Am J Hum Genet 35:21-33, 1983

Genetic Heterogeneity in Acid α-Glucosidase Deficiency

NICHOLAS G. BERATIS,[1] GUNDULA U. LABADIE,[2] AND KURT HIRSCHHORN[2]

SUMMARY

Several clinical forms of acid α-glucosidase deficiency have been described. Our study was planned to identify differences at the molecular level in acid α-glucosidase deficiency. Of nine fibroblast strains derived from patients with the infantile form of the disease, eight were crossreacting material (CRM)-negative and one CRM-positive. This was demonstrated by both agar immunodiffusion and immunotitration. No difference in apparent enzymatic activity was observed between CRM-negative and CRM-positive infantile acid α-glucosidase deficiency fibroblasts. In two fibroblast strains with the adult form of acid α-glucosidase deficiency, rocket immunoelectrophoresis demonstrated a reduction in the amount of enzyme protein, which was directly proportional to the reduction in enzyme activity. In another fibroblast strain obtained from a patient with the adult form of the disease, the activity was within the range of the infantile form and no CRM could be identified. Fibroblasts with phenotype 2 of acid α-glucosidase, considered a normal variant, showed a reduction both in the amount of enzyme protein and in the ability of the enzyme to cleave glycogen. However, the catalytic activity for maltose was normal. The findings demonstrate extensive genetic heterogeneity in acid α-glucosidase deficiency. Molecular differences were identified both between the clinical forms of the disease and within the infantile and the adult forms of acid α-glucosidase deficiency. It remains unknown whether or not the enzyme deficiency in homozygotes for isozyme 2 of acid α-glucosidase will be sufficient to cause glycogen accumulation and lead to the development of muscular dystrophy-like disease later in life.

Received March 3, 1982; revised May 28, 1982.

This study was supported by grant AM-22198 from the National Institutes of Health, a grant from the Muscular Dystrophy Association, and grant I-535 from the March of Dimes Birth Defects Foundation.

[1] Department of Human Genetics, New York State Institute for Basic Research in Mental Retardation, Staten Island, NY 10314.

[2] Division of Medical Genetics, Department of Pediatrics, Mount Sinai School of Medicine of the City University of New York, New York, NY 10029.

INTRODUCTION

Acid α-glucosidase (E.C.3.2.1.20) is a lysosomal enzyme that hydrolyzes maltose and glycogen to release glucose. Deficiency of this enzyme is the basic defect in glycogenosis type II [1]. Several forms of acid α-glucosidase deficiency have been described. The disease is rapidly lethal in the infantile form (Pompe disease), and absence of both acid α-1,4-glucosidase and acid α-1,6-glucosidase activities has been demonstrated [2, 3]. In fibroblasts from patients with the adult form of the disease [4], in which there is a milder progressive course, as much as 22% of normal enzyme activity has been measured. In addition, absence [5–9] or severe reduction [4, 9] of immunologically reactive enzyme protein has been observed in tissues of patients with glycogenosis type II. We identified catalytically inactive enzyme protein in cultured skin fibroblasts from a patient with the infantile type of acid α-glucosidase deficiency [10]. A reduction in the amount of enzyme protein in cultured skin fibroblasts from patients with the adult form of the disease has also been demonstrated [4, 10].

Acid α-glucosidase has been found to be polymorphic [11]. The polymorphism is detectable using starch gel electrophoresis, but not when using agarose or cellogel electrophoresis, indicating reduced affinity for starch. The common isozyme, designated type 1, migrates slowly anodally. About one in 16 Europeans shows a pattern of two isozymes, the slow band plus an additional component, designated type 2, migrating faster toward the anode. The three phenotypes identified, 1, 2-1, and 2, are determined by two alleles at an autosomal locus. Isozymes 1 and 2, purified from a heterozygous placenta, showed the same pH optimum, the same K_m's using maltose substrate, identical thermal denaturation profiles, and shared antigenic identity. However, when glycogen was used as substrate, isozyme 2 showed reduced catalytic activity [12].

Here we present findings that provide evidence for genetic heterogeneity between and within the infantile and adult subtypes of acid α-glucosidase deficiency by immunologic evaluation and quantitative estimation of the residual acid α-glucosidase protein in cultured skin fibroblasts.

MATERIALS AND METHODS

Skin Fibroblasts

Skin fibroblasts derived from nine patients with the infantile form of acid α-glucosidase deficiency were used. Three of these patients (B-4, M-4581, and M-2478) were diagnosed by us. Three fibroblast strains (GM-244, GM-248, and GM-338) were obtained from the Institute for Medical Research, Camden, N.J., and three strains (WG-173, WG-285, and WG-482) were obtained from the Repository for Mutant Cell Strains, Montreal, Canada. Three fibroblast strains derived from patients with the adult form of the disease were also evaluated. One of these patients (M-3773) was diagnosed by us. This patient developed the first symptoms of muscular weakness at age 30. The other two fibroblast strains (GM-443 and GM-1935) were obtained from the Institute for Medical Research. In case GM-443, the first symptoms started during the third decade, and the skin biopsy was obtained at age 30. Case GM-1935, a black female, was first seen at age 25 because of amenorrhea and hypertension. At age 30, she developed the first signs of generalized muscle wasting and weakness, including some facial weakness. A muscle biopsy showed many small vacuoles in the

muscle fibers, and the periodic acid Schiff (PAS) stain was positive, suggesting heavy glycogen deposition. Absence of acid α-glucosidase activity was demonstrated by Dr. B. I. Brown of the Washington University School of Medicine, St. Louis, Mo., in muscle and cultured skin fibroblasts (personal communication, Dr. W. Sly of the St. Louis Childrens Hospital, St. Louis, Mo., 1980). Finally, a fibroblast strain homozygous for isozyme 2 of acid α-glucosidase was studied. This culture was derived from an ovarian dermoid cyst of a woman with the acid α-glucosidase phenotype 2-1. Dermoid cysts are parthenogenic tumors that originate from a single germ cell after the first meiotic division [13]. This culture was donated by Dr. D. Swallow of the Galton Laboratory, University College, London. One dermoid cyst (GM-1306) (phenotype 1), used as a control, was obtained from the Institute for Medical Research. Normal fibroblast strains used as controls were obtained from subjects tested for heterozygosity of various metabolic disorders and found to be normal. The skin biopsies were obtained with informed consent from adults, or, in the case of children, with informed consent from their parents.

Skin fibroblasts were cultivated in RPMI-1640 medium supplemented with 12% fetal calf serum, 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml) (Gibco, Grand Island, N.Y.). Fibroblasts were harvested at early or late (2 weeks after passage) confluency by using a short trypsin treatment followed by EDTA [14]. Harvested cells were washed twice with 0.9% saline and stored at −80°C until used.

Enzyme Assays

The cell pellets were resuspended in water and lysed by freezing and thawing seven times in an acetone/dry ice bath. The lysates were centrifuged at 1,000 *g* for 10 min, and the supernatant fractions were collected. Acid α-glucosidase activity was determined at pH 4.0, using maltose and glycogen (Boehringer-Mannheim, Indianapolis, Ind.) as substrates [9]. Glycogen concentration in the reaction mixture was 120 mg/ml. Released glucose was measured with a modification of the Glucose Colorimetric Enzymatic Method, procedure no. 510 (Sigma, St. Louis, Mo.). Glucose oxidase (10 U/ml) and horseradish peroxidase (4 U/ml) in 0.1 M Tris-HCl buffer, pH 7.0, were mixed with an equal volume of o-dianisidine diHCl (1.25 mg/ml). A 400-μl aliquot of this mixture was added to each tube, and the reaction was terminated with 30 μl 4 N HCl after a 30-min incubation; the optical density was read at 420 nm. One enzyme unit (U) was defined as the activity that released 1 nmol glucose/hr at 37°C. Protein was measured by the method of Lowry et al. [15]. All determinations were performed in duplicate.

Immunochemical Procedures

Double immunodiffusion was carried out on agarose plates containing 1% agarose (Miles, Elkhart, Ind.), 4% polyethylene glycol 6,000 (Gallard, Schlesinger, Carle Place, N.Y.), and 0.02% sodium azide in phosphate-buffered saline, pH 7.0, as described [10]. The plates were stained for enzymatic activity with 4-methylumbelliferyl-α-D-glucopyranoside (Research Products, Elk Grove Village, Ill.), and, subsequently, for protein with Coomassie Blue.

Immunotitration of acid α-glucosidase in cultured skin fibroblast lysates was performed in a final volume of 20 μl. Cells harvested at late confluency were used. An aliquot (15 or 30 μg) of fibroblast protein was incubated with increasing concentrations of partially purified [16] rabbit anti-acid α-glucosidase antibodies prepared by us [10]. Incubation was carried out at 37°C for 30 min and, subsequently, at 4°C for 60 hrs. Enzyme preparations incubated with rabbit nonimmune IgG and saline served as negative controls. Acid α-glucosidase activity was measured using maltose as substrate.

For rocket antigen-antibody electrophoresis [17], fibroblasts. harvested at both early and late confluency, were resuspended in 0.05 M citrate-phosphate buffer, pH 5.0, and the cells were lysed by sonication (5 cycles of 10 seconds duration) using a Branson sonifier, model 200, with a cup horn attachment (Branson Sonic Power, Danbury, Conn.). The lysates were

centrifuged at 12,000 *g* for 20 min and the supernatants collected and assayed for acid α-glucosidase activity and protein.

Rocket immunoelectrophoresis was performed on 6.5 × 8.0-cm plastic plates. A 1% agarose (Seakem, Marine Colloids Div. FMC Corp., Rockland, Me.) solution was prepared in 0.06 M Tris-sodium barbital buffer, pH 8.8 (Gelman high resolution buffer, Gelman Instruments, Ann Arbor, Mich.); 8 × 2-cm "bridges," each containing 4.0 ml of the agarose, were placed at the anode and cathode ends of the plate. The center of the plate (2.5 × 8 cm) was filled with 4.2 ml of the agarose containing 8 μl (approximately 80 μg protein) of partially purified rabbit antiacid α-glucosidase antibodies. Wells were cut at the cathodal end of the antibody-containing gel and filled with 10–25 μl of sample. Electrophoresis (bridge buffer same as the gel buffer) was carried out at 25 mAmp for 4 hrs at room temperature in a horizontal electrophoresis cell (model 1415, Bio-Rad, Richmond, Calif.). The gel was subsequently washed in saline for 8–12 hrs. The antibody-containing portion was overlaid with 0.3 ml sheep antirabbit peroxidase conjugated IgG (Cappel, Cochranville, Pa.), which had been diluted 1:3 with saline. The plate was incubated at room temperature for 8–12 hrs in a humidified chamber. After another 8–12-hr wash, the gel was stained with 50 ml of a solution containing 25 mg diaminobenzidine tetrahydrochloride (Sigma) and 0.15 ml 3% H_2O_2 in 0.1 M Tris buffer, pH 7.6 [18]. After 30 min of staining, the rockets were easily visualized using an indirect light source. The plates were magnified on a view box, and the height of the rocket, from the top rim of the well to the tip of the precipitin arc, was recorded.

RESULTS

On agar double immunodiffusion, lysates from normal skin fibroblasts formed a precipitin line when diffused against antiacid α-glucosidase antibodies. Of the nine fibroblast strains derived from patients with the infantile form of acid α-glucosidase deficiency studied, only one strain (M-4581) demonstrated immunologically cross-reacting material (CRM). The precipitin line was confluent with that of the normal enzyme, indicating immunologic identity (fig. 1*A*). When the gel was stained for acid α-glucosidase, enzymatic activity was detected only in the precipitin line formed with the normal fibroblast lysates (fig. 1*B*). Equal amounts of lysate protein (600 μg) were used from normal cells and from patients' fibroblasts. The apparent acid α-glucosidase activity in the CRM-positive infantile form fibroblast strain was the same as that in the eight CRM-negative strains (84 U/mg protein and 95 ± 24 U/mg protein, respectively). The enzymatic activity in 10 normal fibroblast strains used in these studies was 2,692 ± 742 U/mg protein. For these studies, cell pellets were resuspended in water and lysed by freezing and thawing, as described.

Immunologically CRM was detected in only two of the three fibroblast strains with the adult form of acid α-glucosidase deficiency (M-3773 and GM-443). The precipitin lines obtained were confluent with that of the normal enzyme. Fifteen hundred μg lysate protein from the adult-form fibroblasts was required to obtain a visible precipitin line, whereas 600 μg protein was sufficient for the formation of visible precipitin lines using normal cell lysates (fig. 2*A*). When the gel was stained for acid α-glucosidase, enzymatic activity was observed in the immune complexes of the two adult-form fibroblast strains at high (1,500 μg) and low (600 μg) protein concentrations (fig. 2*B*). No precipitin line was detected in the third case with the adult form of acid α-glucosidase deficiency (GM-1935) when stained either for

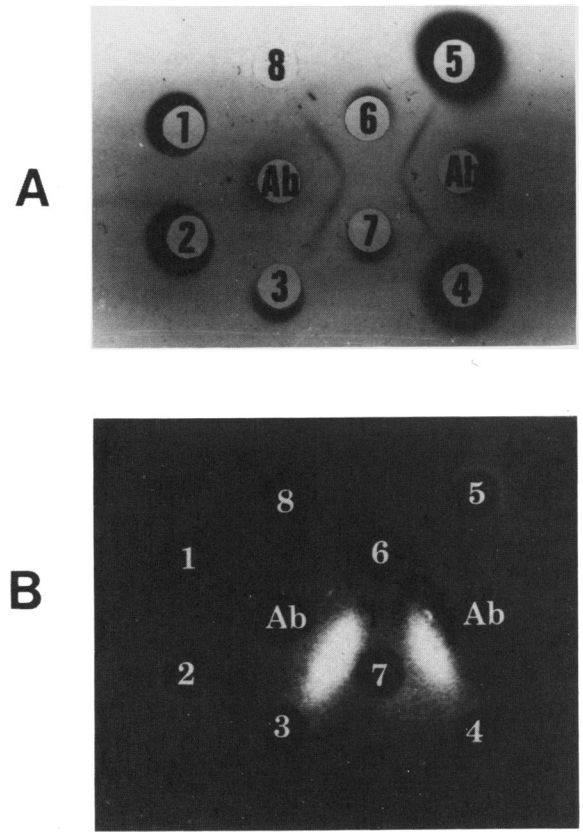

FIG. 1.—Agar double diffusion of six of the nine fibroblast strains with the infantile acid α-glucosidase deficiency studied. *Wells Ab* contain rabbit antiacid α-glucosidase antibodies. *Wells 1–6* contain 600 μg cultured skin fibroblast protein from patients with the infantile form of acid α-glucosidase deficiency (*well 1* = GM-248, *well 2* = GM-338, *well 3* = M-2478, *well 4* = B-4, *well 5* = GM-244, *well 6* = M-4581); *well 7* contains 600 μg protein from normal fibroblasts, and *well 8* contains saline. *A*, Plate stained for protein with Coomassie Blue; *B*, same plate stained for enzymatic activity with the fluorogenic substrate 4-methylumbelliferyl-α-D-glucopyranoside.

activity or for protein. Enzymatic activity in strains M-3773 and GM-443 was 338 U/mg protein and 463 U/mg protein, respectively. Strain GM-1935 had an apparent activity of only 55 U/mg protein.

Immunotitration of acid α-glucosidase in lysates from normal skin fibroblasts with increasing concentrations of partially purified antiacid α-glucosidase antibodies progressively inhibited the enzymatic activity. Antigenic competition was observed in mixtures of lysates (15 μg each) from normal and the CRM-positive infantile acid α-glucosidase deficiency fibroblasts (M-4581). The amount of enzyme activity precipitated in 15 μg normal fibroblast protein was 30% greater than the amount of enzyme activity precipitated in mixtures of 15 μg each of normal fibroblast protein and protein from cells with the CRM-positive infantile form. No change in the amount of enzyme precipitated was detected in mixtures of 15 μg

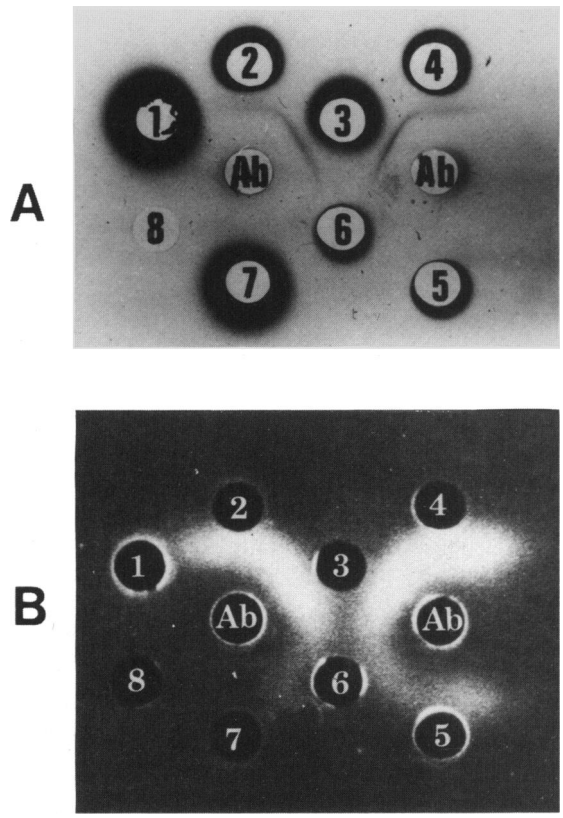

FIG. 2.—Agar double immunodiffusion. *Wells Ab* contain antiacid α-glucosidase antibodies. *Wells 1* (GM-1935), *2* (M-3773), and *4* (GM-443) contain 1,500 μg fibroblast protein from patients with the adult form of acid α-glucosidase deficiency. *Wells 5* (GM-443), *6* (M-3773), and *7* (GM-1935) contain 600 μg fibroblast protein. *Well 3* contains 600 μg protein from normal fibroblasts, and *well 8* contains saline. *A*, Plate stained for protein with Coomassie Blue; *B*, the same plate stained for enzymatic activity with the fluorogenic substrate 4-methylumbelliferyl-α-D-glucopyranoside.

each of normal fibroblast protein and CRM-negative infantile acid α-glucosidase deficiency fibroblast protein (fig. 3).

To determine the amount of specific enzyme protein in fibroblasts from the various subtypes of acid α-glucosidase deficiency studied, quantitative rocket immunoelectrophoresis was performed. For these studies, cells harvested at early and late confluency were lysed by sonication in 0.05 M citrate-phosphate buffer, pH 5.0. At this pH, a large amount of nonenzyme protein precipitated, resulting in an increased specific activity. A typical rocket immunoelectrophoresis gel is shown in figure 4.

When crude fibroblast lysates from normal subjects, from patients with the adult form of acid α-glucosidase deficiency, and from the fibroblast strain with phenotype 2 of the enzyme were electrophoresed into agarose containing specific antibody, the height of the precipitin arc was directly proportional to the number of applied enzyme units, as measured with maltose, indicating the same ability of

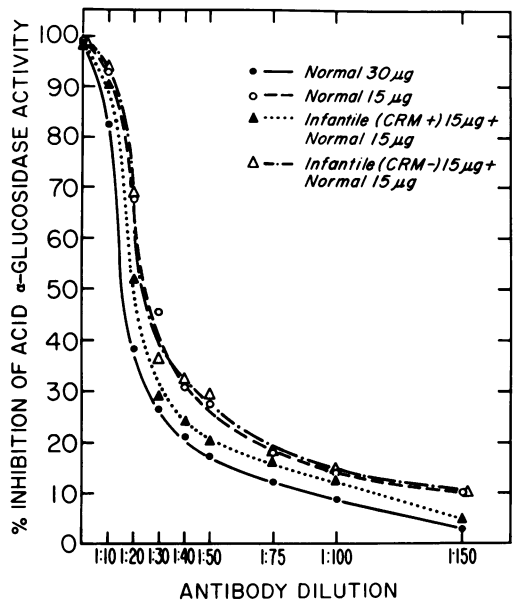

FIG. 3.—Immunotitrations of acid α-glucosidase in cultured skin fibroblasts with partially purified rabbit antiacid α-glucosidase antibodies. No enzyme inhibition was observed when nonimmune rabbit immunoglobulin or saline was substituted for antiacid α-glucosidase antibodies.

the enzyme to cleave this substrate (fig. 5*A*). Similar studies performed with glycogen as substrate showed the reduced ability of isozyme 2 to cleave glycogen (fig. 5*B*). No rocket was obtained with the CRM-negative adult (GM-1935) or the CRM-negative infantile forms.

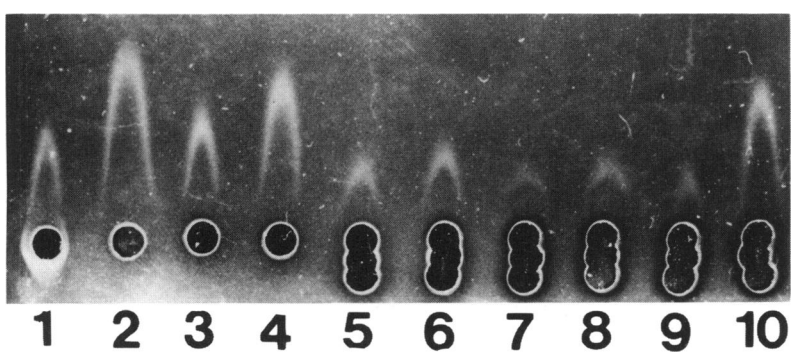

FIG. 4.—Rocket immunoelectrophoresis of cultured fibroblast lysates. *Wells 1* and *2* contain 10 μg fibroblast protein from normal skin fibroblasts with phenotype 1 of acid α-glucosidase, harvested at early and late confluency, respectively; *wells 3* and *4* contain 10 μg protein from fibroblasts of an ovarian dermoid cyst (GM-1306) with phenotype 1 of acid α-glucosidase, harvested at early and late confluency, respectively; *wells 5* and *6* contain 25 μg protein from fibroblast strain M-3773 harvested at early and late confluency, respectively; *wells 7* and *8* contain 25 μg of protein from fibroblast strain GM-443 harvested at early and late confluency, respectively; and *wells 9* and *10* contain 20 μg protein from an ovarian dermoid cyst with phenotype 2 of acid α-glucosidase harvested at early and late confluency, respectively.

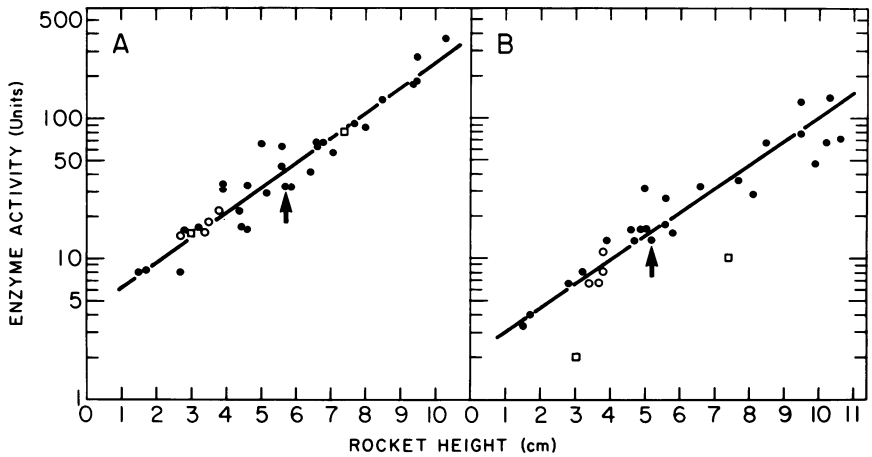

FIG. 5.—Relationship between enzyme units and rocket height obtained by rocket immunoelectrophoresis. Fibroblasts were derived from normal subjects with phenotype 1 of acid α-glucosidase (●), from two patients (M-3773 and GM-443) with the adult form of acid α-glucosidase deficiency (○), and from fibroblasts with phenotype 2 of acid α-glucosidase (□). Cells harvested at both early and late confluency were used. *Arrow* indicates a fibroblast strain with phenotype 1 of acid α-glucosidase obtained from an ovarian dermoid cyst (GM-1306). *A*, Enzymatic activity measured with maltose as substrate; *B*, enzymatic activity measured with glycogen as substrate.

Figure 6 illustrates the amount of enzyme protein measured at early and late confluency in the fibroblast strains studied. Large differences were demonstrated in the amount of enzyme protein per μg cellular protein in the adult form and in the cells with phenotype 2 compared with normal cells. In addition, the data obtained with normal fibroblast lysates segregated into two distinct curves, reflecting an approximate twofold increase in specific activity at late confluency over

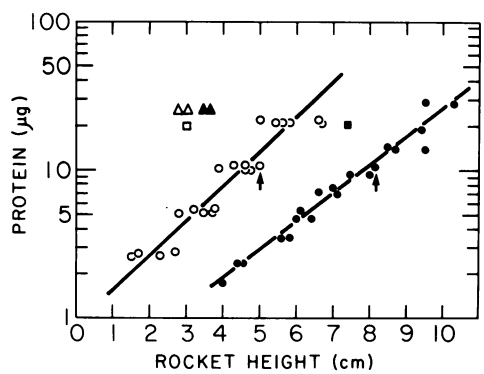

FIG. 6.—Relationship between amount of fibroblast protein used and rocket height obtained by rocket immunoelectrophoresis. Fibroblast lysates from normal subjects with phenotype 1 of acid α-glucosidase (○, ●), from two patients (M-3773 and GM-443) with the adult form of acid α-glucosidase deficiency (△, ▲), and from a fibroblast strain with phenotype 2 of the enzyme (□, ■) were used. *Arrow* indicates a fibroblast strain with phenotype 1 of acid α-glucosidase derived from an ovarian dermoid cyst (GM-1306). *Open symbols* indicate cells harvested at early confluency; *closed symbols* indicate cells harvested at late confluency.

that observed at early confluency. A similar increase in specific activity was also evident in the adult-form fibroblasts. The activity in phenotype 2 cells at early confluency was similar to that observed in cells from patients with the adult form of acid α-glucosidase deficiency. At late confluency, however, the enzymatic activity in phenotype 2 cells increased four- to fivefold over early confluency values and frequently approached near-normal levels (see table 1 and fig. 6).

Quantitative analysis of rocket immunoelectrophoresis gels, such as shown in figure 4, in terms of enzyme units, cellular protein applied, and rocket height obtained (figs. 5 and 6) showed that in two of the adult forms (M-3773 and GM-443), the observed 78%–91% reduction in enzymatic activity corresponded to an equivalent reduction in the amount of enzyme protein present. The direct relationship between enzymatic activity and detectable enzyme protein was observed at both early and late confluency. In fibroblasts with phenotype 2 of acid α-glucosidase, the enzyme activity measured with maltose substrate increased from 10%–20% of normal at early confluency to 40%–60% of normal at late confluency. This increase in activity was accompanied by an identical increase in the amount of enzyme protein detected. Neither the adult-form fibroblasts nor the cells homozygous for isozyme 2 of acid α-glucosidase demonstrated any CRM that was not accountable as enzyme activity (see table 1).

DISCUSSION

The observation that there are two molecular forms of infantile acid α-glucosidase deficiency, CRM-positive and CRM-negative, shows that there is genetic heterogeneity in the infantile form of the disease. This was demonstrated by both immunodiffusion and immunotitration experiments. The finding that the amount of precipitated enzyme in mixtures of lysates from normal and CRM-positive infantile acid α-glucosidase deficiency fibroblasts was decreased by 30% instead of 50% suggests that the amount of inactive enzyme protein present in the deficient fibroblasts is reduced.

It appears that the CRM-negative form of the disease is the more frequent type, and it has been found both in blacks and Caucasians. The CRM-positive form was observed in a black male infant who died at the age of 5 months [10] and whose clinical picture was indistinguishable from that of the CRM-negative patients. Also, the apparent acid α-glucosidase activity in the CRM-positive fibroblasts was within the range of the apparent activity obtained in the CRM-negative cells. We do not know whether this 1%–3% hydrolysis of the substrate is caused by acid α-glucosidase molecules or if it is the result of nonspecific hydrolysis of the substrate. The identification of the two molecular forms of the disease is of potential clinical importance, since attempts at therapy by parenteral administration of purified enzyme may lead to the production of antiacid α-glucosidase antibodies in CRM-negative patients.

It is not known at the present time whether or not double heterozygotes for the CRM-positive and CRM-negative gene will be affected. Fusion between CRM-positive and CRM-negative fibroblasts and subsequent measurement of the acid α-glucosidase activity would indicate whether or not complementation occurs

TABLE 1

ACID α-GLUCOSIDASE ACTIVITY AND PROTEIN IN CULTURED SKIN FIBROBLASTS

FIBROBLAST STRAIN	ENZYME ACTIVITY (MEAN ± SD)*		RATIO LC/EC	RANGE OF RESIDUAL ENZYME ACTIVITY	RANGE OF RESIDUAL ENZYME PROTEIN†
	EC	LC		(% of normal)	(% of normal)
Normal‡	4551 ± 2042	9735 ± 3774	2.1 ± 0.6	100	100
Adult form:					
M-3773‡	494 ± 111	871 ± 77	1.8 ± 0.3	9–19§	10–22§
GM-443‡	522 ± 72	827 ± 155	1.6 ± 0.3	9–22§	16–22§
GM-1935‡	105 ± 77	194 ± 94	1.8 ± 0.1	1–3§	···§
α-G_2‡	888 ± 166	4202 ± 28	4.7 ± 0.5	20–60‖	20–60‖

NOTE: Acid α-glucosidase activity and protein was measured in cultured skin fibroblasts from six normal subjects, from three patients with the adult form of acid α-glucosidase deficiency, and from a fibroblast strain with phenotype 2 of the enzyme (α-G2) harvested at early confluency (EC) and late confluency (LC). Cells were lysed by sonication in 0.05 M citrate-phosphate buffer, pH 5, and assayed with maltose as substrate. The percentage of residual enzyme protein was determined following rocket immunoelectrophoresis.

* U/mg protein.

† Values obtained from the analysis of multiple rocket immunoelectrophoresis gels.

‡ Normal: no. = 6; M-3773, GM-443, and GM-1935: mean of four different preparations; α-G_2: mean of six different preparations.

§ Values obtained at early and late confluency.

‖ 20% of normal at early confluency; 60% of normal at late confluency.

between the two forms. Lack of complementation could be interpreted as an indication that patients carrying both the CRM-positive and CRM-negative genes would be affected.

In two of the three cases with the adult form of acid α-glucosidase deficiency studied, the amount of enzyme protein in the fibroblasts was reduced. Direct measurement of the amount of enzyme protein using rocket immunoelectrophoresis demonstrated that the reduced enzymatic activity was directly proportional to the reduction in the amount of enzyme protein. The enzyme in both the normal fibroblasts and in the fibroblasts with the adult form of acid α-glucosidase deficiency (cases with reduced specific activity) showed the same catalytic activity toward the substrates maltose and glycogen. This was best demonstrated by plotting enzyme units applied against rocket height following antigen-antibody immunoelectrophoresis (see fig. 5).

One of the fibroblast strains with the adult form of acid α-glucosidase deficiency (GM-1935) was different from the other two in that the enzyme activity in these cells was only 1%–3% of normal and no CRM could be detected. This apparent residual activity was the same as that observed in fibroblasts from patients with the infantile form. Lysates of cells prepared immediately after harvesting failed to demonstrate an increase in activity, which would have indicated the presence of a labile enzyme. However, this possibility cannot be ruled out. In the other two fibroblast strains with the adult form of the disease, the activity was 9%–22% of normal. The very low enzyme activity measured in the GM-1935 fibroblasts is not in line with the observation of Reuser et al. [4] that there is an inverse correlation between the severity of the clinical symptoms and the degree of residual enzymatic activity in fibroblasts. The present findings in cultured skin fibroblasts cannot explain why patient GM-1935 developed the adult instead of the infantile form of the disease.

Fibroblasts with phenotype 2 of acid α-glucosidase showed both a reduced amount of enzyme protein and a reduced ability of the enzyme to cleave glycogen, when compared to fibroblasts with phenotype 1 of the enzyme. We demonstrated earlier that isozyme 2 of acid α-glucosidase has a decreased catalytic activity for glycogen [12], but it was not known whether or not the enzyme activity in cells with phenotype 2 of acid α-glucosidase was decreased. The reduction in the amount of enzyme protein in fibroblasts with phenotype 2 was unexpected and could be due to a decreased rate of synthesis or an increased rate of degradation of the enzyme. In vitro studies showed that isozymes 1 and 2 of acid α-glucosidase have the same thermal denaturation profiles [12], a finding that may indicate a reduced rate of synthesis of isozyme 2. However, turnover studies of the enzyme protein are necessary to clarify this issue.

The findings reported here demonstrate the presence of extensive genetic heterogeneity in acid α-glucosidase deficiency. Deficiency of this enzyme may result from: (1) the presence of an enzyme protein with severely reduced catalytic activity (CRM-positive infantile form of acid α-glucosidase deficiency); (2) the absence of the enzyme protein, or the presence of an enzyme protein that is so altered that it has lost both catalytic and immunologic properties (CRM-negative infantile and

CRM-negative adult subtypes); (3) a reduction in the amount of enzyme protein, which leads to a proportional reduction in enzyme activity (adult form of acid α-glucosidase deficiency); and (4) the presence of an enzyme with reduced catalytic activity for glycogen and a parallel reduction in the number of enzyme molecules (cells with phenotype 2 of acid α-glucosidase). Whether or not subjects homozygous for isozyme 2 of acid α-glucosidase will develop muscular weakness late in life, similar to that observed in the adult form of the disease, remains unknown at the present time.

ACKNOWLEDGMENTS

We are grateful to Dr. Eugene Ainbender for his helpful suggestions regarding the development of the rocket immunoelectrophoresis system. We also thank Mrs. Safiana Katz for excellent technical assistance.

REFERENCES

1. Hers HG: Alpha-glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). *Biochem J* 86:11–16, 1963
2. Brown BI, Brown DH, Jeffrey PL: Simultaneous absence of α-1,4-glucosidase and α-1,6-glucosidase activities (pH 4) in tissues of children with type II glycogen storage disease. *Biochemistry* 9:1423–1428, 1970
3. Mehler M, DiMauro S: Residual acid maltase activity in late-onset acid maltase deficiency. *Neurology* 27:178–184, 1977
4. Reuser AJJ, Koster JF, Hoogeveen A, Galjaard H: Biochemical, immunological, and cell genetic studies in glycogenosis type II. *Am J Hum Genet* 30:132–143, 1978
5. deBarsy T, Jacquemin P, Devos P, Hers HG: Rodent and human acid α-glucosidase: purification, properties and inhibition by antibodies; investigation in type II glycogenosis. *Eur J Biochem* 31:156–165, 1972
6. Brown BI, Murray AK, Brown DH: The lysosomal α-glucosidases of mammalian tissues, in *Physiological Effects of Food Carbohydrates*, vol 15, ACS Symposium Series, edited by Jeanes A, Hodge J, Washington, D.C., American Chemical Society, 1975, pp 223–234
7. Koster JF, Slee RG: Some properties of human liver acid α-glucosidase. *Biochim Biophys Acta* 482:89–97, 1977
8. Murray AK, Brown BI, Brown DH: The molecular heterogeneity of purified human liver lysosomal α-glucosidase (acid α-glucosidase). *Arch Biochem Biophys* 185:511–524, 1978
9. Schram AW, Brouwer-Kelder B, Donker-Koopman WE, Loonen C, Hamers MN, Tager JM: Use of immobilized antibodies in investigating acid α-glucosidase in urine in relation to Pompe's disease. *Biochim Biophys Acta* 567:370–383, 1979
10. Beratis NG, LaBadie GU, Hirschhorn K: Characterization of the molecular defect in infantile and adult acid α-glucosidase deficiency fibroblasts. *J Clin Invest* 62:1264–1274, 1978
11. Swallow DM, Corney G, Harris H, Hirschhorn R: Acid α-glucosidase: a new polymorphism in man demonstrable by "affinity" electrophoresis. *Ann Hum Genet* 38:391-406, 1975
12. Beratis NG, LaBadie GU, Hirschhorn K: An isozyme of acid α-glucosidase with reduced catalytic activity for glycogen. *Am J Hum Genet* 32:137–149, 1980
13. Linder D, Kaiser B, Hecht F: Parthenogenic origin of benign ovarian teratomas. *N Engl J Med* 292:63–66, 1975
14. Beratis NG, Turner BM, Weiss R, Hirschhorn K: Arylsulfatase B deficiency in Maroteaux-Lamy syndrome: cellular studies and carrier identification. *Pediatr Res* 9:475–480, 1975

15. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–277, 1951
16. ARNON R, SHAPIRA E: Antibodies to papain. A selective fractionation according to inhibitory capacity. *Biochemistry* 6:3942–3950, 1967
17. LAURELL C-B: Electroimmunoassay. *Scand J Clin Lab Invest [Suppl]* 124:21–37, 1970
18. ALPERT E, CANTON R, PERROTTO J: Immunoenzymatic assay for alphafetoprotein. *Lancet* 1:626, 1974

Erratum

In the paper "Patterns of DNA Replication of Human Chromosomes. II. Replication Model" by M. Camargo and J. Cervenka (*Am J Hum Genet* 34:757–780, 1982), the figure on page 765 with A, B, and C subdivisions should appear on page 762 with the figure 2 legend; the figure on page 762 with only A and B subdivisions should appear on page 765 with the figure 3 legend.