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Characterization of two α-galactosidase mutants (Q279E and R301Q) found in an atypical variant of Fabry disease

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Abstract

The mutant products Q279E (^{279}Gln to Glu) and R301Q (^{301}Arg to Gln) of the X-chromosomal inherited α-galactosidase (EC 3.2.1.22) gene, found in unrelated male patients with variant Fabry disease (late-onset cardiac form) were characterized. In contrast to patients with classic Fabry disease, who have no detectable α-galactosidase activity, atypical variants have residual enzyme activity. First, the properties of insect cell-derived recombinant enzymes were studied. The K_m and V_{max} values of Q279E, R301Q, and wild-type α-galactosidase toward an artificial substrate, 4-methylumbelliferyl-α-D-galactopyranoside, were almost the same. In order to mimic intralysosomal conditions, the degradation of the natural substrate, globotriaosylceramide, by the α-galactosidases was analyzed in a detergent-free-liposomal system, in the presence of sphingolipid activator protein B (SAP-B, saposin B). Kinetic analysis revealed that there was no difference in the degradative activity between the mutants and wild-type α-galactosidase activity toward the natural substrate. Then, immunotitration studies were carried out to determine the

amounts of the mutant gene products naturally occurring in cells. Cultured lymphoblasts, L-57 (Q279E) and L-148 (R301Q), from patients with variant Fabry disease, and L-20 (wild-type) from a normal subject were used. The 50% precipitation doses were 7% (L-57) and 10% (L-148) of that for normal lymphoblast L-20, respectively. The residual α-galactosidase activity was 3 and 5% of the normal level in L-57 and L-148, respectively. The quantities of immuno cross-reacting materials roughly correlated with the residual α-galactosidase activities in lymphoblast cells from the patients. Compared to normal control cells, fibroblast cells from a patient with variant Fabry disease, Q279E mutation, secreted only small amounts of α-galactosidase activity even in the presence of 10 mM NH_4Cl. It is concluded that Q279E and R301Q substitutions do not significantly affect the enzymatic activity, but the mutant protein levels are decreased presumably in the ER of the cells.

Previous Next

Keywords

Atypical variant of Fabry disease; α-Galactosidase

Abbreviations

AcNPV, *Autographa californica* nuclear polyhedrosis virus; Cer, ceramide or *N*-acylsphingosine; ER, endoplasmic reticulum; $GbOse_3Cer$, globotriaosylceramide, Galα1–4Galβ1–4Glcβ1–1Cer; MES, 2-morpholinoethanesulfonic acid; 4MU-α-D, -galactopyranoside, 4-methylumbelliferyl-α-D-galactopyranoside; SAP-B, sphingolipid activator protein B or saposin B

1. Introduction

The degradation of glycolipids is accomplished in lysosomes through the sequential actions of endoglycosidases. An inherited deficiency of the lysosomal enzyme leads to the accumulation of its substrates in lysosomes [1]. The genetic defect of α-galactosidase (EC 3.2.1.22) encoded by a gene localized to the X-chromosomal region, Xq22, results in Fabry disease. This disease is characterized by the systemic intralysosomal accumulation of neutral sphingolipids with terminal α-galactosyl moieties, predominantly globotriaosylceramide ($GbOse_3Cer$), in cells of the heart, kidneys, and vascular endothelial system [2].

Atypical Fabry variants with residual enzyme activity and cardiomyopathy of late-onset have been reported [3], [4], [5], and a screening study revealed that these variants comprised 3% of unrelated male patients with left ventricular hypertrophy referred to a cardiology clinic in Japan [6]. In cases with this cardiac form, four single base substitutions were detected in the upstream region of exon 6 of the α-galactosidase gene, ^{279}Gln to Glu (Q279E) [3], ^{301}Arg to Gln (R301Q) [4], and ^{296}Met to Val (M296V) [5] or Ile (M296I) [6]. Recently, an unrelated Japanese male patient carrying the R301Q mutation who only presented nephropathy, rather than cardiomyopathy, was reported [7].

Since, the lymphoblast cells derived from patients with variant Fabry disease, Q279E and R301Q, transcribed α-galactosidase mRNAs of normal size and normal amounts [3], [4], we wanted to clarify in this paper whether those mutations affect the enzyme's activity toward the natural substrate or result in an abnormal posttranslational decrease of the enzyme level. First, we focused our attention on the degradation of $GbOse_3Cer$ by a recombinant α-galactosidase mutant with the Q279E or R301Q substitution, using a detergent-free-liposomal system, in the presence of sphingolipid activator protein B (SAP-B), since, in vivo hydrolysis of $GbOse_3Cer$ is stimulated by SAP-B [8]. Then, using immunotitration procedures, the amounts of the two mutants and wild-type α-galactosidase naturally occurring in the probands lymphoblast cells were determined. Intracellular processing of mutant α-galactosidase, Q279E, was analyzed in patient's fibroblasts in the presence of either NH_4Cl or leupeptin.

2. Materials and methods

2.1. Materials

Restriction enzymes were purchased from Nippon Gene (Tokyo, Japan). *Spodoptera frugiperda* clonal isolate 9 (Sf9) cells, vector plasmid pAcYM1 [9], and wild-type *Autographa californica* nuclear polyhedrosis virus (AcNPV) were supplied by Dr. A. Takase (National Institute of Neuroscience, Tokyo, Japan). Egg phosphatidylcholine and D,L-α-tocopherol were from Sigma (Deisenhofen, Germany). Protein A–Sepharose, concanavalin A (Con A)–Sepharose, and Mono Q HR 5/5 were from Amersham Pharmacia Biotech (Uppsala, Sweden). 4-Methylumbelliferyl-α-D-galactopyranoside was from Nacalai Tesque (Kyoto, Japan). Lichroprep RP-18 was from Merck (Darmstadt, Germany). Leupeptin was from Peptide Institute (Osaka, Japan). Other reagents were of the highest grade available.

2.2. Cell culture

The Epstein–Barr virus-transformed lymphoblast lines from a normal adult (L-20) and two atypical Fabry patients (L-57 and L-148) were cultured in RPMI-1640 medium (Nissui, Tokyo, Japan) supplemented with 10% fetal calf serum (FCS) at 37°C under 5% CO_2.

Skin fibroblast cell lines derived from a normal adult (F-591) and an atypical Fabry patient (F-374), Q279E mutation, were maintained in Ham's F-10 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% FCS, and were grown to confluency at 37°C under 5% CO_2. Leupeptin was included in the culture medium (3 ml in a 60-mm dish) at a final concentration of 1 mM for 4 days [10]. The normal culture medium above confluent cultures in 10-cm dishes was replaced by FCS-free medium (10 ml) supplemented with NH_4Cl at a final concentration of 10 mM. Four days later, the medium was collected and 30-fold concentrated by ultrafiltration with Centriprep 10 (Amicon, Denvers, MA, USA) and enzyme activity was measured in 30-μl aliquot [11].

Sf9 cells were were cultured in Grace's medium (Gibco BRL) supplemented with 10% FCS at 25°C.

2.3. Expression and purification of recombinant human α-galactosidase

The cDNA for human α-galactosidase (1.3 kb) was subcloned into the *Bam*HI site of pAcYM1, the resultant vector being designated as pAcGal [12]. The expression vectors for the α-galactosidase mutants (Q279E and R301Q) were constructed by directional cloning of fragments containing the mutation site into pAcGal. Clone pBa57 for the Q279E mutant [3] or pBa148 for the R301Q mutant [3] was digested with *Bgl*II and *Kpn*I. The resultant two kinds of 0.5-kb fragments were purified by electrophoresis, and then inserted into pAcGal in place of the corresponding fragment (pAcQ279E or pAcR301Q), respectively. After co-transfection of AcNPV DNA and an expression vector (pAcGal, pAcQ279E, or pAcR301Q) into Sf9 cells, the recombinant viruses were selected by the limiting-dilution method and propagated. Sf9 cells were infected with a recombinant virus and then cultured in serum-free Grace's medium for 4 days. The expressed α-galactosidase mutants were purified from the culture medium by Con A–Sepharose and Mono Q column chromatographies [13]. Electrophoresis (12% polyacrylamide gel) was carried out by the method of Laemmli under reducing conditions [14].

2.4. α-Galactosidase activity toward an artificial substrate and protein concentration determination

α-Galactosidase activity was measured with an artificial substrate as described by Kusiak et al. [15] with the following modification. The reaction mixture (50 μl), comprising 5 mM

4MU-α-D-galactopyranoside in 0.1 M sodium citrate buffer, pH 4.6, was incubated at 37°C for 6 min. The reaction was terminated with 0.7 ml of 0.1 M glycine buffer, pH 10.6. The α-galactosidase activity of cell homogenate or culture medium was assayed under the conditions described above, except for 1–2 h incubation with 120 mM *N*-acetylgalactosamine (Sigma, St. Louis, MO, USA) as an inhibitor of α-*N*-acetylgalactosaminidase [16].

The protein amounts of cell homogenates were determined by means of the Bio-Rad dye-binding assay (Bio-Rad, Hercules, CA, USA), using bovine serum albumin as the standard. The protein concentrations of the recombinant mutants (Q279E and R301Q) and wild-type α-galactosidase were determined as to absorbance at 280 nm with an extinction coefficient of 18.1 [17].

2.5. Thermostability of the recombinant α-galactosidases

Wild-type and the mutant (Q279E and R301Q) α-galactosidases, 1.7–2.0 μg, were incubated at 50°C in 25 mM MES buffer, pH 6.0, the final volume was 100 μl. At various time intervals, samples were removed and assayed for residual activity toward 4MU-α-D-galactopyranoside.

2.6. α-Galactosidase activity toward globotriaosylceramide in a detergent-free liposomal system

α-Galactosidase activity toward $GbOse_3Cer$ was measured according to the method described previously [13]. In brief, liposomes with a total lipid concentration of 2 μmol/ml were prepared from egg phosphatidylcholine, 93% (mol/mol), D,L-α-tocopherol, 2% (mol/mol), and $GbOse_3Cer$, tritiated at the terminal galactose to a specific activity of 7.7 Ci/mol, 5% (mol/mol). To obtain large unilamellar liposomes (80.0±25 nm), the lipid preparation was extruded 19 times through an extrusion device (Liposo Fast-Basic; Avestin, Ottawa, Canada) using 100-nm-pore polycarbonate membranes.

The α-galactosidase assay mixture comprised liposomes (10 μl, 1 nmol of tritiated $GbOse_3Cer$), a mutant or the wild-type α-galactosidase (enzyme activity, 2.4 nmol of artificial substrate hydrolyzed per min), 2 μg of purified human SAP-B [18], 50 mM sodium citrate buffer, pH 4.6, and bovine serum albumin (5 μg), in a total volume of 50 μl. After incubation at 37°C, the reaction was terminated by the addition of 50 μl of methanol and then the reaction mixture was loaded onto a small reverse-phase column of RP-18 (0.25 ml). After washing of the column with 100 μl of a chloroform/methanol/0.1 M aqueous KCl solution, 3:48:47 (v/v/v), the liberated tritiated galactose was eluted with 2 ml of water, and

the radioactivity was measured with a scintillation counter. Control radioactivity was measured by adding SAP-B and the enzyme only after termination of the incubation.

2.7. Immunotitration

Lymphoblast cells were collected by centrifugation, suspended in 25 mM MES buffer, pH 6.0, and then sonicated. Then the cell homogenate (40 μg of protein) was centrifuged at 10 000×g at 4°C for 10 min. The cell supernatant was incubated with 3–500 ng of an IgG preparation (prepared from rabbit antiserum raised against the purified recombinant human α-galactosidase derived from insect-cells, using protein A–Sepharose) [19] in 25 mM MES buffer, pH 6.0, containing bovine serum albumin (1 mg/ml), the final volume being adjusted to 40 μl. After 4 h incubation at 4°C, 30 μl of a protein A–Sepharose suspension (1:1) in the last buffer was added. After 30 min gentle rotation, all tubes were centrifuged at 3000×g for 5 min. The supernatants were assayed for α-galactosidase activity toward 4MU-α-D-galactopyranoside.

3. Results

3.1. Purification and characterization of the α-galactosidase mutants (Q279E or R301Q) expressed in insect cells

The recombinant α-galactosidase mutants (Q279E and R301Q) found in the variant form of the Fabry disease were purified and characterized. The final preparation of each, the two mutants and the wild-type α-galactosidase, gave a slightly broad and single band in SDS–polyacrylamide gel electrophoresis corresponding to 46-kDa (Fig. 1). To assess their thermostability, the recombinant α-galactosidases were incubated at 50°C in a bovine serum albumin-free MES buffer, pH 6.0. As shown in Fig. 2, they were almost completely inactivated after 30 min, while the wild-type α-galactosidase retained 43% of its original activity even after 60 min.

3.2. Degradation of artificial and natural substrates by the wild-type and mutant α-galactosidases

The kinetic properties of two mutants and wild-type α-galactosidase are summarized in Table 1. The pH optimum of the two mutants was 4.6, corresponding to that of the wild-type α-galactosidase. The K_m value toward 4MU-α-D-galactopyranoside was slightly lower for the wild-type α-galactosidase than for the two mutants. The V_{max} value of the Q279E mutant was similar to that of the wild-type α-galactosidase, and the V_{max} of R301Q was

slightly lower than those of Q279E and the wild-type. These results indicated that both the mutations, i.e. Q279E and R301Q, had no significant effects on the enzymatic properties of the α-galactosidase molecule toward the artificial substrate.

Using a detergent-free liposomal system, the degradation of the natural substrate, $GbOse_3Cer$, by the two mutant enzymes was analyzed in the presence of SAP-B, in order to mimic intralysosomal conditions (Fig. 3). There was a linear relationship between enzyme activity and reaction time until 2 h. Time course analysis revealed that there were no significant differences in the degradation activity toward the natural substrate between the two mutants and the wild-type α-galactosidase. In particular, the Q279E mutant and wild-type enzymes exhibited almost the same degradation ability. This indicates that both the two mutations do not affect the enzymatic activity toward the natural substrate.

3.3. Amounts of α-galactosidase mutant enzymes in cultured lymphoblasts from patients with variant Fabry disease

To characterize the naturally occurring α-galactosidase mutant gene products, residual enzyme activities and residual protein levels were studied in cultured lymphoblasts, L-57 (Q279E) and L-148 (R301Q) obtained from patients with variant Fabry disease, and in wild-type lymphoblasts (L-20) obtained from a normal subject. The residual α-galactosidase activity in L-57 was 3% and that in L-148 was 5% of the normal level (L-20), respectively. Additionally, the residual α-galactosidase activity exhibited similar apparent K_m values, 3.2 mM (L-57) and 3.0 mM (L-148) compared to 2.8 mM for the normal enzyme (L-20), toward a fluorogenic substrate. To clarify the relationship between the residual enzyme activity and the protein amounts of the α-galactosidase mutants in these lymphoblast cells, immunotitration experiments were carried out, using antibodies raised against the recombinant wild-type α-galactosidase (Fig. 4). Fifty percent precipitation was observed with 15 ng (IgG per 40 μg protein per assay) for L-57 and 20 ng (IgG per 40 μg protein per assay) for L-148, which were estimated to be 7 and 10% of the normal level, respectively. The relative amounts of immuno cross-reacting materials almost corresponded to the residual α-galactosidase activities observed in the lymphoblast cells. These data suggested that the reduced activities of the mutant α-galactosidases (Q279E and R301Q) in lymphoblasts mainly resulted from decreased amounts of the expressed enzyme proteins.

3.4. Ammonium chloride induced secretion of α-galactosidase

Mutant fibroblasts (Q279E) secreted α-galactosidase activity into the culture medium (-NH_4Cl) only 20% of the normal (wild-type) control level (Table 2). The addition of 10 mM NH_4Cl to the culture medium resulted in a 3–5-fold increase of extracellular α-galactosidase

activity in both cultures. When growing the patient's cells in the presence of 1 mM leupeptin, the intracellular levels of α-galactosidase remained low (8% of wild-type control, Table 2).

4. Discussion

The human α-galactosidase gene located on the X-chromosome is approximately 12 kb long and consists of seven exons. Its cDNA encodes a precursor peptide of 429 amino acid residues including a 31-residue signal peptide [2]. The Q279E [3] and R301Q [4] substitutions are the gene mutations found in two unrelated patients with variant Fabry disease. The two male patients carrying the Q279E and R301Q mutations, respectively, showed mild clinical manifestations at the age of 50–60 years, mainly cardiac symptoms. The residual α-galactosidase activity in mixed leukocytes from these patients was decreased to about 4% of the normal control level [20]. Since, the lymphoblast cells derived from these patients transcribed α-galactosidase mRNAs of normal size and normal amounts [3], [4], we searched for the cause of the decrease of α-galactosidase activity at the protein level. The protein levels of the mutated α-galactosidases were analyzed in the patients' lymphoblasts and fibroblasts, and the properties of the mutant enzymes were investigated after their expression in Sf9 insect cells using a baculo-virus expression system. The transfected Sf9 cells secreted relatively high amounts of the mutant (Q279E and R301Q) and wild-type α-galactosidase. It has been reported that the recombinant wild-type α-galactosidase expressed in insect cells has molecular properties, N-terminal amino acid sequence [17], the extinction coefficient at 280 nm [17], and the molecular mass [13]. However, the recombinant mutant enzymes (Q279E and R301Q) showed a marked decrease in thermostability (Fig. 2) which may be caused by small changes in the protein folding. Significant differences were not observed between mutants and wild-type enzymes, neither in the circular dichronism spectra, the wavelength range of 200–250 nm (data not shown), nor in the binding to Con A–Sepharose during the purification [21], [22], [23]. The latter observation indicates that the recombinant enzymes contain terminal α-D-glucosyl or α-D-mannosyl residues. The presence of mannose oligosaccharide chains on recombinant wild-type α-galactosidase expressed in Sf9 cells is also suggested by their sensitivity to glycopeptidase F [17] and endoglycosidase H [24].

To clarify the pathogenesis underlying the variant form of Fabry disease, we studied the degradative activity of the mutant enzymes toward the natural substrate, $GbOse_3Cer$. As the hydrolysis of $GbOse_3Cer$ is mediated by sphingolipid activator protein B (SAP-B) [8], we used a detergent-free-liposomal system containing SAP-B. Surprisingly, both mutant enzymes, Q279E and R301Q, showed similar degradative activity toward $GbOse_3Cer$ in the presence of

SAP-B as the wild-type α-galactosidase(Fig. 3). The two mutants also showed almost normal K_m or V_{max} for the artificial substrate, 4MU-α-D-galactopyranoside (Table 1). Therefore, the two mutant α-galactosidase gene products, Q279E and R301Q, cannot be regarded as K_m or V_{max} mutant enzyme proteins.

Since the quite normal molecular specific activities of the mutant enzymes cannot explain the α-galactosidase activity deficiencies of the patients, we analyzed the protein levels of the mutant α-galactosidases in the patients' cultured lymphoblasts. The results of immunotitration studies indicate that the steady-state levels of the mutant α-galactosidase proteins decreased to 7 and 10% of the normal level in L-57 (Q279E) and L148 (R301Q), respectively (Fig. 4), which may explain the low residual α-galactosidase activities of 3 and 5% of normal control in L-57 and L-148, respectively. Thus, some unknown defect of posttranslational processing might occur in these cells, allowing only small amounts of the mutant α-galactosidases with enzymatic activity might reach the lysosomes.

Insect cells secrete high amounts of mutant and wild-type proteins. However, the patient's fibroblasts, Q279E mutation, secrete only small amounts of the mutant α-galactosidase, even in the presence of ammonium chloride, indicating that the mutations affect intracellular transport of the patient's enzyme upstream of the *trans*-Golgi network, most likely between ER and the *cis*-Golgi. This notion is also in agreement with the leupeptin experiment which indicates that the mutated α-galactosidase, Q279E, is not degraded by cysteine-proteases in the lysosomes. Proteolytic degradation of mutated protein at the level of the ER has been observed for many proteins, e.g. β-hexosaminidase A [25] or glucocerebrosidase [26].

The accumulation of aggregate was observed previously in COS-1 cells over expressing either Q279E or R301Q [27]. The cells expressing Q279E were treated with brefeldin A or monensin, which inhibit the transport from ER to *cis*-Golgi and from the *medial* to the *trans*-Golgi compartment, respectively. The results suggest that the loss of enzyme activity and accumulation of the aggregates might occur in the ER. This situation has been documented with the protein product of the cystic fibrosis (CF)-associated gene, which is called the CF transmembrane conductance regulator (CFTR) [28]. The most common mutation in CFTR, ΔF508, a deletion of phenylalanine 508, fails to traffic to the correct cellular location. It was proposed that the mutant CFTR is recognized as abnormal by cellular quality control mechanisms and is incompletely processed in the ER where it is subsequently degraded [29], [30].

The threshold theory has been proposed in 1983/84 [31] and has initially been demonstrated to be valid by Leinekugel et al. for GM2 gangliosidoses and metachromatic

leukodystrophy [32], and later on for other storage diseases [1]. The threshold theory correlates onset and severity of a storage disease with the residual activity of the degrading system within the lysosome. This work also indicates that fibroblast cells derived from an atypical Fabry patient, Q279E mutation, also showed residual intracellular α-galactosidase activity about 5% of the normal level when cultured in the medium supplemented with 10% FCS (Table 2). Many more samples are necessary to calculate the critical threshold of Fabry disease, and further analysis may constitute a theoretical model of this disease.

However, there still remains the important point that one patient, carrying the R301Q mutation, presented only nephropathy [7], rather than cardiomyopathy, which is a major symptom in other variant Fabry patients with point mutations in exon 6. The reason for the difference in organ susceptibility to the same point mutation is not clear at present. Different phenotypes of relatives carrying the same mutation have been reported before, e.g. for the W226X mutation [33] and the E341K mutation [34] in a Russian family with Fabry disease. It is unclear, why in some cases the onset of symptoms is delayed for decades and in late-onset cases often a considerable phenotypic variation is observed even among siblings, who must have the same mutation. The nature of the molecular defect alone, however, cannot explain the variability of the resulting clinical phenotype, which is also influenced by the variable genetic background of the respective patients.

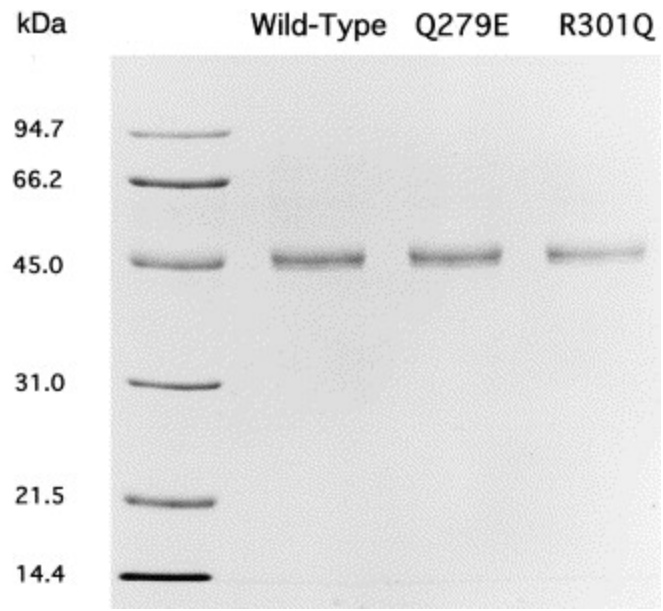

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Fig. 1. Sodium dodecyl sulfate/polyacrylamide gel (12%) electrophoresis of the purified recombinant mutant and wild-type α-galactosidase under reducing conditions. Four μg of each enzyme protein was applied and stained with Coomassie brilliant blue R-250. Wild-

Type, wild-type α-galactosidase; Q279E, Q279E mutant α-galactosidase; R301Q, R301Q mutant α-galactosidase.

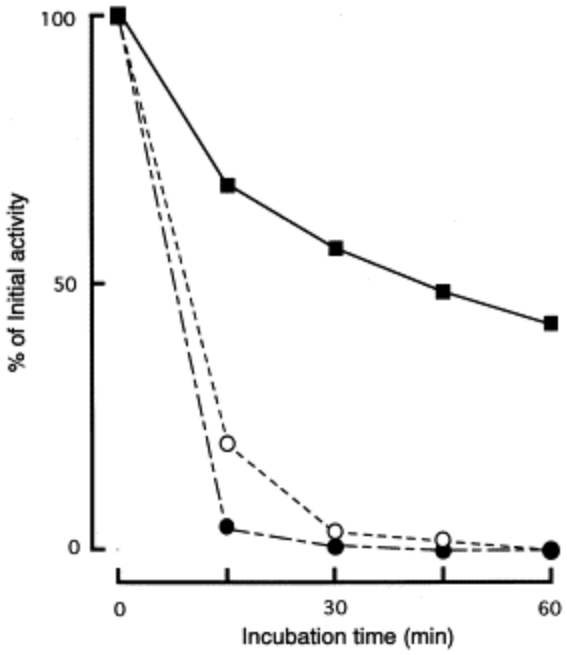

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Fig. 2. Stability of recombinant mutants (Q279E or R301Q) and wild-type α-galactosidase at 50°C, pH 6.0. α-Galactosidase activity was monitored with 4-methylumbelliferyl-α-D-galactopyranoside. The initial enzyme activities (100%) were 4.2, 3.8, and 5.0 nmol/min/100 μl for Q279E (•), R301Q (○), and wild-type α-galactosidase (■), respectively.

Table 1. Kinetic properties of the mutants, Q279E and R301Q, and wild-type α-galactosidase derived from insect cells

K_m (mM)	V_{max} (μmol/min/mg)	
Q279E	2.8	36
R301Q	2.8	27
Wild-type	2.2	34

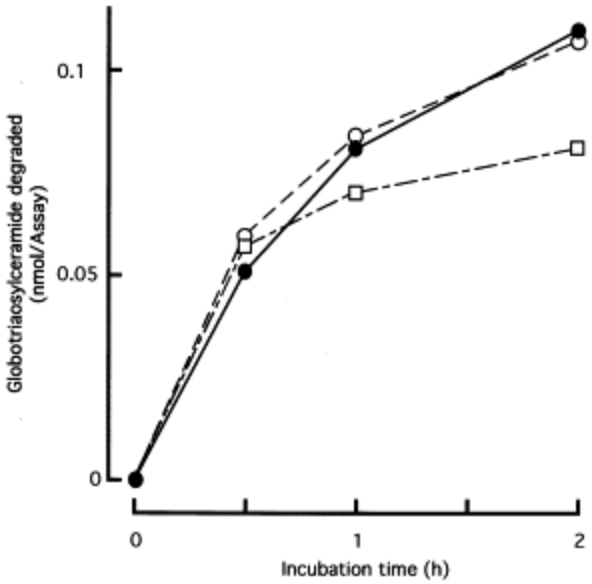

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Fig. 3. The degradation of globotriaosylceramide by a recombinant mutant (Q279E or R301Q) and wild-type α-galactosidase in a detergent-free-liposomal system. Assays were carried out in the presence of 2 μg of sphingolipid activator protein B and mutant or wild-type α-galactosidase, the activity of each corresponded to 2.4 nmol of 4-methylumbelliferyl-α-D-galactopyranoside hydrolyzed per min. The release of radiolabeled galactose was quantified as described in Section 2. Each value is the mean of duplicate determinations. Q279E mutant α-galactosidase (○), R301Q mutant α-galactosidase (□), and wild-type α-galactosidase (•).

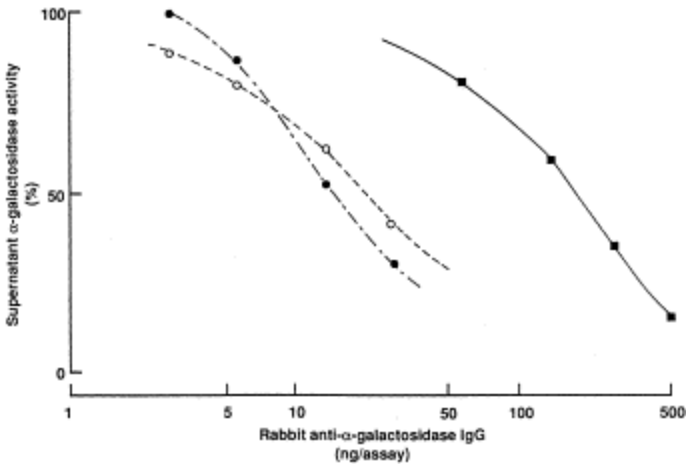

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Fig. 4. Immunotitration of a naturally occurring mutant (Q279E or R301Q) or wild-type α-galactosidase in lymphoblast cells derived from patients with variant Fabry disease or a

normal subject. α-Galactosidase was immunoprecipitated from the cell supernatants with increasing amounts of anti-α-galactosidase IgG, and then the enzyme activity was measured in the supernatants, with 4-methylumbelliferyl-α-D-galactopyranoside as the substrate. Experiments were run in triplicate, the deviations were less than 5%. Lymphoblast cell line L-57, Q279E α-galactosidase gene mutation (•); L-148, R301Q α-galactosidase gene mutation (○); L-20, wild-type α-galactosidase gene (■).

Table 2. Effect of ammonium chloride and leupeptin on the α-galactosidase activity of fibroblasts from a patient with variant Fabry disease and a normal subject

α-Galactosidase mutation	α-Galactosidase activity			
Medium (nmol/h/ml)		Cells (nmol/h/mg protein)		
$-NH_4Cl$	$+NH_4Cl$[a]	−Leupeptin	+Leupeptin[b]	
Q279E	0.2	0.9	2.5	3.9
Wild-type	1.0	2.7	54.7	49.2

α-Galactosidase activity was measured as described in Section 2.

a

10 mM NH_4Cl added to the serum-free medium.

b

1 mM Leupeptin added to the medium supplemented with 10% fetal calf serum.

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potentially significant structural alteration in the enzyme protein, i.e., substitution of histidine for arginine (Table 7). The p.N215S and p.R301Q mutations are known as cardiac variants of Fabry disease with residual enzymatic activity [25,26]. Patients with the p.R363H mutation usually have less severe symptoms.…

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