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A missense mutation ($G^{197} \rightarrow A$) in the α-L-fucosidase gene of fucosidosis patients leads to loss of α-L-fucosidase

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Fucosidosis is an autosomal recessive lysosomal storage disease resulting from the absence of α-L-fucosidase activity. Two natural missense mutations ($G^{197} \rightarrow A$) and ($A^{860} \rightarrow G$) within the α-L-fucosidase gene have been reported to be homozygous in four patients with fucosidosis. Expression of wild-type and mutated α-L-fucosidase cDNAs in COS-1 cells revealed complete deficiency of α-L-fucosidase for the $G^{197} \rightarrow A$ transition and a normal level of enzyme for $A^{860} \rightarrow G$. We therefore conclude that the change of $G^{197} \rightarrow A$ is responsible for fucosidosis in the patients while $A^{860} \rightarrow G$ is a normal polymorphic variant of α-L-fucosidase.

Keywords: α-L-fucosidase, fucosidosis, missense mutation

Introduction

α-L-Fucosidase (α-L-fucoside fucohydrolase; EC 3.2.1.51) catalyzes the hydrolysis of L-fucose from non-reducing termini of fucosylated glycans. In humans, the enzyme is found in virtually all tissues and body fluids with cellular enzyme located in lysosomes [1, 2]. Fucosidosis is an autosomal recessive, inherited disorder. The disease is characterized by lack of α-L-fucosidase activity which leads to a pathologic accumulation of L-fucose-containing compounds, progressive psychomotor retardation and premature death. Lymphoid cells and fibroblasts from a fucosidosis patient (MZ) lacked α-L-fucosidase activity and had reduced immunoreactive α-L-fucosidase protein (3% of normal) [3, 4]. Two missense mutations were reported in the α-L-fucosidase gene of this patient by direct sequencing of α-L-fucosidase cDNA [5] and were homozygous by analysis of genomic DNA (unpublished data). The same mutations were also reported in this patient and in three additional patients [6]. One mutation, $G^{197} \rightarrow A$, changed the codon (GGC) for Gly-60 to the codon (GAC) for Asp. The other mutation, $A^{860} \rightarrow G$, changed the codon (CAG) for Gln-281 to the codon (CGG) for Arg. The $A^{860} \rightarrow G$ mutation was considered a normal polymorphism and not to be responsible for disease because this mutation was homozygous in unaffected family members of fucosidosis patients [6, 7]. It was proposed that the $G^{197} \rightarrow A$ mutation was responsible for deficiency of α-L-fucosidase and disease [6]. However, this has not been demonstrated directly. In this report, the consequences of the $G^{197} \rightarrow A$ and the $A^{860} \rightarrow G$ mutations in the α-L-fucosidase gene on the expression of α-L-fucosidase and thus on disease were assessed by transfection of COS-1 cells with constructs containing these mutations.

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Materials and methods

Human B-lymphoid cell lines included B142 derived from a healthy individual and MZ from a fucosidosis patient. The establishment of B-lymphoid cell lines from peripheral blood, culture conditions and background information have been published [3, 4, 8–11]. COS-1 cells are a transformed simian fibroblast cell line and were cultivated as described [12].

A plasmid containing the full-length, wild-type, α-L-fucosidase cDNA [pSVL-Fuc (wt)] has been described [13]. Site-directed mutagenesis for construction of pSVL-Fuc ($A^{860} \rightarrow G$) and pSVL-Fuc ($G^{197} \rightarrow A$) was performed according to Deng and Nicholoff [14]. The mutagenic oligonucleotides (5′-AATTCAAGCCACGGAGCTTGCCAG-3′) and (5′-ATCCACTGGGACGTGTTCTCGGTG-3′) corresponded to the sequences surrounding the $A^{860} \rightarrow G$ and $G^{197} \rightarrow A$ mutations, respectively, with the underlined nucleotides representing the mutations. The nucleotide numbering system is from Occhiodoro *et al.* [15]. The selection oligonucleotide (5′GTCTAGACTCGAGGAGCTCG GATCC3′) contained the underlined mutations which obliterate a *Sma*I restriction site in the pSVL plasmid. Mutant constructs were confirmed by DNA sequencing [16]. Oligonucleotides were prepared by the Biopolymer Unit at Roswell Park Cancer Institute.

COS-1 cells were transfected with pSVL, pSVL-Fuc (wt), pSVL-Fuc ($G^{197} \rightarrow A$) and pSVL-Fuc ($A^{860} \rightarrow G$) by the DEAE–dextran-mediated procedure [17]. Cells were harvested with a rubber policeman. Cell pellets and media were obtained and extracted as previously described [8]. The media and cell extracts were assayed for α-L-fucosidase and β-D-galactosidase activities at pH 5 using 4-methylumbelliferyl-α-L-fucoside and 4-methylumbelliferyl-β-D-galactoside [8, 9]. α-L-Fucosidase protein was measured by an immunoassay with a polyclonal antibody to human α-L-fucosidase [18]. Cell extracts were assayed for total protein by the Bradford method [19]. A portion of each cell extract was also assayed for plasmid containing the $G^{197} \rightarrow A$ mutation. This mutation creates an *Af*1III restriction site [6]. Oligonucleotide primers S1 and A1 specifically hybridize to α-L-fucosidase cDNA and flank a DNA segment containing nucleotide-197 [7, 16]. These primers were used to amplify α-L-fucosidase cDNA in cell extracts by the polymerase chain reaction [7, 16]. The amplified DNA was digested with *Af*1III as per manufacturer's instructions (New England BioLabs, Beverly, MA) and was analyzed by agarose gel electrophoresis [6]). An *Af*1III-sensitive site was identified in amplified DNA derived from pSVL-Fuc ($G^{197} \rightarrow A$) transfected cells but not in material derived from the other cell extracts. Additionally, transfected cells were assayed for the presence of α-L-fucosidase mRNA derived from pSVL-Fuc ($G^{197} \rightarrow A$). Total RNA was isolated from cells and used for synthesis of α-L-fucosidase cDNA [7, 16]. Primers S1 and A1 were used to amplify this cDNA as described above. An *Af*1III restriction site was identified in amplified DNA derived from RNA of pSVL-Fuc ($G^{197} \rightarrow A$) transfected cells but not in material derived from RNA of the other transfected cells. The presence of the *Af*1III site reflected the presence of α-L-fucosidase mRNA transcribed from pSVL-Fuc ($G^{197} \rightarrow A$).

The secondary structures of normal and mutant

Table 1. Expression of normal and mutant α-L-fucosidase cDNAs in transfected COS-1 cells

Plasmid	α-L-Fucosidase activity		β-D-Galactosidase activity		α-L-Fucosidase protein	
	Cells[a]	Medium[b]	Cells[a]	Medium[b]	Cells[c]	Medium[d]
pSVL	110	54	472	48	75	34
pSVL-Fuc (wt)	464	720	428	62	300	464
pSVL-Fuc ($G^{197} \rightarrow A$)	112	50	401	44	81	37
pSVL-Fuc ($A^{860} \rightarrow G$)	516	847	437	47	403	676

Enzyme activity and protein measurements were made 48 h after transfection of cells with plasmids by the DEAE–dextran method. Each value is the average of two experiments.

[a]nmol/h/mg of cellular protein.

[b]nmol/hr/culture dish.

[c]ng/mg of cellular protein.

[d]ng/culture dish.

α-L-fucosidase were predicted by using the Garnier–Osguthorpe–Robson algorithm [20].

Results and discussion

To determine the effects of the $G^{197} \rightarrow A$ and the $A^{860} \rightarrow G$ mutations in the α-L-fucosidase gene on the expression of α-L-fucosidase, constructs containing these mutations were prepared and transfected into COS-1 cells (Table 1). Transfection of COS-1 cells with pSVL-Fuc ($A^{860} \rightarrow G$) resulted in a 5-fold increase of α-L-fucosidase activity in cells and a 16-fold increase in medium relative to transfection with pSVL plasmid. These increases in α-L-fucosidase activity were comparable to increases resulting from transfection of COS-1 cells with wild-type α-L-fucosidase cDNA [pSVL-Fuc (wt)]. In contrast, transfection of COS-1 cells with pSVL-Fuc ($G^{197} \rightarrow A$) resulted in no increase of

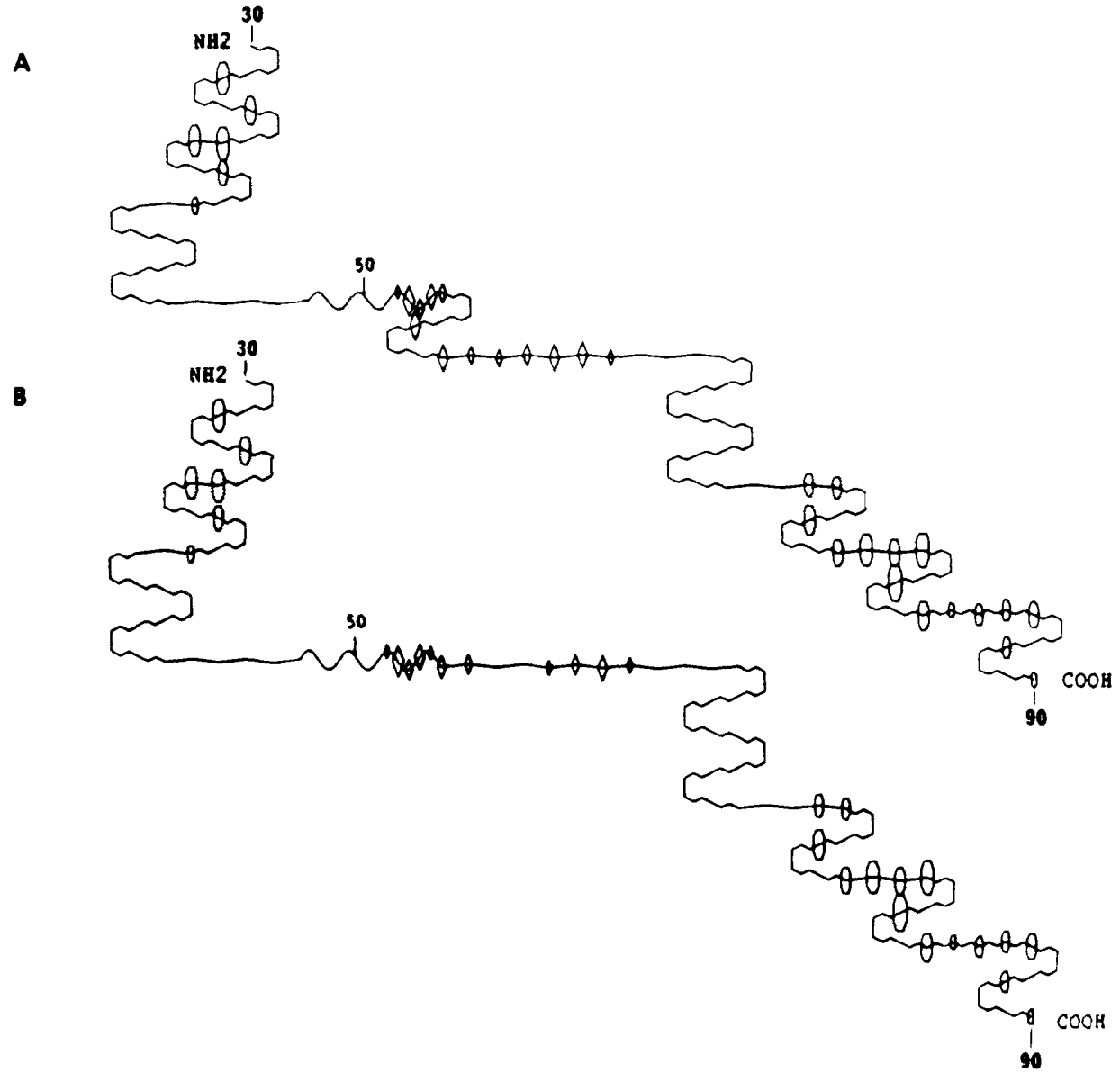

Figure 1. The secondary structure of α-L-fucosidase in the region of amino acids 30–90 as predicted by the Garnier–Osguthorpe–Robson algorithm. A, Normal amino acid sequence; B, Mutant (Gly-60 to Asp) amino acid sequence. The structure of the mutant (Gln-281 to Arg) amino acid sequence was virtually the same as the normal sequence. A sine wave indicates an α-helix; a sharp, saw-tooth wave indicates a β-sheet; changes in direction represent β-turns; and dull, saw-tooth waves represent random coils. The window of integration for hydropathy was set to seven residues and a threshold of 1.3. □, KD hydrophilicity ≥1.3; ◇, KD hydrophobicity ≥1.3.

α-L-fucosidase activity in cells or medium relative to transfection with pSVL plasmid. Expression of immunoreactive α-L-fucosidase protein paralleled α-L-fucosidase activity. No change in β-D-galactosidase activity was observed in cells or media after transfection with any of the plasmids. The successful transfer of pSVL-Fuc ($G^{197} \rightarrow A$) into COS-1 cells and the expression of mutant RNA were verified as described in Materials and methods. These data demonstrate that the $G^{197} \rightarrow A$ mutation, which changes Gly-60 to Asp, causes deficiency of α-L-fucosidase. However, the $A^{860} \rightarrow G$ mutation, which changes Gln-281 to Arg, results in normal expression of α-L-fucosidase. Therefore, we conclude that the $G^{197} \rightarrow A$ mutation is responsible for fucosidosis in patients with this mutation, while $A^{860} \rightarrow G$ is a normal polymorphism of α-L-fucosidase. Additional evidence supports this conclusion. First, the lack of expression of α-L-fucosidase in COS-1 cells transfected with the $G^{197} \rightarrow A$ mutation reflected the lack of α-L-fucosidase activity in cells and serum of a fucosidosis patient, MZ, and the drastically reduced level of immunologically reacting α-L-fucosidase protein (3% of normal) in lymphoid cultures and fibroblasts of MZ [3, 4, 9–11]. Second, normal expression of α-L-fucosidase in cells transfected with the $A^{860} \rightarrow G$ mutation was consistent with reports that homozygosity of $A^{860} \rightarrow G$ did not cause fucosidosis [6, 7]. Third, the alteration of Gly-60 to Asp in the α-L-fucosidase peptide chain predicts a major change of secondary structure, the elimination of two consecutive β-turns; whereas no major change is predicted for Gln-281 to Arg (Figure 1). Fourth, the amino acid sequence at positions 53–68 in human α-L-fucosidase is evolutionally conserved among rat and slime mold with identity at Gly-60; whereas Gln-281 in human α-L-fucosidase is not conserved [21, 22].

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