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A specific acid α-glucosidase in lamellar bodies of the human lung

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In the present investigation, we have demonstrated that three lysosomal-type hydrolases, α-glucosidase, α-mannosidase and a phosphatase, are present in lamellar bodies isolated from adult human lung. The hydrolase activities that were studied, all showed an acidic pH optimum, which is characteristic for lysosomal enzymes. The properties of acid α-glucosidase in the lamellar body fraction and that in the lysosome-enriched fraction were compared. Using specific antibodies against lysosomal α-glucosidase from human placenta, two α-glucosidases could be distinguished in the lamellar body fraction: one with a high affinity to the antibodies as found in the lysosome-enriched fraction and another with a much lower affinity. Both forms showed an acidic pH optimum. The same heterogeneity of α-glucosidase in the lamellar body fraction could be observed using immobilized concanavalin A. The lectin was able to precipitate nearly all α-glucosidase activity of the lysosome-enriched fraction. In contrast, 30% of the α-glucosidase activity in the lamellar body fraction was not precipitable. Furthermore, the lamellar body α-glucosidase with the low antibody affinity could not be bound to concanavalin A. The results suggest that lamellar bodies contain at least two acid α-glucosidases: one similar to the lung lysosomal α-glucosidase, and another lamellar body-specific isoenzyme with a different immunoreactivity and lectin affinity. The lamellar body-specific α-glucosidase should prove useful as a lamellar body-specific marker enzyme.

Introduction

The alveoli of the lung are lined with pulmonary surfactant, a lipid-protein complex that prevents alveolar collapse and transudation by lowering the surface tension at the interface of the air and the liquid covering the epithelial cells [1]. This surfactant is produced by the alveolar type II epithelial cells [1,2]. Prior to its secretion by these cells the surfactant is stored in lamellar bodies [3,4]. Dipalmitoylphosphatidylcholine is considered to be the most important surface-active constituent of pulmonary surfactant [5]. Lipids comprise approx. 95% by weight of purified surfactant, while the remaining 5% is protein. In many biochemical studies it has been shown that hydrolytic enzyme activities are present in lamellar body preparations [6,7] as well as in the extracellular layer lining the alveoli [8]. In addition, hydrolases in lamellar bodies have been demonstrated histochemically [9]. However, it is not known what the relationship is between these activities and similar activities in the lysosomes. To throw more light on this question, we compared the properties of the hydrolase activities in lung lysosomes with those found in preparations of lamellar bodies. Special attention was paid to acid α-glucosidase because of the availability of specific antibodies against this enzyme. It is

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known that lysosomal enzymes [10,11], including acid α-glucosidase from human liver [12,13], are glycoproteins. Therefore, lectin binding assays were performed to obtain information about possible differences in the oligosaccharide moiety of lysosomal and lamellar body α-glucosidase. In this paper we present evidence that isolated lamellar bodies contain acid hydrolase activities which are not due to lysosomal contamination and that lamellar bodies contain a specific lysosomal-type α-glucosidase.

Materials and Methods

Materials. 4-Methylumbelliferyl-α-D-glucoside and 4-methylumbelliferyl-α-D-mannoside were obtained from Sigma Chemical Co., St. Louis, MO, *p*-nitrophenylphosphate from Koch-Light, Suffolk, U.K., silica gel G from Merck, Darmstadt, F.R.G., and glycogen from United States Biochemical Corporation, OH. Sepharose 4B, Protein A-Sepharose CL-4B, concanavalin A-Sepharose and Sephacryl were purchased from Pharmacia, Uppsala, Sweden. Goat anti-rabbit and goat anti-mouse IgG horseradish peroxidase conjugates were bought from Bio-Rad Laboratories, Richmond, CA.

Isolation of subcellular lung fractions. Fresh human lung parenchyma was obtained from patients who had undergone a lung resection or lobectomy to remove a malignancy. The tissue used for isolation of the different subcellular fractions was tumor free. The lung tissue was rinsed with 0.9% NaCl, minced and homogenized in 0.33 M sucrose/0.01 M Tris-HCl (pH 7.0) (5 ml/g lung tissue) using a motor-driven Potter Elvehjem homogenizer with a Teflon pestle and, subsequently, a hand-driven homogenizer with a ground-glass pestle (clearance 0.25 mm). Lamellar bodies, a lysosome-enriched fraction and microsomes were isolated from the homogenate as described in Refs. 14 and 15. The lamellar body fraction had the same high phospholipid phosphorus-to-protein ratio and similar high contents of phosphatidylcholine and phosphatidylglycerol as reported earlier [14]. In addition, electronmicroscopic studies in this earlier work [14] had shown that the lamellar body fraction isolated by this procedure consists predominantly of intact lamellar bodies. All preparations were sonicated six times for 30 s at 0°C with an MSE ultrasonic disintegrator at 21 kcycles/s and an amplitude of 6 μm peak-to-peak.

Preparation of placenta and liver homogenates. Human placentas were obtained as soon as possible after delivery and frozen at −20°C until use. Human liver tissue was obtained at autopsy, frozen immediately at −20°C, and stored at this temperature until used. Placenta and liver homogenates (10%, w/v) in phosphate-buffered saline (150 mM NaCl, 10 mM phosphate (pH 7.4)) were made with a Potter-Elvehjem homogenizer.

Lipid analyses. Lipids of the subcellular fractions were extracted according to the method of Bligh and Dyer [16]. Phospholipids were separated by two-dimensional thin-layer chromatography on silica gel G plates impregnated with boric acid [17] using chloroform/methanol/water/concentrated NH_3 (15 M) (70 : 37 : 4 : 6, v/v) and chloroform/methanol/water (65 : 35 : 5, v/v) as developing solvents. After detection by brief exposure to iodine vapor, phospholipids were extracted from the silica gel [16]. Phospholipid phosphorus was estimated according to the method of Bartlett [18].

Enzyme assays. Activities of α-glucosidase and α-mannosidase were measured fluorimetrically using methylumbelliferyl glycosides as substrates. The fluorescence of the liberated 4-methylumbelliferone was measured in an Eppendorf fluorimeter with a 313–366 nm filter for excitation and a 420–3000 nm secondary filter. Acid phosphatase was determined using *p*-nitrophenylphosphate as substrate. The liberated *p*-nitrophenol was estimated spectrophotometrically at 405 nm, a molar extinction coefficient of $18.5 \cdot 10^6$ cm^2/mol being used. The substrate concentrations in the reaction mixtures were: 0.44 mM 4-methylumbelliferyl-α-D-glucoside, 0.44 mM 4-methylumbelliferyl-α-D-mannoside or 2.7 mM *p*-nitrophenylphosphate. In addition to substrate, the incubation mixtures contained 0.1 ml McIlvaine's buffer (pH 3.0–8.0) [19], varying amounts of protein (50–100 μg) and water to a final volume of 0.5 ml. After incubation at 37°C for 1–2 h, the reaction was stopped by adding 2 ml 0.3 M glycine/NaOH buffer (pH 10.6). To determine α-glucosidase activity towards glycogen as substrate, the reaction mixture contained 100 mM sodium acetate (pH 4.0), 10 mg/ml glycogen, appropriate aliquots of lung preparations and water to a total volume of 0.5 ml. In

control incubations either lung preparation or substrate was omitted. After incubation at 37°C for 1 h, the reaction was stopped by heating the mixture at 100°C for 2 min. The liberated glucose was estimated using hexokinase and glucose-6-phosphate dehydrogenase [20]. All subcellular fractions were sonicated again just before use.

Antibody and lectin binding studies. Acid α-glucosidase was purified from human placenta by the procedure described for liver [21]. Rabbits were injected with the purified enzyme in Freund's adjuvant followed by three booster injections with incomplete Freund's adjuvant to obtain anti-α-glucosidase antibodies [21]. A control immunoglobulin preparation (control Ig) was obtained from rabbits injected with Freund's adjuvant only. Varying amounts of antibody were mixed with 50 μl packed gel of Protein A-Sepharose Cl-4B and phosphate-buffered saline to a total volume of 250 μl. The suspension was stirred well for 1 h at room temperature. The immobilized anti-α-glucosidase was centrifuged down at $2000 \times g$ for 2 min. Subsequently, the immunobeads were washed five times with phosphate-buffered saline. To obtain a 1 : 1 diluted gel suspension, 50 μl phosphate-buffered saline were added to 50 μl packed immunobeads. Incubations of lung preparations or a placental glycoprotein fraction with immobilized anti-α-glucosidase or concanavalin A-Sepharose 4B were performed at 37°C for 1 h. Then, the reaction mixture was centrifuged at $2000 \times g$ for 2 min. The enzymic activities in the supernatant and the activity bound to the immunobeads or concanavalin A-Sepharose 4B beads were assayed as described above.

Electrophoretic blotting procedure. Electrophoresis on a 10% polyacrylamide slab gel in the presence of SDS was performed using the discontinuous system described by Laemmli [22]. The proteins were transferred electrophoretically from the gels to nitrocellulose filters according to Towbin et al. [23]. The filters were washed three times with a 0.05% (w/v) solution of Tween 20 (polyoxyethylene sorbitanmonolaurate) in phosphate-buffered saline (hereafter indicated as phosphate-buffered saline/Tween). After washing, the filters were immersed in 1% bovine serum albumin in phosphate-buffered saline/Tween for 30 min, washed again with phosphate-buffered saline/Tween and subsequently incubated overnight with polyclonal anti-α-glucosidase antibodies raised in a rabbit as described above or with a mixture of three monoclonal anti-α-glucosidase antibodies produced as described by Hilkens et al. [24]. The filters were then washed six times with phosphate-buffered saline/Tween. Following this, the blots treated with polyclonal antiserum were incubated for 1 h with goat anti-rabbit IgG horseradish peroxidase conjugate, whereas the blots treated with the mixture of monoclonal antibodies were incubated for 1 h with goat anti-mouse IgG horseradish peroxidase conjugate. Both peroxidase conjugates were diluted in phosphate-buffered saline/Tween (1 : 3000). The filters were then washed four times with phosphate-buffered saline/Tween and twice with 20 mM Tris (pH 7.0) −50 mM NaCl. Immune complexes were visualized by incubating the filters with peroxidase color development reagent (Bio-Rad) according to the manufacturer's instructions. The reaction was terminated by washing in distilled water. The whole procedure was performed at room temperature with gentle shaking of the filters.

Enzyme-linked immunosorbent assay. The specificity of polyclonal anti-α-glucosidase was checked by an enzyme-linked immunosorbent assay as follows. A 96-well microtiter plate was coated with lung homogenate, lamellar body fraction, human IgA or human IgG for 1 h at 37°C. Each well contained 30 ng–60 pg of protein in 150 μl phosphate-buffered saline. After washing the plate with phosphate-buffered saline/Tween, polyclonal anti-α-glucosidase was added to each well and the plates were incubated at 37°C for 1 h. After washing with phosphate-buffered saline/Tween, 150 μl of a 1 : 3000 dilution of goat anti-rabbit IgG horseradish peroxidase conjugate was added to each well. It should be stressed that the presence of Tween 20 was essential in order to prevent non-specific adsorption of protein. After 1 h at 37°C, the plate was washed five times with phosphate-buffered saline/Tween and 150 μl of a freshly prepared solution containing 11 mM *o*-phenylenediamine dihydrochloride, 0.6 mM H_2O_2 and 0.1 M phosphate buffer (pH 6.0) was added to each well. The reaction was stopped by addition of 50 μl of 2 M H_2SO_4 and $A_{492\,nm}$ was measured in a Titertek spectrophotometer.

Other methods. A placental and a urine glycoprotein fraction were isolated as described for liver [21]. Protein was determined by the method of Lowry et al. [25].

Results

pH optima of hydrolases in lamellar bodies of human lung

Several studies have shown that hydrolase activities are frequently associated with the lamellar body fraction [6–8,26–28]. However, except in one study on phospholipase A_1 and A_2 in lung lamellar bodies from rabbit [26], the pH optima of the hydrolase activities were not investigated. It is well established now, that the intralysosomal pH is low compared to that of the surrounding medium (see Refs. 29 and 30 for reviews). Therefore, it is to be expected that in lysosomes the degradation of macromolecules is catalyzed by hydrolases with an acid pH optimum [31]. However, several non-lysosomal hydrolases without an acid optimum are able to hydrolyze the same substrates as the lysosomal isoenzymes do. For example, at least two isoenzymes of α-glucosidase are known in human liver: a lysosomal acid-α-glucosidase [32,33] with a pH optimum at about 4.5 and a cytoplasmic neutral α-glucosidase [32,34] with a pH optimum at 6.5. Thus, the neutral α-glucosidase may interfere in the determination of the lysosomal α-glucosidase even at a low pH as a consequence of the considerable activity of this cytoplasmic enzyme at acidic pH.

If the hydrolases in the lamellar bodies have a lysosomal character, a low pH optimum can be expected. Fig. 1 shows that the lamellar body fraction does, indeed, contain hydrolase activities with an acid pH optimum.

Binding of α-glucosidase to immobilized concanavalin A and immobilized anti-α-glucosidase antibodies

In all current hypotheses on the mechanisms involved in the delivery of lysosomal enzymes, which are glycoproteins [10,11], to the lysosomal apparatus an important role is assigned to the oligosaccharide moiety [35]. To the best of our knowledge none of the authors who claimed that lamellar bodies contain lysosomal of lysosomal-type enzymes investigated whether these enzymes are glycoproteins. In order to obtain information on this point we made use of the lectin concanavalin A, which binds molecules containing α-D-mannopyranosyl or α-D-glucopyranosyl residues.

Fig. 2 shows the effect of preincubation with concanavalin A bound to Sepharose 4B on the acid α-glucosidase activity in preparations of human lung. As a standard we used lysosomal α-glucosidase in a glycoprotein fraction from human placenta. Upon increasing the amount of immobilized concanavalin A the activity of the placental enzyme remaining in the supernatant decreased abruptly. Addition of 10 μl (1 : 1) of the suspension of immobilized concanavalin A enabled the precipitation of more than 95% of the acid α-glucosidase activity. Most of the α-glucosidase activity was also precipitated from lung homogenate and the lysosome-enriched fraction from lung. A rather different curve was observed with the lamellar body fraction. At low concanavalin A concentrations, the α-glucosidase activity in the supernatant fell rapidly. However, ultimately, a plateau

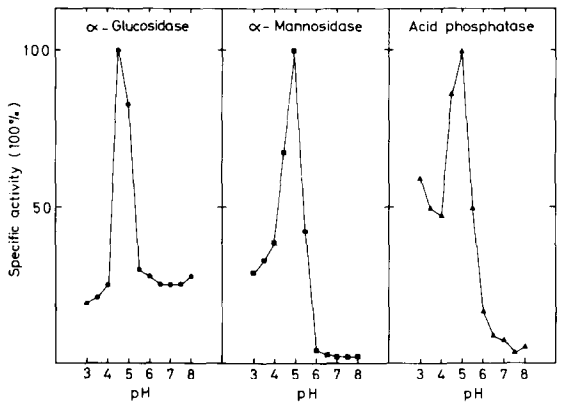

Fig. 1. The effect of pH on the activity of α-glucosidase, α-mannosidase and acid phosphatase in the lamellar body fraction from human lung. The activities of α-glucosidase and α-mannosidase were determined with 4-methylumbelliferyl derivatives as substrates. Acid phosphatase was assayed with *p*-nitrophenylphosphate. The optimal specific activities were taken as 100%. The optimal specific activities of α-glucosidase, α-mannosidase and acid phosphatase were 0.5, 1.8 and 71.5 nmol of substrate hydrolyzed/min per mg protein, respectively. Results of a typical experiment from a series of three are shown.

was reached, which was caused by 30% non-precipitable α-glucosidase activity. These data suggest that the lamellar body fraction contains at least two α-glucosidases: one with a high affinity to concanavalin A (like the activity in the other preparations) and another with no affinity to concanavalin in the test used. This shows the existence of at least two α-glucosidases in the lamellar body fraction. Binding to uncoated Sepharose beads was insignificant (Fig. 2).

As Table I shows, the highest specific activity of the α-glucosidase not binding to immobilized concanavalin A was observed in the lamellar body fraction. Whereas in whole lung homogenate, and in the lysosome-enriched and microsomal lung fractions specific activities below 10 pmol of substrate hydrolyzed/min per mg protein were measured, the specific activity in the lamellar body fraction was at least an order of magnitude higher.

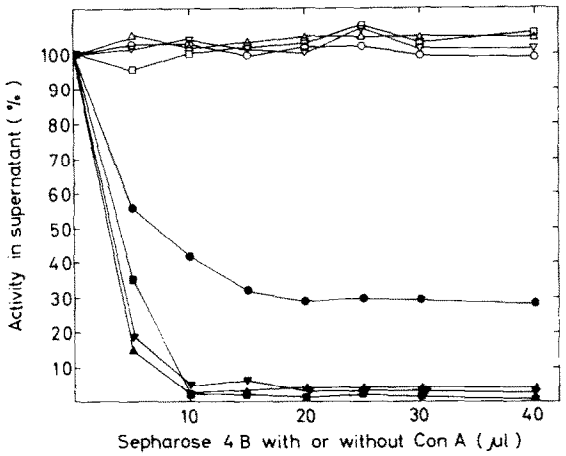

Fig. 2. Effect of titration with concanavalin A-Sepharose 4B on the acid α-glucosidase activity in preparations from human lung and placenta. The sonicated preparations were preincubated with concanavalin A-Sepharose 4B as described in Materials and Methods. After centrifugation, α-glucosidase activity at pH 4.5 was determined with methylumbelliferyl-α-D-glucoside as the substrate in the supernatant from the lamellar body fraction (●) the lung homogenate (▼) the lysosome enriched fraction (▲) and a glycoprotein fraction isolated from placenta (■). The same procedure was followed with uncoated Sepharose beads and the control values obtained in this way are indicated by the corresponding open symbols. All incubations with the lectin were performed with the same total amount of α-glucosidase activity. Results of a typical experiment from a series of four are shown.

It should be noted that the concanavalin A-negative α-glucosidase activity in the microsomal fraction is not only low compared to that in the lamellar body fraction (Table I), but moreover has no acid pH optimum and no affinity to glycogen (data not shown). The latter is in contrast to the concanavalin A-negative α-glucosidase activity in the lamellar body fraction. Therefore, the non-binding α-glucosidase activity with affinity to glycogen and with an acid pH optimum seems to be lamellar body specific. Furthermore, this activity seems to be specific for lung. Whereas in whole lung homogenate the non-binding activity could be estimated to be between 5 and 10 pmol of substrate hydrolyzed/min per mg protein, no non-binding activity could be detected in homogenates from human liver and placenta (data not shown).

A further investigation of the properties of α-glucosidase in the various subcellular fraction of human lung was performed with a polyclonal antiserum raised against lysosomal α-glucosidase from human placenta. These antibodies were immobilized by binding them to Protein A-Sepharose 4B. The purified placental α-glucosidase preparation showed a high affinity to the immobilized antibodies (Fig. 3). We found no significant differences in

TABLE I

TOTAL α-GLUCOSIDASE ACTIVITY AND α-GLUCOSIDASE ACTIVITY WITHOUT BINDING AFFINITY TO IMMOBILIZED CONCANAVALIN A IN PREPARATIONS FROM HUMAN LUNG

The α-glucosidase activity was determined with 4-methylumbelliferyl-α-D-glucoside at pH 4.5. For experimental details see Materials and Methods. Data represent mean ± S.E. The numbers in parentheses are the number of preparations used.

Preparation	α-Glucosidase activity (pmol methylumbelliferone/min per mg protein)	
	total	non-precipitable
Lung homogenate	106.4 ± 6.0(6)	7.2 ± 3.3(6)
Lung lysosome-enriched fraction	483.3 ± 26.3(3)	5.4 ± 3.6(3)
Lung lamellar body fraction	371.1 ± 18.3(4)	115.8 ± 12.0(4)
Lung microsomal fraction	59.2 ± 6.6(3)	8.1 ± 1.2(3)

percent immunoprecipitable activity between this placental preparation, the lung homogenate and the pulmonary lysosome-enriched fraction (Fig. 3). With the lamellar body fraction a biphasic precipitation curve was obtained (Fig. 3). This heterogeneity in the α-glucosidase activity indicates again the existence of at least two α-glucosidases in the lamellar body preparation, one with an affinity to the antibodies similar to that of the placental enzyme and another with a much lower affinity to anti-α-glucosidase. As a control, non-specific rabbit immunoglobulins immobilized to Protein A-Sepharose 4B were used. Binding to these control beads was insignificant (Fig. 3).

Both the experiments with immobilized concanavalin A and those with immobilized anti-α-glucosidase antibodies show that a lamellar body-specific type of α-glucosidase exists. The following experiment was carried out to investigate the relationship between the isoenzyme with low affinity towards concanavalin A and that with low affinity towards anti-α-glucosidase. The pH dependence of

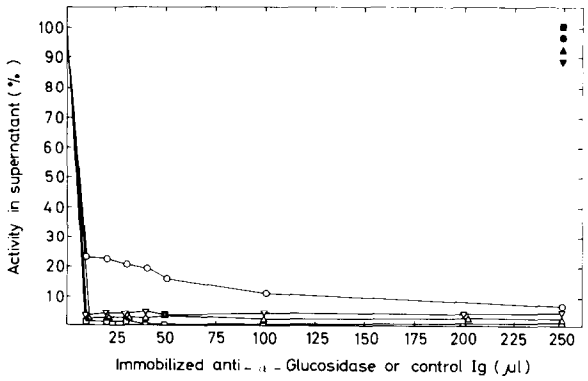

Fig. 3. Effect of titration with polyclonal anti-α-glucosidase bound to Protein A-Sepharose 4B on the acid α-glucosidase activity in preparations from human lung and placenta. After preincubation of the sonicated preparations with immobilized anti-α-glucosidase for 1 h at 37°C the suspension was centrifuged and the α-glucosidase activity with methylumbelliferyl-α-D-glucoside as substrate was measured at pH 4.5 in the supernatant from the lamellar body fraction (○) the lung homogenate (▽) the lysosome-enriched fraction (△) and a glycoprotein fraction isolated from placenta, (□). Controls (corresponding closed symbols) were obtained by incubation with beads coated with preimmune serum. The same total amount of α-glucosidase activity was used in all incubations performed. Results of a typical experiment from a series of three are shown.

the α-glucosidase activities present in the lamellar body fraction was measured after a preincubation with immobilized concanavalin A, immobilized anti-α-glucosidase antibodies or uncoated control beads. After the preincubation the beads were spun down. We determined the pH dependence both of the unbound α-glucosidase activity remaining in the supernatant and of the activity bound to the beads. The results of a typical experiment from a series of three are shown in Fig. 4. Upon preincubation with Protein A-Sepharose 4B (control beads), optimal activity was observed at pH 4.5. More than 90% of the enzymic activity did not bind to these control beads. Hence, aspecific binding was less than 10%. After preincubation with anti-α-glucosidase coupled to Protein A-Sepharose (immunobeads), about one third of the activity was found in the supernatant, while the rest could be detected on the immunobeads. Both isoenzymes, that with a high affinity and that with a low affinity to anti-α-glucosidase, show an optimum at pH 4.5. Fig. 4 also shows that the α-glucosidase with a low affinity to the antibodies cannot be precipitated by immobilized concanavalin A. This strongly suggests that the α-glucosidase with a low affinity to the antibody and the one without affinity to concanavalin A are identical. The α-glucosidase activity which is not bound to Protein A-Sepharose can be partly bound to immobilized concanavalin A. The amount of α-glucosidase activity which is not bound to concanavalin A beads is equal to the amount of activity unbound to immobilized anti-α-glucosidase alone. This strongly suggests that the α-glucosidase with a low affinity to anti-α-glucosidase and the one without affinity to concanavalin A are the same. Fig. 4 also shows that the α-glucosidase activity that can be removed by the second incubation with concanavalin A beads is about equal to the amount of activity bound by anti-α-glucosidase beads. These observations also suggest that the α-glucosidase activities with a high affinity to concanavalin A and the antibodies are identical. It should be emphasized that both isoenzymes identified have a low pH optimum.

The concanavalin A-positive and -negative α-glucosidases in the lamellar body fraction, like the α-glucosidase activity in the lysosome-enriched fraction, were able to hydrolyse not only the artifi-

cial substrate 4-methylumbelliferyl-α-glucoside but also the natural substrate glycogen (data not shown). There is evidence from studies in other tissues that neutral α-glucosidase has little, if any, activity towards glycogen [36].

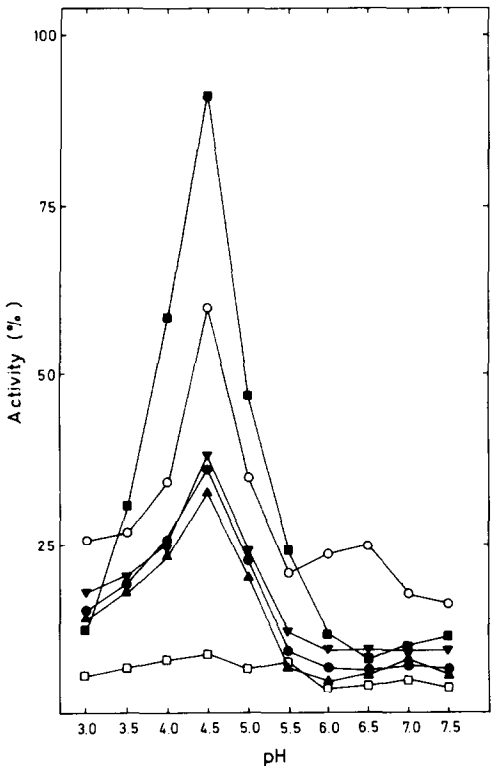

Fig. 4. Effect of pH, immobilized concanavalin A and anti-α-glucosidase antibodies on α-glucosidase activities in the lamellar body fraction. α-Glucosidase activities were determined with methylumbelliferyl-α-D-glucoside as substrate after different preincubations. Lamellar body sonicate was preincubated with Protein A-Sepharose 4B beads. After centrifugation the unbound activity was measured in the supernatant (■) and the bound activity on the beads (□). Part of the supernatant of the first incubation was subjected to a second incubation with concanavalin A-Sepharose 4B beads. After centrifugation the activity in the resulting supernatant was assayed (▼). The same procedure with immunobeads (polyclonal anti-α-glucosidase coupled to Protein A-Sepharose 4B) yielded a bound α-glucosidase activity (○) and an unbound activity in the supernatant (●). Subsequently, part of this supernatant was incubated with concanavalin A-Sepharose 4B beads and the unbound activity determined in the resulting supernatant (▲). The same total amount of α-glucosidase activity was used in all incubations performed. Results of a typical experiment from a series of three are shown.

SDS-polyacrylamide gel electrophoresis and immunoblotting

The different preparations were also analyzed by SDS-polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol as a reducing agent. The proteins were transferred to nitrocellulose sheets using the electrophoretic procedure. Fig. 5 shows an immunoblot treated with monoclonal mouse anti-α-glucosidase antibodies. Several immunoreactive bands were detectable. A glycoprotein fraction from human urine was used as a reference. The urine preparation (lane 6) shows one main band of $M_r = 110000$, a second of $M_r =$

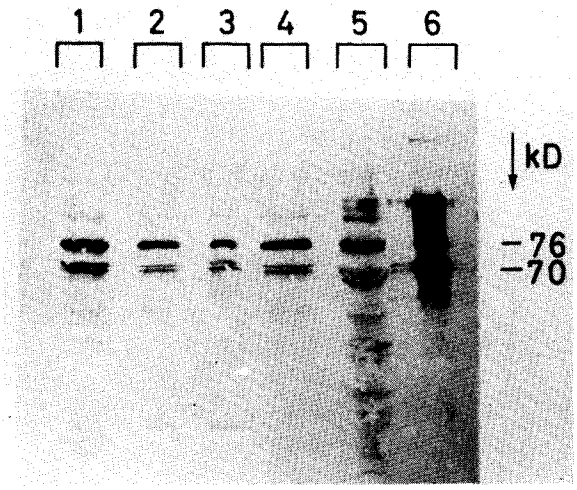

Fig. 5. Immunoblot with monoclonal anti-α-glucosidase antibodies. The samples, containing 25 μg of protein, were added to the SDS and 2-mercaptoethanol-containing sample buffer described by Laemmli [22] and proteins were separated on a 10% SDS-polyacrylamide slab gel. The proteins were electrophoretically transferred to nitrocellulose paper. The immunoblot was treated with a mixture of three monoclonal antibodies against lysosomal α-glucosidase. The antigen-antibody complexes were visualized using horseradish peroxidase-labelled goat antibodies to mouse IgG. The molecular weights of the protein standards used were: phosphorylase b, 94000; bovine serum albumin, 67000; ovalbumin, 43000; carbonic anhydrase, 30000; trypsin inhibitor, 20100, α-lactalbumin, 14400 (not visible on the immunoblot). Lane 1: lysosome-enriched lung fraction. Lane 2: lamellar body proteins without binding affinity to concanavalin A-Sepharose 4B. Lane 3: lamellar body fraction with binding affinity to concanavalin A-Sepharose 4B. Lane 4: total lamellar body fraction. Lane 5: whole lung homogenate. Lane 6: a glycoprotein fraction from human urine.

76000 and a doublet band with M_r of about 70000. The molecular weights of the protein bands are in accordance with those reported earlier for lysosomal α-glucosidase from human urine [37]. The major bands of M_r 70000 and 76000 are detectable in all lung preparations (lanes 1–5). The pattern observed for the concanavalin A-Sepharose-treated lamellar body fraction (lane 2) remained the same when the concanavalin-Sepharose treatment was repeated to remove possibly remaining traces of the concanavalin A-positive α-glucosidase (not shown). The band observed in urine (lane 6) with a molecular mass of 110000 Da, which represents a precursor form of a α-glucosidase, is also observed in homogenate of whole lung (lane 5). The polyclonal antiserum could not be used in the immunoblotting experiment with lung material because of the presence of traces of anti-IgG and anti-IgA antibodies as shown by an enzyme-linked immunosorbent assay.

Discussion

Several studies have shown that considerable hydrolase activities are present in lamellar body fractions isolated from the lungs of various species [6,8,26–28]. However, with the exception of phospholipase A_1 and A_2 [26], the pH optima of these hydrolases were not investigated. In the present investigation we demonstrate that purified human lamellar bodies contain hydrolase activities with an acid pH optimum. The present observations suggest that it is unlikely that the acid hydrolase activity in the lamellar body fraction is due to contamination with acid hydrolases from lysosomes.

We paid special attention to the α-glucosidase activity in lamellar bodies. The availability of polyclonal and monoclonal anti-acid α-glucosidase antibodies offered us the opportunity to characterize this enzyme in a highly specific way. No differences were found in immunoprecipitability between the acid α-glucosidase in lung lysosomes and that from placenta, which was used as a reference. The results indicated that the lysosomal fraction contained an α-glucosidase with high affinity towards both concanavalin A and anti-α-glucosidase. The lamellar body fraction also contained this protein but, in addition, an isoenzyme was present which had a low affinity towards both concanavalin A and anti-α-glucosidase. Both α-glucosidases are characterized by low pH optima and are able to degrade glycogen, the natural substrate of lysosomal α-glucosidase.

Immunoblotting using monoclonal anti-α-glucosidase antibodies shows that the lamellar body fraction contains lysosomal α-glucosidase. The two immunoreactive main bands of $M_r = 70000$ and 76000 as described earlier for placenta [11] were observed not only in the concanavalin A-binding fraction but also in the fraction that does not bind to concanavalin A. Thus, we cannot distinguish between the concanavalin A-negative α-glucosidase in the lamellar body fraction and the normal lysosomal α-glucosidase by this immunoblotting technique. We conclude that a lamellar body fraction from human lung contains an α-glucosidase identical to lysosomal α-glucosidase from lung, and a lamellar body-specific acid α-glucosidase with a different immuno- and lectin affinity. The absence of a significant concanavalin A-negative α-glucosidase activity in the lysosome-enriched fraction strongly indicates that at least one acid hydrolase activity in the lamellar body fraction is not the result of lysosomal contamination caused by the isolation procedure. In addition, it is very unlikely that the concanavalin A-negative α-glucosidase in the lamellar body fraction is caused by mitochondrial or microsomal contamination. An earlier study showed that the lamellar body fraction contains no mitochondria and less than 5% microsomal contamination [14]. We found that although the microsomal fraction contains a significant amount of concanavalin A-negative α-glucosidase, this activity differs from that in the lamellar body fraction by not having an acid pH optimum and not having affinity to glycogen. Therefore, the concanavalin A-negative acid α-glucosidase in the lamellar body fraction is probably endogenous to these organelles. This makes it useful as a lamellar body-specific marker enzyme; until now, no marker enzyme has been available for lamellar bodies. In placenta and liver tissue we could not measure any concanavalin A-negative α-glucosidase activity at all. This suggests that the non-binding enzyme found in lamellar bodies is lung specific.

Specific functions of lamellar body-associated

hydrolases in general and lamellar body-specific α-glucosidase in particular are not known. Bourbon and Jost [38] suggest that in fetal lung, lysosomal α-glucosidase is involved in glycogen breakdown and that lamellar bodies may play a role in this process. However, the role of acid hydrolases in general in adult lamellar bodies remains unclear at present. The presence of acid hydrolytic enzymes within these surfactant secretion organelles implies that the lamellar body-specific α-glucosidase can be expected in lung surfactant. Indeed, hydrolytic enzymes have been found in the extracellular lining of the terminal airways and alveoli [8]. However, further identification and characterization of these enzymes need to be done to shed light on their physiological role.

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