ful for recombinant vaccine preparation considering this specificity. Two of our Mabs were used as immunoaffinity chromatography ligands yielding high purity recombinant HBsAg.

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BACTERIAL EXPRESSION, ISOLATION AND PURIFICATION OF A DIAGNOSTIC RECOMBINANT PROTEIN REPRESENTATIVE OF HIV-1 gp120 (TAB1).

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INTRODUCTION

The production of viral proteins by genetic engineering is by all means essential for the development of new diagnostic methods. The recombinant protein TAB1 is an artificial construction comprising an antigenic determinant common to HIV-1 gp120 plus several V3 loops from different viral isolates (1). Here we report a detailed process for the production and purification of this recombinant protein expressed as cytoplasmic insoluble inclusion bodies in *E. coli.*

EXPERIMENTAL PROCEDURES

The TAB1 insert was cloned into the pPF-15 vector (1), bearing a tryptophan promoter that drives expression of cloned genes as fusion proteins with 58 aa of human IL-2 in their N-terminus. The *E. coli* strain K-12 W-3110 was used as host. All fed-batch process was done in 50 L fermenters (B.E. Marubishi, Japan), at 37°C, 1 vvm, and 600 rpm. A complex media supplemented with 0.50 g/L of ampicillin was used. In the fed-batch step, the feed solution (yeast extract 12 g/L, glucose 200 g/L) was added when a cell weight of 0.37 g/L was achieved. In all cases the culture time was 12 h, and the induction of the tryptophan promoter was made by depletion of the initial tryptophan concentration of the culture medium. Cellular mass was reported as dry weight (g/L). Expression was estimated by SDS-PAGE densitometry. Cells were harvested by centrifugation. Cell pellets were suspended in TE buffer and lysed by sonication on ice. The isolated inclusion bodies were washed with different buffers and caotropic solutions (2). Finally, the inclusion bodies were completely solubilized in TE buffer containing 8 M guanidine and purified by RP-HPLC, using a C_4 10x250 mm, 0.015 mm BAKERBOND (USA) reverse phase semipreparative column. For analysis, a C_4 4.6

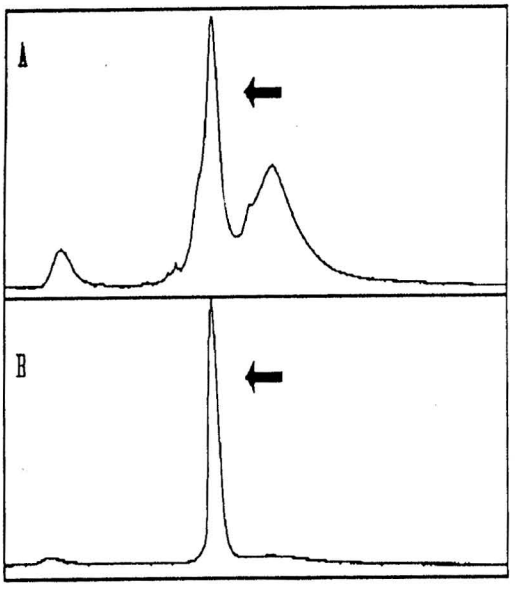

Fig.1. Chromatography profile of TAB 1 (gp120-r) by RP-HPLC. (A)- Initial soluble extract in a semipreparative column. (B). Final purified material in an analytical column.

x 100 mm, 0.005 mm BAKERBOND (USA) reverse phase column was used. All purifications were done using linear elution gradients.

RESULTS AND DISCUSSION

TAB1 is expressed in *E. coli* as insoluble inclusion bodies lacking a distinct membrane. After 8 hours of cultivation the expression of the recombinant protein started due to depletion of the initial tryptophan concentration. The highest yield was obtained after 12 h of culture. At this time, 10 g of cells/L were obtained, with TAB1 being 20% of the total cellular protein. The intact granules separated from the cellular lysates by centrifugation were semipurified by a wash pellet cell procedure. Washing the pellet with different solutions removes a great quantity of insoluble impurities and some of the cellular debris. Using this procedure a material with a purity higher than 70% was obtained. The purification of the TAB1 was done by reverse-phase HPLC. The larger collected fraction in figure 1A corresponds with TAB1, and is easily separated from other proteins. The purified antigen (figure 1B; 97% purity) has been used in the development of a diagnostic assay system, showing good specificity and sensitivity.

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A RECOMBINANT TMPA FUSION PROTEIN. EFFECT OF IL-2 AND HISTIDIN DOMAINS ON EXPRESSION

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INTRODUCTION

Treponema Membrane Protein A (TMPA) is one of the most successful diagnostic markers for Syphilis (1). A recombinant TMPA has been expressed by us in *E. coli* as a cytoplasmic soluble protein, using the pPF-15 vector (2). The resulting protein is a fusion of the 345 aa of TMPA and 58 aa of human IL-2 at its N-terminus (3). Its nature and characteristics have made difficult to scale-up the purification process. In this report we explore the addition of histidine domains to the protein, as a way to use immobilized Metal Affinity Chromatography for purification. We also evaluate the importance of the IL-2 fragment in expression.

MATERIALS AND METHODS

Plasmid construction

The pPF-15 plasmid (2), bearing the tryptophan promoter, the T4 terminator, and the N-terminus IL-2 fragment was modified so as to: (a) include 6-histidine domain encoding sequences either at the amino- or carboxi terminus of the expressed protein, (b) remove the IL-2 sequence, while including the aforementioned histidine domains,. The TMPA encoding DNA sequence, obtained by PCR, was cloned into the five different plasmids, and used to transform the *E. coli* strain W3110. The cells were plated in 2XYT medium with 0.1 mg/mL of ampicillin, and colonies screened by PCR. Selected positive clones were checked by sequence to determine correct frame for the amino and carboxiterminal histidine domains.

Expression

Positive clones were grown in 10 mL 2XYT medium with 0.1 mg/mL of ampicillin for 8 h, followed by the inoculation at 0.1 O.D. of 50 mL of M9 medium, supplemented with 0.5% of glucose and 0.5% of casaminoacids. Four hours later, expression was induced by adding 0.02 mg/mL of 3-β indoleacrylic acid and the culture was allowed to grow for four more hours. Expression was checked by using SDS-PAGE and Western blot (W.B.) using a reactive sera pool obtained from ten infected patients.

RESULTS AND DISCUSSION

All constructions gave positive clones, as shown by PCR and sequence. In every case, at least 5 clones were used to test expression levels. No expression could be demonstrated by SDS-PAGE or W.B. when the N-terminus IL-2 fragment encoding sequence was absent. If