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

the IL-2 sequence was present, but preceded by a six-histidine domain, expression was very low. Only the construction with the IL-2 fragment N-terminus sequence, an the six histidine domain at the carboxiterminus showed high expression levels of TMPA (20% of total bacteria protein), and the protein was recognized by the specific serum in W.B.

We, and others, have discussed that the presence of a human IL-2 encoding sequence, 5' to the protein of interest, provides an excellent stabilizer for protein expression in *E. coli*, either by conferring high stability to mRNA, or by reducing the probability of proteolysis (2, 4-5). This same effect has been found with the five other different viral proteins, and immunoglobulin fragments we have cloned into pPF-15, and its modified versions lacking the IL-2 fragment (unpublished results). Now we also show that the effect of the IL-2 sequence at the N-terminus can be partially affected by a preceding six-histidine domain.

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HIGH EXPRESSION LEVEL IN E. coli OF HTLV-I CORE PROTEINS

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INTRODUCTION

The Human T cell Leukemia Lymphoma virus type 1 (HTLV-I) was the first human retrovirus to be discovered (1). It is associated to adult T cell leukemia/lymphoma (ATLL) and a progressive neuromyelopathy named Tropical Spastic Paraparesis (TSP) (2). The antibodies of infected individuals are predominantly against the core proteins (p19 and p24) and the envelope protein gp46 (3). These proteins have been cloned in *E. coli*, but the expression levels obtained have been very low, less than 1% (4), in the specific case of p19-p24 and under the lactose promoter the expression level was 0.3% (5). In this work we cloned and expressed the core protein p19 and p24 fussed to IL-2 fragment, under tryptophan promoter, and the expression level obtained was 10% of total bacterial protein.

MATERIALS AND METHODS

The DNA fragment of 1042 bp corresponding to p19 and p24 proteins was amplified by PCR using specific primers. The fragment was cloned in the expression vector pR2M6 and transformed in the *E. coli* strain GC366. All the procedures for PCR, cloning and the analysis of the clones have been previously described (6). For the sequence analysis the Pharmacia sequencing Kit was used, following the manufacturer instructions. In one case, the induction of the tryptophan promoter was done in minimal media supplemented with glucose and casaminoacids at 37°C, by adding 0.012 mg/mL of ß-Indoleacrilyc acid after 2 h of the inoculation and growing the culture for 4 h at 37°C. Also, recombinant cells were inoculated in Luria Broth supplemented with 0.1 mg/mL of tryptophan and the culture was growth for 8 h at 37°C. The expression analysis was made by SDS-PAGE and Western blot (W.B) (7). In the latter, a sera from an infected patient diluted 1/10 in PBS-5% skim-milk and free of anti-E. coli antibodies, was used.

RESULTS AND DISCUSSION

The Plasmid named pGAG1, was obtained from the cloning of the PCR product corresponding to the p19-p24 gene in the pR2M6 expression vector. The clone was analyzed using the restriction enzymes XbaI and BamHI and by partial sequence of the ends of the gene, corroborating in frame insertion and absence of any mutation and stop codons. The induction of the clone in the E. coli strain GC366 by the two methods evaluated gave the following results. With minimal media and ß-Indoleacrilyc acid as inducer the expression was less than 1%, as previously reported for other cases. With rich media and the depletion of the repressor as induction method we obtained an increase in the expression