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Purification of human alpha-L-fucosidase precursor expressed in *Escherichia coli* as a glutathione S-transferase fusion protein

Alejandro de Carlos*, Dolores Montenegro, Ana Alonso-Rodríguez, María Páez de la Cadena, Francisco Javier Rodríguez-Berrocal, Vicenta Soledad Martínez-Zorzano

Department of Biochemistry, Genetics and Immunology, Faculty of Sciences, University of Vigo, 36200 Vigo, Spain

Abstract

Alpha-L-fucosidase (FUC) is a glycosidase involved in the degradation of fucose-containing glycoconjugates. A cDNA representing the complete sequence of human FUC was inserted into the prokaryotic expression vector pGEX-2T. High levels of the glutathione S-transferase (GST) fusion protein were detected in *Escherichia coli* cells after induction with isopropyl thio-beta-D-galactopyranoside. The GST-FUC protein was mostly found as inclusion bodies and attempts to optimise its expression as a soluble form were unsuccessful. Nevertheless, the recombinant protein was purified by affinity chromatography on glutathione-sepharose and its fucosidase activity was characterised. After thrombin cleavage of the GST tag, the FUC precursor protein was purified by electro-elution.

Keywords: *Escherichia coli*; α-L-Fucosidase precursor; Glutathione S-transferase fusion protein

1. Introduction

Alpha-L-fucosidase (FUC) (EC 3.2.1.51) is a glycosidase that cleaves fucose from the non-reducing end of oligosaccharides, glycoproteins and glycolipids. The human enzyme has been purified from a variety of cells, fluids and tissues, and its kinetic, electrophoretic, and immunological properties have been studied extensively (for review see Ref. [1]). Human alpha-L-fucosidases are sialoglycoproteins which contain ~10% carbohydrate, by weight. Although the exact functions of the carbohydrate component are unknown, it has been suggested that glycosylation affects the stability and kinetic properties of the enzyme as well as protein folding, intracellular transport, secretion and the generation of multiple molecular forms. Alpha-L-fucosidase is a multimeric protein which is usually derived from two closely related polypeptide subunits of ~50 000–60 000 which differ in carbohydrate composition and possibly other post-translational modifications [1]. Cloning studies of the human fucosidase gene provide evidence for only one structural gene, denominated *FUCA1*, which includes eight exons spanning 23 kb [2], and has been assigned to the distal region of 1p34 by in situ hybridisation [3]. A number of cDNA clones containing the coding sequence and poly (A) region of human alpha-L-fucosidase have been isolated [4,5]. The open reading frame encodes a protein of 461 amino acids consisting of a 22 amino acid signal peptide and a processed mature enzyme of 439 amino acids [5]. Although expression

*Corresponding author. Tel.: +34-986-812-576; fax: +34-986-812-556.
E-mail address: adcarlos@uvigo.es (A. de Carlos).

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of an active human fucosidase has been achieved in eukaryotic systems [6,7], there have been no reports of successful expression of alpha-L-fucosidase using prokaryotic vectors. In this investigation, human alpha-L-fucosidase precursor was expressed in *Escherichia coli* cells as an enzymatically active glutathione S-transferase (GST) fusion protein. After thrombin cleavage of the GST tag, purified recombinant human alpha-L-fucosidase precursor was obtained.

2. Experimental

2.1. Recombinant DNA technology

Restriction endonuclease digestions, DNA ligations, and plasmid DNA preparations were performed following standard procedures [8]. Modifying enzymes were obtained from Amersham Biosciences (Barcelona, Spain). DNA was amplified by the polymerase chain reaction (PCR) using AmpliTaq FS DNA polymerase from Perkin-Elmer Biosystems (Foster City, CA, USA). The reactions were carried out in a final volume of 100 μl with 0.5 μ*M* of each oligonucleotide, 0.2 m*M* of each of the four dNTPs, 2.5 U of the enzyme, and 500 ng of a double stranded plasmid DNA, which includes the human fucosidase coding sequence, as template. Amplifications were performed in a GeneAmp PCR System 2400 from Perkin-Elmer (Foster City, CA, USA) with an initial denaturation step at 94 °C, 5 min followed by 30 cycles of 94 °C, 1 min; 65 °C, 1 min and 72 °C, 3 min; and a final extension at 72 °C for 7 min.

2.2. Bacterial strains

The *E. coli* strain TG2 (*supEhsd*Δ5 *thi* Δ(*lac-proAB*) Δ(*srl-recA*)306::Tn*10* (*tet*^{r}) F′ (*traD*36 *proAB*$^{+}$ *lacI*^{q}*lacZ* ΔM15)), a TG1 derivative deficient in recombination [8], was used routinely in all the DNA manipulation procedures. The *E. coli* strain BL21 (*hsdSgal* (λ*cIts*857 *ind*1 Sam7 *nin*5 *lac*UV5-T7 gene *1*)) was used as host for the expression of the human alpha-L-fucosidase. Unless otherwise stated, bacteria were grown at 37 °C in Luria-Bertani (LB) broth [8] supplemented with 0.1 mg/ml ampicillin (Roche Diagnostics, Barcelona, Spain) for the selection of transformants.

2.3. Human alpha-L-fucosidase cDNA

A full length cDNA clone coding for the human alpha-L-fucosidase inserted into the *Eco*R I site of the plasmid pUC19 was a generous gift from Dr Donald S. Anson (Women's and Children's Hospital, North Adelaide, South Australia). The cDNA molecule was ~1.4 kb in size, and it contained an internal *Eco*R I site close to the 3′ end, 11 bp of 5′ untranslated sequence, and 11 bp of 3′ untranslated sequence as well as the complete reading frame. The alpha-L-fucosidase coding sequence was amplified by PCR and inserted into the *Bam*H I site of the expression vector pGEX-2T to form plasmid pGEX-FUC. The complete coding sequence for human alpha-L-fucosidase from cDNA clones is available in the GenBank database with the accession number M29877.

2.4. DNA sequencing

In order to verify the insertion and the orientation of the alpha-L-fucosidase cDNA molecule, the nucleotide sequence of the pGEX-2T vector and the cDNA insert junction were confirmed by DNA sequencing. DNA from plasmid pGEX-FUC was purified using the Flexiprep kit (Amersham Biosciences). Cycle sequencing was performed by the dideoxy chain termination method using the Cy5 Thermo Sequenase Dye Terminator kit and the 5′ pGEX sequencing primer (Amersham Biosciences). The resulting reactions were analysed in a ALFexpress II DNA Analysis System using the ALFwin Sequence Analyser 2 software (Amersham Biosciences).

2.5. Expression of the GST-fucosidase fusion protein in E. coli

Plasmid pGEX-FUC was used to transform competent *E. coli* BL21 cells and transformants were selected on LB plates supplemented with ampicillin according to standard protocols [8]. A colony carrying the pGEX-FUC plasmid DNA was grown overnight in LB medium containing ampicillin and 2%

(w/v) of glucose (Sigma, Madrid, Spain). This culture was diluted 1:30 in 200 ml of fresh medium, grown with vigorous agitation to mid logarithmic phase (absorbance detection value at 600 nm of 0.6–0.8), and induced at 25 or 30 °C by the addition of isopropyl thio-beta-D-galactopyranoside (IPTG, Amersham Biosciences) to a final concentration of 0.1 m*M*. At the appropriate times, aliquots of 3 ml of the culture were collected, further centrifuged, and the bacterial pellets re-suspended in 0.5 ml of phosphate-buffered saline (PBS; 140 m*M* NaCl, 2.7 m*M* KCl, 10 m*M* Na_2HPO_4, 1.8 m*M* KH_2PO_4, pH 7.3). Then 400 μl of each re-suspended bacterial pellet were lysed by sonication in the presence of a protease inhibitor cocktail (Complete, Roche Diagnostics) using a Sonifier Cell Disruptor 450 (Branson) with a 2.5-mm probe. Soluble (supernatant) and insoluble (pellet) fractions were separated by centrifugation at 12 000 *g* for 15 min at 4 °C and pellets were re-suspended in PBS. The protein concentration of the samples was determined by the Bradford method [9], using bovine serum albumin as standard.

2.6. Purification of the GST-fucosidase fusion protein

The GST-FUC fusion protein was affinity purified using a glutathione sepharose 4B matrix (GS4B; Amersham Biosciences) following a batch method previously described [10]. The appropriate volume of a 50% GS4B beads suspension was added to an aliquot of a re-suspended bacterial pellet and mixed gently for 1 h at 4 °C, followed by a brief centrifugation (500 *g*, 5 min at 4 °C) and the removal of the supernatant. After extensive washing with PBS, the bound proteins were eluted from the beads by an overnight incubation in one bed volume of 100 m*M* Tris–HCl, pH 8.5, with 7.5 m*M* reduced glutathione (Amersham Biosciences). The supernatant containing the purified GST-FUC protein was recovered after centrifugation in the same conditions as described above.

2.7. Polyacrylamide gel electrophoresis

Crude lysates, bacterial pellets and supernatants, fractions obtained in the GS4B chromatography and purified samples were subjected to polyacrylamide gel electrophoresis with SDS (SDS–PAGE) in a discontinuous pH system [11], using a Miniprotean II apparatus (Bio-Rad) with a 4% stacking gel and a 10% separating gel. A set of low molecular mass protein standards (Amersham Biosciences) containing phosphorylase b (97 000), bovine serum albumin (66 000), ovalbumin (45 000), carbonic anhydrase (30 000), trypsin inhibitor (20 000) and alpha-lactalbumin (14 000), was included. Total proteins were visualised by staining the gels for 30 min in fixative solution (40% (v/v) methanol (Merck, Darmstadt, Germany) and 10% (v/v) acetic acid (Merck)) containing 0.1% (w/v) Coomassie Brilliant Blue R-250 (Sigma), and destaining by several changes in the same solution without Coomassie Brilliant Blue R-250.

2.8. Western blotting

After SDS–PAGE, gels were allowed to equilibrate for 30 min in transfer buffer (25 m*M* Tris–HCl, pH 8.3, 192 m*M* glycine, 20% (v/v) methanol). Proteins were transferred to 0.45-μm pore size polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore) using a Mini trans blot electrophoresis cell (Bio-Rad), following a standard method [12], at 100 V for 60 min at 4 °C. A set of broad range pre-stained molecular mass standards from Bio-Rad: myosin (208 000), beta-galactosidase (105 000), bovine serum albumin (80 000), ovalbumin (50 000), carbonic anhydrase (35 000), trypsin inhibitor (28 000) and lysozyme (20 000), was included in the electrophoresis gels and transferred to the PVDF membranes.

For the detection of the GST-FUC protein, the membranes were processed using, as primary antibody, an anti-GST antiserum (Amersham Biosciences) diluted 1000-fold in blocking solution (5% (w/v) non-fat dry milk in PBS). An anti-goat IgG alkaline phosphatase conjugate (Roche Diagnostics), diluted 1:2500 in blocking solution, was used as second antibody. For alpha-L-fucosidase protein detection, a rabbit antiserum raised against an alpha-L-fucosidase from human placenta (Sigma) was used as primary antibody (at a 1:500 dilution), followed by a 2000-fold dilution goat anti-rabbit IgG alkaline phosphatase conjugate (Bio-Rad). Protein bands, to which antibodies were bound, were visualised by

incubation of the membranes in a solution containing a mixture of nitroblue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate toluidine salt (BCIP) in 100 m*M* Tris–HCl, pH 9.5, following the manufacturer's suggestions (Roche Diagnostics).

2.9. Analysis of gels and membranes

Dried polyacrylamide gels and PVDF membranes were processed using a Scanjet 5370C desk-top scanner (Hewlett-Packard) and subjected to computerised image analysis. The relative molecular mass (M_r) of each protein band was calculated according to the migration rates of the protein standards, using the Image Master 1D Elite software version 2.01 (Amersham Biosciences).

2.10. Thrombin digestion

An aliquot containing 200 μg of the purified GST-FUC protein was mixed with 20 cleavage units of thrombin (Amersham Biosciences) and incubated overnight at room temperature. The degree of enzymatic cleavage was followed by SDS–PAGE.

2.11. Electro-elution

The thrombin digestion reaction mixture was submitted to preparative SDS–PAGE. After visualising the protein bands, the gel slice containing the FUC protein was excised and cut into small pieces, which were placed into the elution chamber of a model 422 Electro-Eluter (Bio-Rad). Alpha-L-fucosidase protein was eluted at 100–150 V for 2 h using elution buffer (25 m*M* Tris–HCl, pH 8.3, 192 m*M* glycine, containing 0.1% (w/v) SDS) and following the manufacturer's instructions. In order to remove SDS (Sigma) from the preparation, the elution procedure was continued for another 2 h using fresh elution buffer without SDS. After the elution was completed the FUC protein was recovered in a minimal amount of the same buffer and its purity checked by SDS–PAGE.

2.12. Assay of fucosidase activity

Enzyme assays were always performed under linear conditions as far as the amount of protein and time of incubation were concerned. Alpha-L-fucosidase activity was determined using the fluorogenic substrate 4-methyl-umbelliferyl-α-L-fucopyranoside (4-MU-fucoside; Sigma), following a method previously described [13]. Alpha-L-fucosidase activity was expressed as specific activity (U/mg). One unit of enzyme (U) was defined as the amount of enzyme required to hydrolyse 1 μmol of substrate per minute at 37 °C under optimal conditions. Optimal pH was determined using citrate-phosphate and phosphate buffers at different pH values ranging from 3.0 to 5.5 and from 5.0 to 8.0, respectively. Kinetic studies were performed using the substrate 4-MU-fucoside at final concentrations ranging from 0.12 to 2.60 m*M*. Apparent Michaelis-Menten constant (K_m) and maximal velocity (V_{max}) values were calculated from the Hanes-Woolf representation using the linear regression data analysis program Enzpack 3.0 (Biosoft, 1989).

3. Results

3.1. Construction of the recombinant plasmid pGEX-FUC

For the expression of the human alpha-L-fucosidase in *E. coli*, the prokaryotic vector pGEX-2T was used. *Bam*H I restriction sites were created at both ends of the alpha-L-fucosidase cDNA molecule by PCR, using the oligonucleotide primers CCGGATCCATGAGGTCGCGGCCG (5′ primer) and CCGGATCCTTACTTCACTCCTGTCAG (3′ primer) and a pUC19 plasmid DNA carrying the alpha-L-fucosidase cDNA insert (see Experimental section) as template. The PCR product was digested with *Bam*H I and ligated to *Bam*H I-digested, alkaline phosphatase-treated pGEX-2T. The resulting ligation mixture was used to transform *E. coli* TG2 competent cells. Restriction endonuclease analysis allowed the selection of bacterial clones containing the insert in the appropriate orientation. The identity of one of the clones was confirmed by DNA sequencing of the plasmid-insert junction. The construct obtained was therefore designated as pGEX-FUC (Fig. 1). Messenger RNA transcripts initiated at the P*tac* promoter of pGEX-FUC would encode a fusion protein consisting of 226 amino acids from

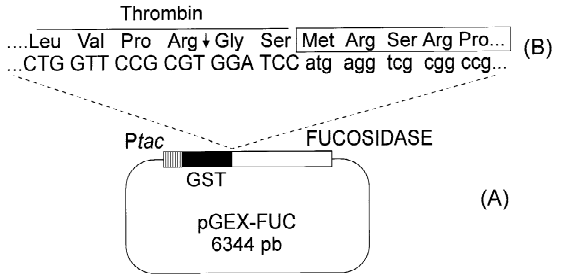

Fig. 1. Structure of the recombinant plasmid pGEX-FUC. (A) Scheme of the pGEX-FUC plasmid containing the fucosidase cDNA under transcriptional regulation of the *tac* promoter (P*tac*). (B) Detail of the fusion region between the nucleotide coding sequences of GST (uppercase) and fucosidase (lowercase) proteins. Amino acids are indicated in three letter code. Fucosidase amino acids are boxed. Thrombin protease recognition sequence is indicated and the arrow shows the cleavage site.

the N-terminus of the GST protein fused with the entire human alpha-L-fucosidase protein (461 amino acids).

3.2. Expression of the GST-fucosidase fusion protein in E. coli

The human alpha-L-fucosidase coding sequence was expressed as a GST fusion protein by transformation of *E. coli* BL21 cells with plasmid pGEX-FUC and induction with IPTG. The time course of the GST-FUC protein synthesis was followed by removing aliquots of a culture at different intervals after IPTG addition, lysing the cells in protein sample buffer for 5 min at 100 °C, and analysing the lysates by SDS–PAGE (Fig. 2). The synthesis of a protein of ~76 000, consistent with the sum of the molecular masses of GST (26 000) and FUC precursor (51 000) as predicted by translation of the corresponding nucleotide sequences, was induced in *E. coli* cells containing plasmid pGEX-FUC (lanes 1–6), but not in cells containing the parental vector pGEX-2T (lane 7). The amount of GST-FUC protein synthesised increased with prolonged incubation, such that at 3 h after induction (lane 6) it constituted ~20% of the total cellular proteins, as estimated from the Coomassie stained polyacrylamide gel by image analysis.

3.3. Alpha-L-fucosidase activity in E. coli cells expressing the GST-FUC fusion protein

To determine whether the GST-FUC protein was enzymatically active an in vitro assay was used to measure the levels of alpha-L-fucosidase activity in lysates of *E. coli* cells. As it can be seen in Fig. 3, *E. coli* cells transformed with the expression vector pGEX-2T exhibited no alpha-L-fucosidase activity. In contrast, in IPTG-induced cells containing the plasmid pGEX-FUC increasing levels of alpha-L-fucosidase activity along the induction time were detected.

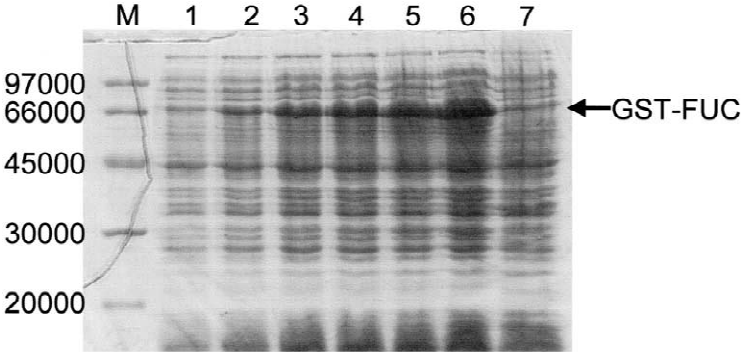

Fig. 2. Expression of the GST-FUC fusion protein in *E. coli* cells. Lanes 1–6 show lysates from *E. coli* BL21 cells transformed with pGEX-FUC and induced with 0.1 m*M* IPTG for 0 (lane 1), 30 (lane 2), 60 (lane 3), 90 (lane 4), 120 (lane 5) and 180 (lane 6) min. Lane 7 shows a lysate from *E. coli* BL21 cells transformed with pGEX-2T. Lane M corresponds to the protein standards with their molecular mass values indicated on the left side.

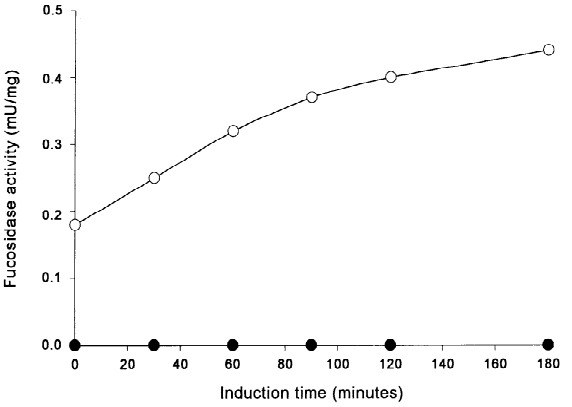

Fig. 3. Fucosidase activity in *E. coli* cells expressing the GST-FUC fusion protein. The fucosidase activity was assayed in *E. coli* BL21 cell lysates along the fusion protein induction period. The specific activity values in IPTG induced cells transformed with pGEX-2T (filled circles) or with pGEX-FUC (open circles) are shown.

3.4. Purification of the GST-fucosidase fusion protein

Bacterial lysates obtained from cultures grown at 30 °C and induced with IPTG for 180 min (Fig. 4, lane 1) were separated by centrifugation in soluble (supernatant) and insoluble (pellet) fractions. Although some amount of the induced fusion protein was found in the supernatant (Fig. 4, lane 2), most of it was localised in the pellet fraction (Fig. 4, lane 3). This accumulation of the GST-FUC protein in the insoluble fraction suggested that it was sequestered within inclusion bodies inside the cells. In order to reduce the formation of insoluble aggregates the expression conditions were varied. Shorter expression periods, lower expression temperature and increased aeration of the cultures were tried but none of these conditions resulted in an increased solubility of the fusion protein (data not shown). Therefore, the GST-FUC protein was purified from bacterial pellets by affinity chromatography on glutathione sepharose 4B. Proteins bound to GS4B beads were eluted from the matrix by the addition of reduced glutathione and analysed on SDS–PAGE. As shown in Fig. 4, lane 4, a major band migrating at the expected size of the GST-FUC protein (76 000) was observed. In addition, several minor bands of lower molecular mass were also present. These bands could correspond either to proteolytic degradation products of the GST-FUC fusion protein or to carboxy-terminal truncated forms.

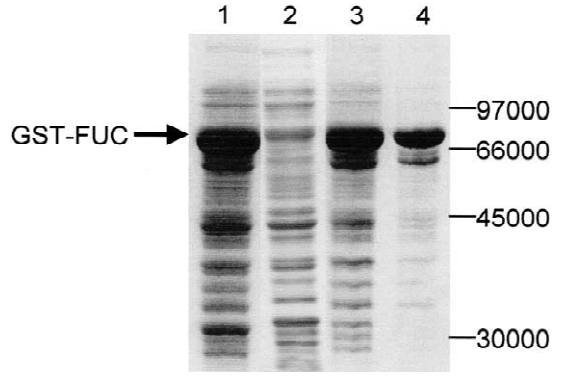

Fig. 4. Purification of the GST-FUC fusion protein synthesised in *E. coli* cells. Lane 1, lysate of *E. coli* cells grown at 30 °C and induced with IPTG for 180 min. Lanes 2 and 3, supernatant and pellet, respectively, obtained from the bacterial lysate. Lane 4, GST-FUC fusion protein purified by GS4B affinity chromatography. Molecular mass values of the protein standards are shown on the right.

By the one-step purification method described, 65 mg of GST-FUC protein were obtained from 1 l of bacterial culture.

3.5. Characterisation of the alpha-L-fucosidase activity of the purified GST-FUC fusion protein

The fucosidase activity of the affinity-purified GST-FUC protein was characterised by investigating the effects of pH and substrate concentration on its ability to hydrolyse the substrate 4-MU-fucoside. The GST-FUC protein showed a broad pH activity curve (Fig. 5A) with a maximum at pH 5.0 and activity levels higher than 60% at pH values between 4.5 and 6.5. The apparent K_m and V_{max} values for the mentioned substrate, determined by the Hanes-Woolf plot (Fig. 5B), were estimated to be 0.18 ± 0.01 m*M* and 7.90 ± 0.19 U/mg, respectively (mean $\pm$ SD for three independent experiments).

3.6. Purification of the recombinant human alpha-L-fucosidase precursor

For the purification of the recombinant human alpha-L-fucosidase precursor, the existence in the pGEX-2T vector of a target sequence for thrombin

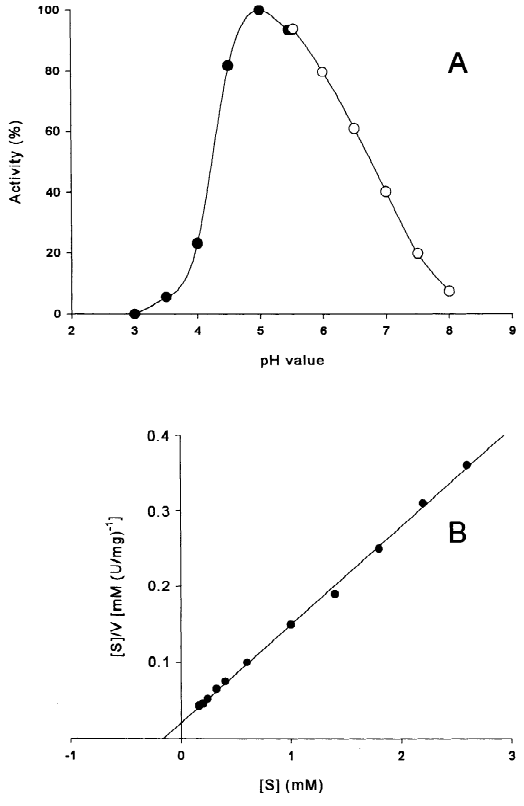

Fig. 5. Characterisation of the alpha-L-fucosidase activity of the purified GST-FUC protein. (A) Effect of pH. Enzyme activity was determined using citric-phosphate (closed circles) and phosphate (open circles) buffers. (B) Effect of 4-MU-fucoside concentration. Hanes-Woolf representation is shown.

was taken advantage of, so that the GST-FUC fusion protein was digested with this protease. Fig. 6 shows the results of SDS–PAGE and immunodetection with an anti-GST antiserum of the thrombin digestion of the GST-FUC protein. Cleavage of the GST-FUC protein led to the formation of one band of 26 000 (lane 2), which was recognised by the antiserum (lane 4) and corresponds to the GST protein, and another band with a M_r value of 51 000 (lane 2) corresponding to the recombinant human alpha-L-fucosidase precursor protein, which consequently did not react with the anti-GST antiserum (lane 4). In addition, some amount of undigested GST-FUC fusion protein was still present (lanes 2 and 4).

The digestion mixture of the GST-FUC protein was subjected to preparative electrophoresis and alpha-L-fucosidase was recovered by electro-elution

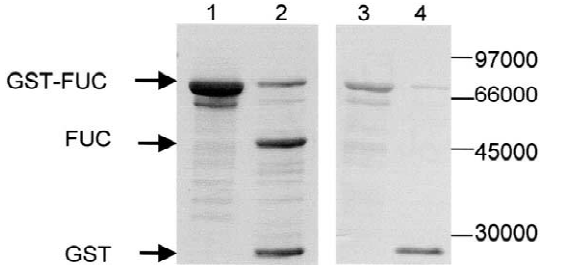

Fig. 6. Thrombin digestion of the GST-FUC fusion protein. Lanes 1–2, SDS–PAGE. Lanes 3–4, Western blot. Lanes 1 and 3, purified GST-FUC protein. Lanes 2 and 4, products from the thrombin digestion. Numbers on the right correspond to the molecular mass values of the protein standards.

from the corresponding gel slice. Fig. 7 shows the purified recombinant human alpha-L-fucosidase precursor as a single protein band of ~51 000 in size (lane 1), which was able to react with an antiserum raised against human placenta alpha-L-fucosidase (lane 2).

4. Discussion

Although the expression of biologically active human alpha-L-fucosidase has been demonstrated in transfected COS cells [6] and human fibroblasts [7], there are no reports to our knowledge about the expression of this enzyme in bacterial systems.

In this investigation, a cDNA representing the complete sequence of human alpha-L-fucosidase (FUC) was cloned into pGEX-2T, an *E. coli* expres-

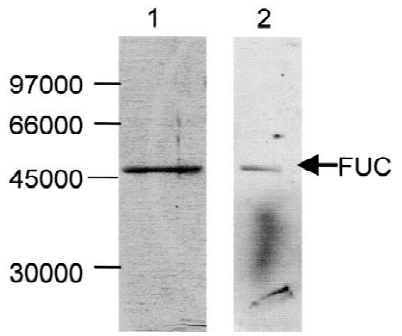

Fig. 7. Purified recombinant human alpha-L-fucosidase precursor. Lane 1, CBB staining. Lane 2, immunodetection with an anti-human alpha-L-fucosidase antiserum. Numbers on the left correspond to the molecular mass values of the protein standards.

sion vector containing the glutathione S-transferase (GST) coding region of *Schistosoma japonicum* followed by a thrombin cleavage site, to generate the recombinant plasmid pGEX-FUC. Lysates from *E. coli* cells transformed with plasmid pGEX-FUC and induced with IPTG revealed the presence of the GST-FUC protein with an apparent molecular mass of 76 000, in accordance with that expected for a fusion protein containing GST (26 000) [10] and an unprocessed FUC protein (51 000) [5].

Despite the GST expression system having been designed for inducible, high level intracellular expression of genes as soluble proteins [10], most of the expressed GST-FUC fusion protein was found in inclusion bodies. Similar results have been described for the expression of other GST fusion proteins such as canine protein phospholamban [14], human myelin oligodendrocyte glycoprotein [15] and porcine interleukin-2 [16]. Attempts to optimise the expression of GST-FUC as a soluble form, including changes in temperature and induction time, were unsuccessful. Negative results on the suppression of inclusion bodies formation have also been reported [14,15].

Purification of the GST-FUC protein from the insoluble protein fraction using glutathione sepharose 4B affinity chromatography was attempted. By this method, a major band migrating at the expected size of the GST-FUC protein (76 000) was recovered. In addition, a small proportion of lower molecular mass protein bands was also detected. These bands could correspond to proteolytic degradation products of the fusion protein that were not eliminated by the use of protease inhibitors as happened to others [15,17,18]. However, it would also be possible to consider the presence of carboxy-terminal truncated GST-FUC forms [19], as a consequence of translation multiple stops.

Purified GST-FUC protein (65 mg) was obtained from 1 l of bacterial culture. This yield is comparable to those described for other GST fusion proteins [16,20,21].

The purified GST-FUC protein showed alpha-L-fucosidase activity over a broad range of pH values, exhibiting its maximal activity at pH 5.0. This result is in agreement with data reported for most human alpha-L-fucosidases which present pH optima in the range 4.0–6.0. However, the recombinant enzyme did not show the second optimum between pH 6.0 and 7.0 which is present in the pH-activity curve of several human fucosidases (reviewed in Ref. [1]). The apparent K_m value of GST-FUC for the substrate 4-MU-fucoside (0.18 ± 0.01 m*M*) was in the range reported for most of the studied human alpha-L-fucosidases (reviewed in Ref. [1]). Since bacterial systems, in general, are not able to perform glycosylation of proteins, GST-FUC fusion protein expressed in *E. coli* will lack carbohydrate residues. Our findings suggest that the absence of glycosylation modifies the pH dependence of the alpha-L-fucosidase activity of the GST-FUC fusion protein, whereas it has no effect on its substrate affinity. Similar results were obtained when the effect of carbohydrate removal on the properties of human liver alpha-L-fucosidase was investigated [22]. However, the mentioned modified pH-dependence exhibited by the alpha-L-fucosidase activity of the GST-FUC fusion protein, characterised by the reduced activity levels at pH values between 6.0 and 7.0, could not be entirely attributed to the lack of glycosylation since this recombinant protein includes the signal peptide which is not present in the fully processed human alpha-L-fucosidase.

The cleavage of the purified GST-FUC protein, using the site specific protease thrombin, allowed the separation of the GST tag from the recombinant human alpha-L-fucosidase precursor protein, which was further recovered by electro-elution. The apparent molecular mass of the purified protein (51 000) corresponds to an unprocessed non-glycosylated protein of 461 amino acids, including 22 amino acids of the signal peptide. This size is in agreement with the reported values for the *N*-glycanase-treated alpha-L-fucosidase from human liver (45 000–48 000) [22] and from human seminal plasma (~45 000) [23], considering that these eukaryotic enzymes are mature forms which have lost their signal peptides.

5. Conclusions

Human alpha-L-fucosidase precursor was expressed in *E. coli* cells as an enzymatically active GST fusion protein which was purified by using glutathione sepharose affinity chromatography. After thrombin cleavage of the GST tag and electro-elu-

tion, purified recombinant human alpha-L-fucosidase precursor was obtained. This protein could be useful as a highly purified biological reagent for the preparation of polyclonal and monoclonal antibodies. In addition, because bacterially expressed human alpha-L-fucosidase is unglycosylated, studies investigating the role of oligosaccharides on the structure and function of this enzyme would be possible.

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