons are reached (8). Changes in the codon usage to fit with the codon bias of the host organism could be an appropriate strategy to improve the expression of a heterologous gene (6, 15), presumably by securing to a straightforward ribosome traffic.

Titration of the hemolytic activity of the purified material was assayed side by side with native SLO (Wiener Lab, Rosario, Argentina) in reduced conditions, nonreducing conditions (without cysteine), and in oxidizing conditions using FeCl₃ (0.012%) as previously described (1). The hemolytic activity of native and recombinant SLO were similar, showing biological activity in reducing conditions (1,600 and 25,600, respectively) that was abrogated in nonreducing or oxidizing conditions as expected (4). Specific hemolytic activity of purified native SLO has been re-

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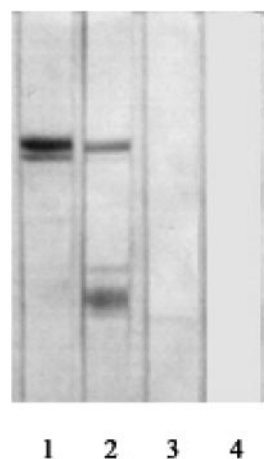


FIG. 1. Analysis of the purified extract by immunoblotting. The material eluted from the glutathione-Sepharose column was separated according to molecular weight on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immediately transferred to nitrocellulose for analysis by antigenic recognition. Nitrocellulose strips were assessed with the following: lane 1, anti-SLO human serum 1/500 and developed with an anti-human IgG conjugated to alkaline phosphatase 1/1,000; lane 2, mouse anti-GST serum 1/200 and developed with an anti-mouse IgG conjugated to alkaline phosphatase 1/1,000; lane 3, mouse anti-GST serum previously incubated with GST (0.0075 mg/ml) and developed with anti-mouse IgG conjugated to alkaline phosphatase 1/1,000; lane 4, negative control.

ported as 8×10^5 IU/mg of protein (1), and for a different rSLO a value of 1×10^5 IU/mg has been reported (9). Our preparation had a specific hemolytic activity of 1×10^8 IU/mg. This finding was surprising, since GST does not have hemolytic activity and it represents approximately one third of the total molecular weights of the fusion.

Thirty-four human sera obtained from patients with recent *S. pyogenes* infection (collected at the University Hospital, School of Medicine, Universidad de la República, Uruguay) were assessed using either a specially designed enzyme-linked immunosorbent assay (ELISA) that uses the GST-rSLO and a latex agglutination test (Rheumagen ASO, Biokit Inc., Barcelona, Spain) that uses native SLO (3). Titers obtained by latex agglutination technique have discontinuous distribution, and those obtained by ELISA are continuously distributed; thus, the ELISA results were made discrete for comparison. Compared in this way, anti-SLO titers obtained by both methods showed good correlation ($r = 0.9646$; Fig. 2). However, when we applied the Wilcoxon rank sign test for a paired experiment (7), we did not find significance to the null hypothesis that proposes that results were the same by either method. This lack of correlation could be attributed to the intrinsic error of the latex technique (\pm one dilution) and to differences in the dilution schemes employed by the two assays.

In summary, our results suggest that the GST-rSLO obtained is a suitable antigen for the specific detection of anti-SLO antibodies in human serum samples without any further processing, which could replace the native molecule in diagnostic assays. Also, these results suggest that the ELISA method could be an acceptable alternative for quantification of anti-SLO antibodies. Work aimed at scaling up the production

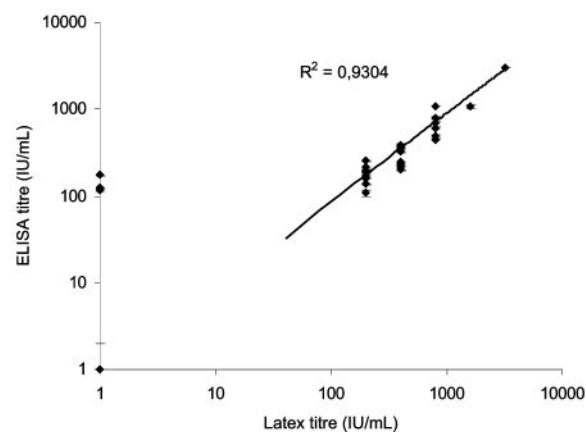


FIG. 2. Correlation between anti-SLO antibody titers measured by ELISA and by latex agglutination. ELISA and latex results were plotted, and their statistical relationships were assessed using log-transformed values.

as well as at improving the purity of the protein is currently under way in our laboratory.

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