Characterization of lysosomes and lysosomal enzymes from Chediak–Higashi-syndrome cultured fibroblasts

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Chediak–Higashi-syndrome cultured skin fibroblasts were used to study the possible involvement of lysosomal enzymes and lysosomal dysfunction in this disorder. Our evidence indicated that Chediak–Higashi fibroblasts displayed a significant decrease in the specific activity of the acidic α -D-mannosidase (pH 4.2) compared with normal controls. Additional studies revealed a small, but significant, decrease in the rate of degradation of ^{125}I -labelled β -D-glucosidase that had been endocytosed into Chediak–Higashi cells.

INTRODUCTION

Chediak–Higashi syndrome, a rare human autosomal recessive genetic disease, is clinically characterized by partial albinism, recurrent bacterial infections, neuropathies and early death (Chediak, 1952; Higashi, 1954; White, 1966, 1967; Padgett *et al.*, 1969). One of the diagnostic criteria for this disorder is the appearance of abnormally large cytoplasmic inclusions in circulating white blood cells, platelets, thyroid cells and neural tissues (Davis *et al.*, 1971a,b; Root *et al.*, 1972; Costa *et al.*, 1976; Spicer *et al.*, 1981). Subsequent histochemical and immunocytochemical studies have shown that these inclusions contain acid phosphatase and peroxidase activities (Hirschhorn & Weissman, 1965; Essner & Oliver, 1973; Kimball *et al.*, 1975; Rausch *et al.*, 1978) and comprise primary, heterophagic secondary or autophagic secondary lysosomes (White & Clawson, 1980; Spicer *et al.*, 1981). Furthermore, the interaction of the Chediak–Higashi lysosomes with phagosomes containing bacteria is accompanied by a continual incorporation, or fusion, leading to the formation of ‘giant’ lysosomes. This results in a lower specific activity of several lysosomal enzymes and a decrease in the bactericidal index (Weissman, 1964; White, 1966; Padgett *et al.*, 1969; Kimball *et al.*, 1975; Clawson *et al.*, 1979; Tanaka, 1980). It has been proposed that Chediak–Higashi disease represents a ‘lysosomal disorder’. However, the molecular relationship of the genetic defect to the morphological findings of ‘giant’ lysosomes, the possible involvement of lysosomal enzymes, and the predisposition of Chediak–Higashi patients to pyrogenic infection, has not been resolved.

Skin fibroblasts from patients with Chediak–Higashi syndrome contain phosphatase-positive granules or inclusions analogous to those seen in their leucocytes (Danes & Bearn, 1967; Ostlund *et al.*, 1979). The present study uses cultured skin fibroblasts from patients with Chediak–Higashi disease as a model system to compare certain properties of their lysosomal enzymes and lysosomal function with normal controls. Our results revealed a significant decrease in the acid α -D-mannosidase specific activity from Chediak–Higashi samples compared with normal controls. In addition, the Chediak–Higashi cells exhibited a decrease in the rate of degradation of endocytosed β -D-glucosidase. A preliminary report of these findings has appeared (Stein *et al.*, 1983).

EXPERIMENTAL

Protein determination

Proteins were determined as described by Lowry *et al.* (1951), with bovine serum albumin as a standard. All procedures were carried out at 0–4 °C unless otherwise stated.

Culture conditions

Chediak–Higashi-syndrome skin fibroblast cells were obtained from two unrelated patients. The first cell line (passage 3) was derived from an 18-month-old female and supplied by the Human Genetic Mutant Cell Repository, Camden, NJ, U.S.A. (GM2075). The second sample (passage 5) represented cells from a 4-year-old male patient (no. 154) sent by Dr. Erik Harms, Universitäts-Kinderklinik, Im Neuenheimer Feld 150, 6900 Heidelberg, West Germany. The control cells represent cell lines (passages 2–10) derived from eight normal individuals who were in an age range similar to the affected patients. All cell lines had been stored under liquid N₂ before culture for the experiments detailed in this paper. The Chediak–Higashi and normal skin fibroblast cells were cultured in glass roller bottles or plastic flasks in Coon’s modified (Coon & Weiss, 1969) or Ham’s F-12 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum plus penicillin/streptomycin, Fungizone and glutamine. The cells were then harvested at confluency by scraping in phosphate (10 mM)-buffered saline, pH 7.4, and collected by centrifugation for 10 min at 450 g (Miller *et al.*, 1981). The cells were resuspended in 10 vol. of glass-distilled water, and homogenates were obtained by sonicating the harvested cells (Kontes Scientific Instruments, San Leandro, CA, U.S.A.) with four 5 s bursts in an ice bath.

Assay for acid hydrolases

All acid hydrolase activities were assayed at 37 °C with the corresponding fluorescent 4-methylumbelliferyl substrates (Koch–Light Laboratories, Colnbrook, Bucks., U.K.) as reported elsewhere (Kress *et al.*, 1980; Miller *et al.*, 1981). Briefly, up to 20 μl of cell homogenate containing 2–3 mg of protein/ml was added to 50 μl of buffer/substrates solutions containing: 6 mM-4-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside in 40 mM-citrate/phosphate, pH 4.4; 2 mM-4-methylumbelliferyl α -D-glucopyranoside in 200 mM-sodium acetate, pH 4.0; 5 mM-4-methylumbelliferyl α -D-man-

nopyranoside in 100 mM-citrate/phosphate, pH 4.2; 5 mM-4-methylumbelliferyl α -D-mannopyranoside in 200 mM-citrate/phosphate, pH 5.6, and 10 mM- CoCl_2 ; 1 mM-4-methylumbelliferyl α -L-fucopyranoside in 1 mM-sodium citrate, pH 5.0, and 10 mg of bovine serum albumin/ml; 5 mM-4-methylumbelliferyl β -D-glucuronide in 200 mM-sodium acetate, pH 4.1; 0.5 mM-4-methylumbelliferyl β -D-galactopyranoside in 22 mM-citrate/phosphate, pH 4.35, and 100 mM-NaCl; 5 mM-4-methylumbelliferyl β -D-glucopyranoside in 200 mM-citrate/phosphate, pH 5.4, 8 mg of Triton X-100/ml and 10 mg of sodium taurocholate/ml; 2 mM-4-methylumbelliferyl α -D-glucopyranoside in 0.2 M-sodium acetate, pH 4.0; or 0.5 mM-4-methylumbelliferyl α -D-glucopyranoside in 250 mM-Tris/HCl, pH 6.8.

Assay for cathepsin D

Cathepsin D activities were determined by incubating 50 μ l of cell homogenate with a mixture containing 50 μ l of 1 M-sodium formate, pH 3.3, and 50 μ l of 8% (w/v) bovine haemoglobin in a 45 °C water bath for 15 or 30 min. The assay mixtures were subsequently chilled and 1 ml of cold 3% (w/v) trichloroacetic acid was added to each tube. The mixture was centrifuged for 10 min at 450 g and the A_{280} of the supernatant was determined. One unit of activity is defined as the amount of enzyme that produced an increase in A_{280} of 1.0/h in the assay (Barrett, 1970).

Differential assay for acidic and intermediate-pH α -D-mannosidases

Sonicated cell homogenates were divided into two tubes and sealed. One tube was incubated at a 60 °C water bath for 30 min to inactivate the α -D-mannosidase activity at pH 5.6 (intermediate), and the other homogenate was kept at 0–4 °C for 30 min. Both tubes were then placed into an ice bath for 5 min and sonicated. The α -D-mannosidase activities were assayed at pH 5.6 (intermediate) with 10 mM- CoCl_2 or at pH 4.2 (acidic). The specific activities of the two enzymes were calculated as described by Winchester *et al.* (1976). Co^{2+} stimulates the intermediate-pH α -D-mannosidase (Hirani & Winchester, 1979), but has no effect on the acidic form of the enzyme.

Colloidal-silica-gradient isolation of lysosomes

Two roller bottles of cultured normal or Chediak-Higashi cells were grown for 2 weeks to confluency by the procedures described above. The growth medium was poured off and the cells were rinsed twice with 20 ml of 0.9% NaCl. Then 5 ml of 2.5% (w/v) trypsin and 20 ml of 0.9% NaCl were added to each roller bottle. After rotating the bottles for 10 min at 37 °C, the cells were collected by centrifugation at 1500 g for 20 min. The supernatant was discarded and the cell pellet suspended in 20 ml of growth medium containing 15% (v/v) bovine serum for 15 min at room temperature. The cells were then centrifuged as before, washed with 0.9% NaCl and re-centrifuged. The resulting cell pellet was resuspended in 2.0 ml of 0.25 M-sucrose. The suspension was slowly passed through an 18-gauge syringe needle. Unbroken cells and cell debris were pelleted by centrifugation in an SS34 rotor at 3500 rev./min for 10 min. The supernatant was kept and the pellet suspended in 2.0 ml of 0.25 M-sucrose. The suspension was slowly passed through a 20-gauge syringe needle. The sample was

centrifuged as above and the supernatant kept. The pellet was again resuspended in 2.0 ml of 0.25 M-sucrose. The suspension was passed slowly through a 22-gauge syringe needle and centrifuged in a SS34 rotor at 3500 rev./min for 10 min. The resulting pellet was discarded and the combined supernatants were fractionated on a colloidal-silica gradient. The preparation of the gradient and the centrifugation procedure were identical with those used by Rome *et al.* (1979).

Radioiodination of purified β -D-glucosidase

A 25 μ g portion of purified β -D-glucosidase from *Dictyostelium discoideum* (Freeze *et al.*, 1983) was radioiodinated with 1 mCi of carrier-free ^{125}I (Amersham/Searle) by the chloramine-T procedure (Greenwood *et al.*, 1963). The procedure yielded 1.25×10^7 c.p.m./ μ g of protein, and 95% was precipitable with 10% trichloroacetic acid. The radiolabelled β -D-glucosidase was analysed by autoradiography as described by Elder *et al.* (1977).

Uptake of ^{125}I -labelled β -D-glucosidase

This was performed as described by Freeze *et al.* (1983), with normal and Chediak-Higashi fibroblasts grown to confluency in 24-well multi-test dishes which contained 40–50 μ g of protein/well. Briefly, growth medium containing 10% fetal bovine serum was removed and replaced with 250 μ l of minimal essential medium (9.5 g/litre), 0.02 M-Mes and 1 mg of human serum albumin/ml (uptake medium). Uptake was allowed to proceed as indicated for each experiment. After uptake, the unbound ^{125}I -labelled ligand was removed by aspiration and the cells were washed with 6 $\times$ 1 ml of phosphate-buffered saline at 0–4 °C. The cells were lysed by adding 200 μ l of 0.25% (w/v) sodium deoxycholate in 15 mM-sodium phosphate, pH 6.5 (lysis buffer), to each well. After 15–30 min at room temperature, samples were removed for protein determination and radioactivity counting in a Beckman gamma counter. After subtraction of background radioactivity (20–30 c.p.m.), the uptake was calculated as c.p.m./mg of cell protein. Non-specific uptake in the presence of 2 mM-mannose 6-phosphate accounted for about 5% of total radioactivity.

Binding measurements using ^{125}I -labelled β -D-glucosidase

These were performed for 2 h at 0–4 °C in multi-test plates as described for uptake measurements. Non-specific binding was determined by performing incubations in the presence of 10 mM-mannose 6-phosphate and usually amounted to 8–25% of the total radioactivity. The higher concentration of mannose 6-phosphate was required to prevent specific binding.

Stability of endocytosed ^{125}I -labelled β -D-glucosidase

Cultured fibroblasts were allowed to take up iodinated β -D-glucosidase in uptake media for 2 h at 37 °C. At times indicated, intra- and extra-cellular trichloroacetic acid-soluble and -precipitable radioactivity was counted.

Inhibition by chloroquine of uptake of ^{125}I -labelled β -D-glucosidase

Various concentrations of chloroquine were tested in uptake medium at pH 7.4 for its effect on the endocytosis of ^{125}I -labelled β -D-glucosidase into normal and Chediak-Higashi cells. These studies were performed in 24-well

Table 1. Acid hydrolase activities of normal and Chediak-Higashi-syndrome cultured skin fibroblasts

Up to 20 μ l of cell homogenate containing 2–3 mg of protein/ml was added to 50 μ l of substrate/buffer solution containing: 5 mM-4-methylumbelliferyl α -D-mannopyranoside in 100 mM-citrate/phosphate, pH 4.2; 5 mM-4-methylumbelliferyl β -D-glucuronide in 200 mM-sodium acetate, pH 4.1; 2 mM-4-methylumbelliferyl α -D-glucopyranoside in 200 mM-sodium acetate, pH 4.0; 5 mM-4-methylumbelliferyl β -D-glucopyranoside in 200 mM-citrate/phosphate, pH 5.4, 8 mg of Triton X-100/ml and 10 mg of sodium taurocholate/ml; 6 mM-4-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside in 40 mM-citrate/phosphate, pH 4.4; 1 mM-4-methylumbelliferyl α -L-fucopyranoside in 1 mM-sodium citrate, pH 5.0, and 10 mg of bovine serum albumin/ml; or 0.5 mM-4-methylumbelliferyl β -D-galactopyranoside in 22 mM-citrate/phosphate, pH 4.35. The assays were run for 15 and 30 min at 37 °C. Data for normals are the results of multiple determinations using eight different cell lines ranging from 14 to 26 days in age, except cathepsin D and β -D-glucosidase, where a smaller number of normal samples were used.

Enzyme	Specific activity (nmol/h per mg)				
	Normals		Chediak-Higashi syndrome		% of normal activity
	Mean	Range	Mean	Range	
Acid α -D-mannosidase	129	54–207	30	18–42	23
β -D-Glucuronidase	299	126–394	348	246–404	116
α -D-Glucosidase	94	51–282	150	126–174	160
N-Acetyl- β -D-hexosaminidase	10307	4423–15355	10800	8580–12960	105
α -D-Fucosidase	210	60–307	150	114–180	71
β -D-Galactosidase	941	72–1428	1134	960–1308	121
β -D-Glucosidase*	720	476–725	600	348–798	83
Cathepsin D†	2.44	2.05–2.99	4.36	3.25–5.74	179

* Average of multiple determination using four normal cell lines.

† Average of four determinations of one normal line; in units/mg, where 1 unit = change of 1.0 A_{280} unit/h.

multi-test plates by using the procedure outlined above for the uptake of 125 I-labelled β -D-glucosidase.

RESULTS

Lysosomal enzyme activities in cultured fibroblasts

The specific activity of acid α -D-mannosidase in normal fibroblasts was 4-fold greater than that of the enzyme from Chediak-Higashi-syndrome cells (GM2075) (Table 1). Similar results were obtained with a second Chediak-Higashi cell line (154), where the α -mannosidase activity was 19% of normal, whereas β -D-glucuronidase was in the normal range. Additional enzymes could not be measured, owing to the continued poor growth of this cell line. In contrast, the specific activity of α -D-glucosidase and cathepsin D were higher in Chediak-Higashi cells. The specific activities of the other acid hydrolases tested showed no significant differences between the normal and Chediak-Higashi cells. The lower α -D-mannosidase activity is not due to a lowered growth rate of the Chediak-Higashi cells, since the number of cells per flask for both cell lines was comparable when cultured for 14–20 days (results not shown).

Properties of α -D-mannosidase in Chediak-Higashi fibroblasts

The pH-activity curves of α -D-mannosidase indicate that cultured human skin fibroblast cells contain at least two forms of α -D-mannosidase (Figs. 1a and 1c). One form exhibited optimum activity at pH 4.2, and was not stimulated by 10 mM- CoCl_2 . A second form of α -D-

mannosidase displayed optimum activity at pH 5.6, was stimulated by 10 mM- CoCl_2 , and was inactivated by incubation at 60 °C for 30 min (compare all four panels of Fig. 1). There were no differences in the pH optima for the two mannosidases from normal and Chediak-Higashi cell lines. However, the Chediak-Higashi cells demonstrated a significant decrease in the α -D-mannosidase activity at pH 4.2.

This observation was further supported after examination of the specific activities of acidic and intermediate-pH α -D-mannosidases after differential heat inactivation (Table 2). The specific activities of the intermediate-pH α -D-mannosidase for both normal and Chediak-Higashi fibroblasts were the same; however, Chediak-Higashi fibroblasts showed a significant decrease in the activity of the acidic α -D-mannosidase. The activity ratio of acidic to intermediate forms was 3-fold greater for normal cells than for Chediak-Higashi cultured cells, indicating that the lower specific activity of α -D-mannosidase was the result of an effect on the acidic lysosomal enzyme. Substrate-saturation studies revealed that the remaining Chediak-Higashi α -D-mannosidase activity at pH 4.2 retained normal affinity for its 4-methylumbelliferyl substrate (Table 3).

Isolation of lysosomes from Chediak-Higashi cells

By colloidal-silica density-gradient centrifugation, primary (light) and secondary (heavy) lysosomal populations were isolated from normal and Chediak-Higashi cultured fibroblasts. On the basis of assay of marker lysosomal enzymes (Miller *et al.*, 1981), no differences in specific activities were found between normal and Chediak-Higashi lysosomes (Fig. 2). Overall recoveries

Fig. 1. pH-activity curves of α -D-mannosidase activity from normal and Chediak-Higashi fibroblasts

Samples of dialysed supernatants from normal and Chediak-Higashi fibroblast homogenates were assayed at various pH values from 2.1 to 7.3 in the presence (●) or absence (○) of Co^{2+} .

Table 2. Differential assay of acid and intermediate α -D-mannosidase in normal and Chediak-Higashi-syndrome cultured skin fibroblasts

All homogenates were divided into two fractions; one was heat-inactivated at 60 °C for 30 min, and the other was incubated at 0–4 °C for the same period of time. α -D-Mannosidases were assayed at pH 4.2 or 5.6 in the presence of 10 mM- CoCl_2 for both fractions. Specific activities for acid and intermediate α -D-mannosidase were calculated as described by Winchester *et al.* (1976). The results are calculated on the basis of three duplicate determinations.

α -D-Mannosidase	Specific activity (nmol/h per mg)			
	Normal		Chediak-Higashi syndrome	
	Mean	Range	Mean	Range
Acidic	90	78–108	30	18–36
Intermediate	36	30–42	48	24–66
Ratio acidic/intermediate	2.4	2.3–2.7	0.7	0.3–1.3

Table 3. Apparent K_m and V_{max} values for α -D-mannosidase from normal and Chediak-Higashi-syndrome cultured skin fibroblasts

All homogenates were dialysed for 3 h against 200 vol. of 10 mM-phosphate buffer, pH 6.5, and assayed at substrate concentrations of 0.25, 0.75, 1.0, 1.5 and 2 mM by the assay systems described in the Experimental section. Values for K_m and V_{max} were obtained by the least-squares method. Data represent the results of two duplicate determinations.

Assay condition	Apparent K_m (mM)				Apparent V_{max} (nmol/h per mg)			
	Normal		Chediak-Higashi syndrome		Normal		Chediak-Higashi syndrome	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
pH 4.2	1.43	1.14–1.71	1.56	1.13–1.76	289	146–431	118	56–179
pH 5.6, 10 mM- CoCl_2	0.44	0.32–0.56	0.23	0.08–0.37	152	61–242	122	64–180

Fig. 2. Distribution of marker enzyme activities for the separation of lysosomes from (a) normal and (b) Chediak-Higashi fibroblasts

Two lysosome fractions were obtained by colloidal-silica gradient centrifugation as described by Rome *et al.* (1979). Abbreviation: INT, *p*-iodonitrotetrazolium violet.

of *N*-acetyl- β -D-hexosaminidase activity applied to the gradient were > 95%. Experiments testing the structure-linked latency of *N*-acetyl- β -D-hexosaminidase and acid α -D-mannosidase activities present in the lysosomal fractions from normal and Chediak-Higashi cultured cells were found to exhibit over 90% latency, by using the method of Sellinger *et al.* (1960), which was modified by Baccino & Zuretti (1975), indicating that the structural integrity of the lysosomal membrane in both cell types was similar (results not shown).

Receptor-mediated endocytosis by Chediak-Higashi cells

These studies were carried out to ascertain if the path-

Fig. 3. Uptake measurements of ^{125}I -labelled β -D-glucosidase (purified from *Dictyostelium discoideum*) into normal and Chediak-Higashi fibroblasts

Normal (●) and Chediak-Higashi (○) fibroblast cells were grown to confluency in 24-well multi-test dishes. Receptor-mediated endocytosis was carried out for 2 h at 37 °C in a 5%-CO₂ incubator. The ^{125}I -labelled ligand was removed by aspiration and the cell monolayers were washed with phosphate-buffered saline, pH 7.4, at 4 °C. The cells were lysed by addition of 200 μl of 0.25% sodium deoxycholate in 15 mM-sodium phosphate, pH 6.5. After 15–30 min at room temperature, 50 μl was removed for protein determinations, and 100 μl was assayed for radioactive-ligand incorporation with a gamma counter. Uptake was calculated as c.p.m./mg of cell protein. The inset shows double-reciprocal plots of the results.

way of receptor-mediated endocytosis and subsequent intracellular routing and degradation of a labelled ligand is functioning normally in Chediak-Higashi cells. It has been previously established that highly purified extracellular β -D-glucosidase from the culture medium of *Dictyostelium discoideum* is efficiently endocytosed into normal human fibroblasts through a receptor-mediated, saturable and mannose 6-phosphate-inhibitable uptake system, with a K_{uptake} of 1.0×10^{-9} M and V_{max} of 0.38 pmol/h per mg of cell protein (Freeze *et al.*, 1983). When cultured skin fibroblast cells from Chediak-Higashi patients were compared with normal cell lines in similar endocytosis experiments using ^{125}I -labelled β -D-glucosidase, little, if any, difference in K_{uptake} was observed between normal and Chediak-Higashi cells over a 15-fold variation of ligand concentrations (Fig. 3). The binding of ^{125}I -labelled β -D-glucosidase to normal and Chediak-Higashi fibroblasts at 0 °C was also saturable, with a K_d of 1.1×10^{-9} M, and was nearly complete by 2 h (Fig. 4). We have estimated the number of specific binding sites (receptors) per cell, assuming that one molecule of ligand is bound to one receptor, to be 12800 per cell and 13500 per cell for normal and Chediak-Higashi cells respectively. Studies to examine the fate of the endocytosed ^{125}I -labelled β -D-glucosidase revealed that Chediak-Higashi cells exhibited a slower degradation rate of the endocytosed ligand, as monitored by the slower appearance of trichloroacetic acid-soluble

Fig. 4. Binding of different concentrations of ^{125}I -labelled β -D-glucosidase to normal (a) and Chediak-Higashi-syndrome (b) fibroblasts. The specific binding (●, ○) is obtained by subtracting non-specific binding in the presence of 10 mM-mannose 6-phosphate (■, □) from apparent binding (▲, △). (c) represents a Lineweaver-Burk plot of specific binding to normal (●) and Chediak-Higashi (○) cells. One unit of β -glucosidase equals 0.26 nmol. The binding sites for normal and Chediak-Higashi-syndrome cells were calculated as 12800/cell and 13500/cell respectively.

Fig. 5. Stability of ^{125}I -labelled β -D-glucosidase after uptake

Cultured normal (●, ▲) and Chediak-Higashi-syndrome (○, △) fibroblasts were allowed to take up 0.13 nmol of ^{125}I -labelled β -D-glucosidase in uptake medium for 2 h at 37 °C; the culture medium was removed and the cell surfaces were washed with 10 mM-mannose 6-phosphate at 37 °C for 15 min. Then 250 μl of fresh uptake medium was added back to each well and re-incubated for various times. At indicated times, 200 μl of uptake medium was removed and an equal amount of chilled 20% trichloroacetic acid was added. The trichloroacetic acid-non-precipitable fractions from the medium (▲, △) were counted for radioactivity. The remaining uptake medium was removed by aspiration and 150 μl of 0.25% deoxycholate was added to each well. A 100 μl sample was mixed with an equal volume of human serum albumin (5 mg/ml), and precipitable fractions (●, ○) were counted for radioactivity. Trichloroacetic acid-precipitable fractions from the medium (about 3%) and cell-associated trichloroacetic acid-non-precipitable fractions (about 1%) were also counted for radioactivity, and the results were expressed as a percentage of total radioactivity.

material in the growth medium (Fig. 5). Additional studies demonstrated that the lysosomotropic amine chloroquine inhibited receptor-mediated endocytosis of the labelled β -D-glucosidase into normal and Chediak-Higashi cells. The apparent K_i of uptake of the ligand was 10–20 μM -chloroquine for both normal and Chediak-Higashi cell lines (results not shown). Subsequent studies revealed no significant differences between the receptor-mediated endocytosis of normal or Chediak-Higashi lysosomal *N*-acetyl- β -D-hexosaminidase into cultured Sandhoff-syndrome cells, indicating that the mannose 6-phosphate marker was not altered on Chediak-Higashi lysosomal enzymes (results not shown).

DISCUSSION

The primary defect responsible for Chediak-Higashi syndrome is unknown. Evidence suggests that an alteration in lysosomal function may be involved, but the exact nature of this involvement is not clear. The present studies represent the first report utilizing cultured human fibroblasts as a model system to evaluate selected properties of lysosomal function and lysosomal enzymes in an effort to provide information about their participation in the disease mechanism responsible for Chediak-Higashi syndrome. The experiments utilizing labelled β -D-glucosidase from *Dictyostelium discoideum* revealed that the processes of receptor-mediated endocytosis, binding and uptake of labelled β -D-glucosidase into Chediak-Higashi cultured fibroblasts and inhibition of the receptor-mediated endocytosis by chloroquine are not significantly different from those observed with normal cells (Figs. 3 and 4). The first indication of lysosomal dysfunction in Chediak-Higashi cells was seen in a slower degradation rate of the endocytosed β -D-glucosidase and a delayed appearance of trichloroacetic acid-soluble material into the media (Fig. 5). Additional evidence supporting an alteration of lysosomal function came from our isolation of Chediak-Higashi lysosomes by free-flow electrophoresis (Miller *et al.*, 1981). These latter studies indicated that the 'giant' secondary lysosomes from Chediak-Higashi cultured fibroblasts were significantly more sensitive to the

procedure of electrophoretic separation than the corresponding normal controls (results not shown). Recoveries for marker lysosomal enzymes averaged less than 1%, compared with 16–20% for normal controls (results not shown). These results are in agreement with the suggestion that the impaired killing of bacteria by Chediak-Higashi neutrophils is attributed to an alteration in properties of their lysosomes (Clawson *et al.*, 1979). However, the relationship of these altered properties to the primary defect is unknown at present.

Evidence suggests that the decreased bactericidal index may be related to decreases in selected lysosomal enzyme activities, including α -D-mannosidase, α -D-galactosidase, α -L-fucosidase, myeloperoxidase and β -D-glucuronidase, in a variety of circulating white blood cells from Chediak-Higashi patients (Padgett, 1967; Kimball *et al.*, 1975; Tanaka, 1980). Our data with cultured Chediak-Higashi fibroblasts indicated that the only significant difference from normal controls was a consistent decrease in the expression of acidic α -D-mannosidase activity in the Chediak-Higashi samples (Table 1).

The cause of the decreased acidic α -D-mannosidase activity and decreased turnover of endocytosed ligand in Chediak-Higashi cells and tissues is not known, but possible explanations include a change in the rate of biosynthesis or degradation of the enzyme, an alteration in the post-translational modification of the enzyme which leads to changes in its intracellular stability or the presence of an inhibitor. The last possibility was eliminated by a series of mixing experiments between normal and Chediak-Higashi cultured cell extracts (results not shown). Evidence does exist in other autosomal recessive disorders that a structural gene mutation can result in an alteration of post-translational modifications leading to subsequent activity changes (Burditt *et al.*, 1978). Our current studies do not yet allow us to distinguish between the first two possibilities.

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