Biochem. J. (1983) **210**, 795–802
Printed in Great Britain

Inhibition by cyanate of the processing of lysosomal enzymes

Andrej HASILIK, Regina POHLMANN and Kurt VON FIGURA
Physiologisch-Chemisches Institut der Universität Münster, Waldeyerstrasse 15, D 4400 Münster, West Germany

(*Received 5 August 1982/Accepted 19 November 1982*)

In cultured human fibroblasts, maturation of the lysosomal enzymes β-hexosaminidase and cathepsin D is inhibited by 10mM-potassium cyanate. In cells treated with cyanate the two enzymes accumulate in precursor forms. The location of the accumulated precursor is probably non-lysosomal; in fractionation experiments the precursors separate from the bulk of the β-hexosaminidase activity. The secretion of the precursor of cathepsin D, but not that of β-hexosaminidase precursor, is enhanced in the presence of cyanate. The secreted cathepsin D, as well as that remaining within the cells, contains mostly high-mannose oligosaccharides cleavable with endo-β-*N*-acetylglucosaminidase H. After removal of cyanate, the accumulated precursor forms of the lysosomal enzymes are largely released from the pretreated cells. It is concluded that cyanate interferes with the maturation of lysosomal-enzyme precursors by perturbing their intracellular transport. Most probably cyanate affects certain functions of the Golgi apparatus.

Lysosomal enzymes are synthesized as larger precursors. Within several hours the precursors are converted intracellularly into mature or intermediate forms of smaller size (reviewed by Hasilik, 1980; Neufeld, 1981). The transport of the enzymes into the lysosomes depends on phosphorylated oligosaccharides in these enzymes (reviewed by Sly & Creek, 1981).

The subcellular location of the phosphorylating system (Varki & Kornfeld, 1980; Waheed *et al.*, 1981), formation of complex oligosaccharides (Hasilik & von Figura, 1981) and cytochemical observations in certain cells (Novikoff, 1976; Bainton & Farquhar, 1970; Bentfeld-Barker & Bainton, 1982) indicate that the enzyme precursors pass through the Golgi apparatus. During or after the transport of the precursors to lysosomes, a proteolytic processing takes place.

It has been claimed that in Nil hamster fibroblasts cyanate reversibly inhibits cathepsin B-like enzymes and arrests turnover of a hexose-carrier system (Christopher & Morgan, 1981). Since thiol-dependent cathepsins have been suggested to be involved in processing of lysosomal enzyme precursors (Frisch & Neufeld, 1981), we have become interested in the effect of cyanate on the processing of the precursors. Our results show that cyanate perturbs the transport of lysosomal enzymes.

Materials and methods

Materials

Foetal-calf serum was purchased from Boehringer Mannheim, Mannheim, W. Germany, and dialysed foetal-calf serum was from Gibco, Paisley, Scotland, U.K. L-[^{35}S]Methionine (sp. radioactivity 1196Ci/mmol) and standard [^{14}C]methylated proteins were from NEN, Dreieich, W. Germany. [2-^{3}H]Mannose (sp. radioactivity 22Ci/mmol) was from Amersham Buchler, Braunschweig, W. Germany, endo-β-*N*-acetylglucosaminidase H (specific activity 30units/mg) was from Seikagaku, Tokyo, Japan, and alkaline phosphatase (type III-R from *Escherichia coli*; specific activity 38units/mg) from Sigma Chemical Co., München, W. Germany. Instagel was purchased from Packard Instruments, Frankfurt, W. Germany, potassium cyanate 'for synthesis' was from Merck, Darmstadt, W. Germany, and sodium dodecyl sulphate was from Sigma (cat. no. L-5750).

Culture and labelling of cells

Human skin fibroblasts were maintained at 37°C in 5% CO_2 in air in Eagle's minimal essential medium supplemented with non-essential amino acids and 7.5% foetal-calf serum (Boehringer Mannheim) as described by Cantz *et al.* (1972). Confluent cultures grown (unless otherwise stated)

0306-3283/83/030795-08$2.00

in six-well dishes (9 cm^2) were radioactively labelled as described by Hasilik & Neufeld (1980), by using 20 μCi of L-[^{35}S]methionine and 2 ml of methionine-free Waymouth medium supplemented with 4.5% dialysed foetal-calf serum, which was preincubated at pH 10.4 (Hasilik & von Figura, 1981). The labelling was terminated by adding 1–2% (v/v) of L-methionine (5 mg/ml). At 4 h before labelling, cells were supplemented with 2 ml of Eagle's minimal essential medium with 7.5% foetal-calf serum with or without potassium cyanate. The methionine-free medium used to wash and preincubate the cells for 1 h before labelling (Hasilik & von Figura, 1981) also contained cyanate, except in controls.

Preparation of samples for immunoprecipitation

The dialysed concentrate of medium was prepared as described by Hasilik & von Figura (1981) and frozen. The cells were scraped off the dish, resuspended in 0.5 ml of 50 mM-Tris/HCl, pH 7.4, supplemented with 25 μl of foetal-calf serum and frozen. After thawing, the suspensions were sonicated and incubated at 4°C for 16 h. This incubation was also performed with samples of medium. It considerably improved the specificity of the immunoprecipitation. For treatment with endo-β-*N*-acetylglucosaminidase H, the cell homogenates and concentrates of medium were prepared as above, but in phosphate-buffered saline [10 mM-sodium phosphate (pH 6.0)/0.15 M-NaCl], and divided into two portions. One of each pair was supplemented with 50 munits of endo-β-*N*-acetylglucosaminidase H/ml, and all samples were incubated at 37°C for 15 h. The addition of the enzyme was repeated and the incubation continued for 20 h. A stock solution of the endo-β-*N*-acetylglucosaminidase H (1 unit/ml) was prepared in phosphate-buffered saline, pH 6.0. It was supplemented with 1 mM-phenylmethanesulphonyl fluoride, incubated for 30 min at room temperature and stored at −20°C. After the incubation the cell homogenates were supplemented with 0.03% (v/v) Triton X-100 and 0.01% protamine sulphate. Soluble fractions of cell homogenates and concentrates of medium were prepared by centrifugation at 45000 *g* for 1 h. When subcellular fractions were analysed the procedure was varied (see the corresponding Figure legends).

Immunoprecipitation

β-Hexosaminidase was immunoprecipitated by using an antiserum against human placental β-hexosaminidase A (Hasilik & von Figura, 1981). The soluble fractions were mixed with carrier antigen, antiserum and detergent solution described previously (Hasilik & von Figura, 1981), incubated for 30 min at room temperature and for 36 h at 4°C at a final dilution of 1:27 in the presence of purified β-hexosaminidase A (Hasilik & von Figura, 1981) as carrier (0.2 and 0.4 μg in the cell and medium samples). The immunoprecipitates were isolated, washed and solubilized in presence of sodium dodecyl sulphate and dithiothreitol as before (Hasilik & von Figura, 1981) and kept frozen till analysed. From the immunoprecipitation supernatants cathepsin D was immunoprecipitated with placental cathepsin D as carrier (0.3 and 0.6 μg in the cell and medium samples) and a specific antiserum (final dilution 1:8) under the above conditions. The antiserum was raised in rabbits against human placental cathepsin D and had a titre of 70 μg of antigen/ml.

Analysis of the immunoprecipitates

The solubilized immunoprecipitates were subjected to polyacrylamide-gel electrophoresis (Laemmli, 1970) as modified by Hasilik & von Figura (1981), and the radioactivity in gels was detected by fluorography (Laskey & Mills, 1975).

Fractionation on Percoll gradients

Fibroblast cultures (75 cm^2 flasks) were grown for 5 h in the presence of 10 mM-potassium cyanate, followed by labelling with 0.2 mCi of [^{35}S]methionine in the presence of 10 mM-potassium cyanate for 16 h. In controls potassium cyanate was omitted. Cells were harvested and homogenized as described by Remacle *et al.* (1980). The postnuclear supernatant obtained after centrifugation for 10 min at 600 *g* was fractionated on a continuous self-generating gradient of colloidal silica gel (Percoll in 0.25 M-sucrose). The starting density was 1.065 g/cm^3. The silica mixture (30 ml) was underlaid with a 2 ml cushion of 2.5 M-sucrose, followed by sequential over-layering of 1 ml of postnuclear supernatant and of 1 ml of sample buffer. Centrifugation was for 1 h at 20000 rev./min in a Beckman VTi 50 rotor. Fractions (2 ml) were collected from the bottom of the tubes by pumping paraffin oil into the top. The fractions were analysed for β-hexosaminidase and radioactivity. The fractions were pooled, freed from Percoll and immunoprecipitated as described in the legend to Fig. 3.

Other methods

Oligosaccharides were isolated as described by Hasilik *et al.* (1980). Cells were labelled with 0.28 mCi of [2-^{3}H]mannose by using 2 ml of glucose-free Waymouth medium supplemented with 4.5% dialysed foetal-calf serum inactivated at pH 10.4, in the presence or absence of 10 mM-cyanate for 6 h. The inhibitor was added to the cells 4 h before labelling. Details of the treatment with mild acid, alkaline phosphatase and of paper electrophoresis were described by Hasilik *et al.* (1980). The activity of β-hexosaminidase was determined with *p*-nitro-

phenyl β-*N*-acetyl-D-glucopyranoside (von Figura, 1977). The radioactivity was determined by liquid-scintillation counting using Instagel.

Results

In a preliminary experiment we observed that fibroblasts radioactively labelled for 16h in the continuous presence of 10mM-cyanate contain increased amounts of immature polypeptide chains of cathepsin D and to a larger extent those of β-hexosaminidase. The maturation of β-hexosaminidase proceeds more slowly than that of cathepsin D; thus the result indicated that, in order to inhibit completely the maturation of lysosomal enzymes, preincubation of fibroblasts with cyanate may be necessary. Fig. 1 shows that fibroblasts treated with 10mM-cyanate for 6h and incubated for an additional 2, 4 or 6h with radioactive methionine in the presence of 10mM-cyanate synthesize both cathepsin D and β-hexosaminidase in a time-dependent manner. In the treated cells the maturation of the two enzymes is largely inhibited. Furthermore, in the presence of cyanate the secretion of the cathepsin D precursor is increased, whereas that of the β-hexosaminidase precursor polypeptides is rather inhibited. In the presence of 10mM-cyanate, the labelling by [^{35}S]methionine of the total cellular material as well as of the two lysosomal enzymes is diminished by 50–60% as compared with controls.

In order to study the location of the precursors accumulating in cells treated with cyanate, we have labelled the cells with [^{35}S]methionine, disrupted them gently in the presence of 0.25M-sucrose and subjected the homogenates to differential centrifugation. The results (Fig. 2) show that with increasing concentrations of cyanate the maturation of the two lysosomal enzymes studied is progressively inhibited and the accumulation of the precursors increased. Once again the labelling of the enzymes is inhibited, and this inhibition parallels that of the labelling of the total cellular material. Most significantly, the intracellular precursor forms of the enzymes distribute about equally between the sedi-

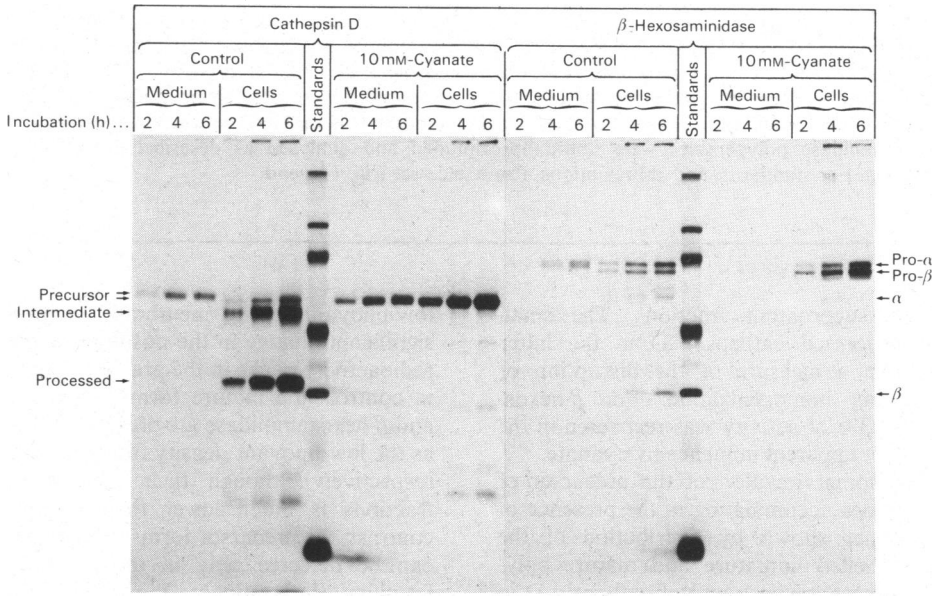

Fig. 1. *Effect of cyanate on synthesis and maturation of cathepsin D and β-hexosaminidase*
Fibroblasts were preincubated in the presence of 10mM-cyanate for 6h and with [^{35}S]methionine in the presence of 10mM-cyanate for 2, 4 and 6h as indicated. The labelling was terminated by adding an excess of non-radioactive methionine. Controls were incubated in the same way but in the absence of cyanate. Cathepsin D and β-hexosaminidase were immunoprecipitated from the soluble fractions of cell homogenates and media and the immunoprecipitates were analysed by gel electrophoresis and fluorography. Cathepsin D was synthesized as a M_r-53000 polypeptide. In control cells the precursor was processed to intermediate (M_r 47000) and mature (M_r 31000) forms, and a small portion of the precursor was secreted and accumulated unchanged in the medium. β-Hexosaminidase was synthesized as precursor polypeptides of M_r 67000 (Pro-α) and 63000 (Pro-β). In control cells mature α (M_r 54000) and β (M_r 29000) polypeptides of β-hexosaminidase were found. A portion of the precursors accumulated in the medium. The radioactive standards are, from the bottom, cytochrome *c* (M_r 12300), carbonic anhydrase (M_r 30000), ovalbumin (M_r 69000) and phosphorylase *b* (M_r 92500). In this and the other gels shown the bottom was located next to the anode during the electrophoresis.

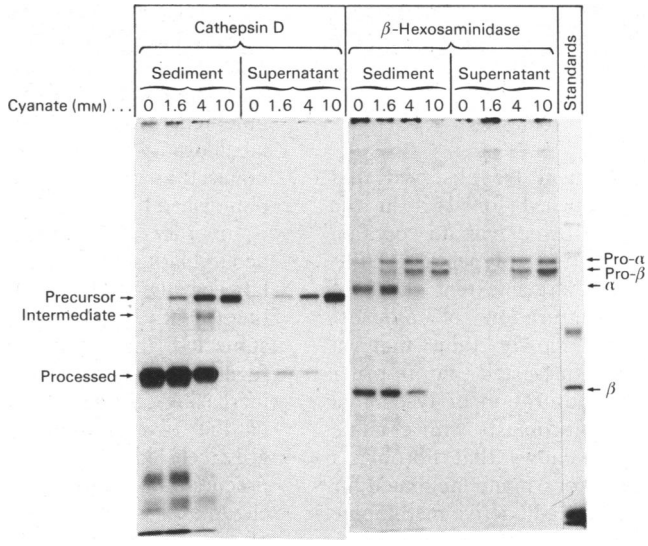

Fig. 2. *Distribution of precursor and processed forms of cathepsin D and β-hexosaminidase in subcellular fractions of control and cyanate-treated fibroblasts*

Confluent cultures in 25 cm^2 flasks were treated with 1.6 mM-, 4 mM- or 10 mM-cyanate as indicated for 6 h. Subsequently the cells were labelled with 35 μCi of [^{35}S]methionine for 16 h. The cells were rinsed twice with 2.5 ml of phosphate-buffered saline, pH 7.4, and once with 0.25 M-sucrose/1 mM-EDTA, pH 7.4, then scraped and washed out with 1.2 ml of the latter solution, spun down at 600 *g* for 10 min, resuspended in 0.5 ml of 0.25 M-sucrose/3 mM-imidazole/HCl, pH 7.4, and homogenized in a tight Dounce homogenizer (20 strokes). The homogenates were transferred to 1.6 ml capped plastic test tubes and centrifuged at 15000 *g* for 10 min. The sediments were resuspended in 450 μl of the homogenization medium and both fractions were supplemented with 0.2% Triton X-100, frozen, thawed and incubated for 16 h at 4°C. The soluble material was isolated by centrifugation, and cathepsin D and β-hexosaminidase polypeptides were immunoprecipitated and analysed as described in the Materials and methods section. For standards, and abbreviations 'Pro-α' etc., see Fig. 1 legend.

ment and the supernatant fractions. The small amounts of processed cathepsin D in the latter fraction may be a measure of the disruption of lysosomes during homogenization. For β-hexosaminidase, 85–93% of activity was recovered in the sediment, without apparent influence by cyanate.

The non-lysosomal location of the precursor of lysosomal enzymes accumulating in the presence of cyanate is further shown by distribution of the radioactively labelled immature and mature polypeptides of β-hexosaminidase and of cathepsin D in Percoll-gradient centrifugation, shown in Figs. 3 and 4.

Fig. 3 shows that the activity of β-hexosaminidase is found predominantly in the region of higher buoyant density, fractions 1–5. Minor portions of the activity are found in fractions 10–14, which contain most of the membrane material and of the particle-associated radioactivity, and in fractions 15–17, which contain the soluble constituents of the homogenate, including contents of damaged lysosomes and other organelles. There is a slight increase in the activity of β-hexosaminidase in the low-buoyant-density membrane fraction, without a significant change in the positions of the enzyme or radioactivity peaks in the gradient. Fig. 4 shows that in controls the mature forms of both cathepsin D and β-hexosaminidase are present in the high- as well as the low-buoyant-density fractions (pools I and III respectively), though their content in the latter fractions is much lower than in the former. In contrast, the precursor forms of the enzymes studied can be detected only in the low-buoyant-density fractions. Interestingly, the small amount of the M_r-47000 processing intermediate of cathepsin D present in the control cells can be detected only in the high-buoyant-density fractions. The homogenates prepared from cyanate-treated cells contained solely the precursor forms of the two enzymes, and these were located exclusively in the low-buoyant-density fractions.

The fate of the precursors accumulated in a non-lysosomal compartment in cyanate-treated cells is studied in the experiment shown in Fig. 5. As expected, control cells labelled with [^{35}S]methionine secrete a small amount of β-hexosaminidase during

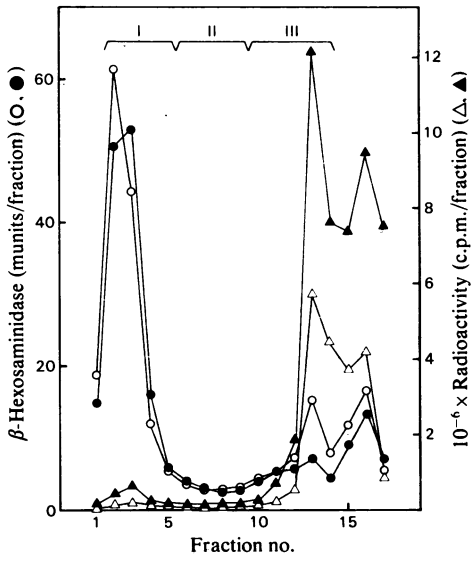

Fig. 3. *Distribution of β-hexosaminidase and of radioactivity in Percoll-gradient fractions of control and cyanate-treated cells*

The treated cells were preincubated for 6h with 10mM-cyanate. The labelling was performed with 0.2mCi of [^{35}S]methionine in 75cm^2 cultures for 16h. The cells were disrupted as described in the legend of Fig. 2. Post-nuclear supernatants were centrifuged in a Percoll gradient, and samples of the fractions were analysed for β-hexosaminidase activity (O) and for radioactivity (△). The closed and the open symbols correspond to control and cyanate-treated cells respectively.

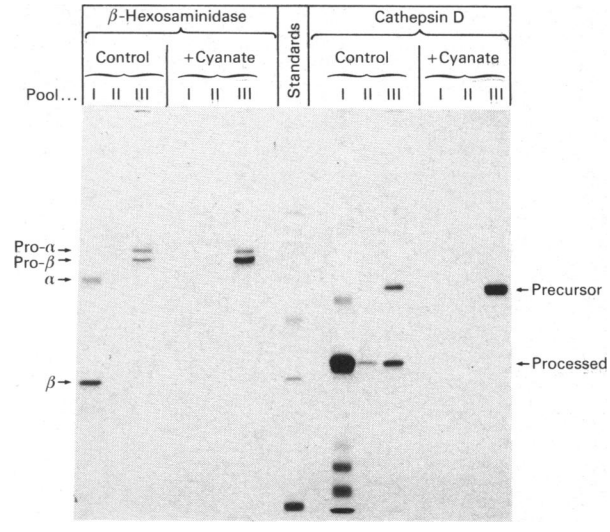

Fig. 4. *Distribution of radioactively labelled β-hexosaminidase and cathepsin D polypeptides in the Percoll gradients shown in Fig. 3*

Three pools were prepared across the gradient as indicated in Fig. 3. The pools were centrifuged at 105000*g* in a Beckman 50 Ti rotor for 3.5h. The organic material (about 1ml, which contained 69–87% of total recovered β-hexosaminidase) above the Percoll pellet was collected, supplemented with 2% (v/v) foetal-calf serum and 0.2% Triton X-100 and frozen. After thawing, the samples were centrifuged in an Airfuge [Beckman Instruments; 138kPa (20lb/in^2) for 20min] and the supernatants (75% of the applied volume) were subjected to immunoprecipitation. The radioactive polypeptides of β-hexosaminidase and cathepsin D were analysed by gel electrophoresis and detected by fluorography. In the control cells, between the precursor (M_r 53000) and the processed (M_r 33000) forms of cathepsin D an intermediate (M_r 47000) was observed. For standards, and abbreviations 'Pro-α' etc., see Fig. 1 legend.

the labelling as well as during a chase period of 39h. If the chase is conducted in the presence of 10mM-NH_4Cl, the secretion of the precursor chain is enhanced (to an extent limited by their availability) and, as expected, the mature forms remain within the cells. Cells treated with cyanate secrete little β-hexosaminidase during the labelling period. During the extensive chase period, however, most of the precursors of β-hexosaminidase appear in the medium, but a minor portion remains in the cells and can be converted into the mature enzyme. NH_4Cl was included in this experiment, because it was not expected that cells prelabelled in the presence of cyanate would secrete most of the accumulated precursors on transfer into a cyanate-free medium. In the presence of NH_4Cl the secretion of precursors previously accumulated during the incubation with cyanate is not significantly altered. The formation of the mature β-chain, however, is abolished and the cells contain small amounts of radioactivity only in the precursor polypeptides of β-hexosaminidase and at the position coinciding with the mature α-chain or the intermediate β-chain (M_r 54000 and 52000 respectively). Similar results were obtained for cathepsin D (not shown).

The phosphorylation of the immature lysosomal enzymes accumulating in cells treated with cyanate was examined in the experiment shown in Fig. 6. Paper electrophoresis of oligosaccharides isolated from cathepsin D and β-hexosaminidase from cells labelled with [^{3}H]mannose indicates that there is little if any effect of cyanate on the distribution of high-mannose oligosaccharides cleavable with endo-β-*N*-acetylglucosaminidase H between the neutral, mono- and di-phosphorylated fractions. The mobilities of the acidic oligosaccharides in the electrophoretogram are similar to those found

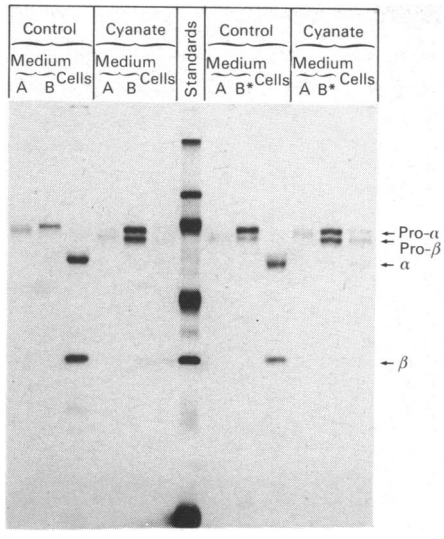

Fig. 5. *Secretion of precursor polypeptides of β-hexosaminidase from cells treated with cyanate after removal of the inhibitor*

Four cultures were subjected to a pulse–chase labelling with [^{35}S]methionine for 4 and 12h, respectively. In two cultures treatment with 10mM-cyanate was started 4h before the labelling, and continued through the chase period. At this time the medium was collected (medium A) and replaced by Eagle's minimal essential medium containing 7.5% foetal-calf serum. The medium added to one of each of the control and the treated cells contained 10mM-NH_4Cl. After 39h the media (B without ammonia, B* with ammonia) were collected, the cells were harvested and β-hexosaminidase was immunoprecipitated from all samples. The radioactive polypeptides in the immunoprecipitates were analysed by gel electrophoresis and fluorography. For standards, and abbreviations 'Pro-α' etc., see Fig. 1 legend.

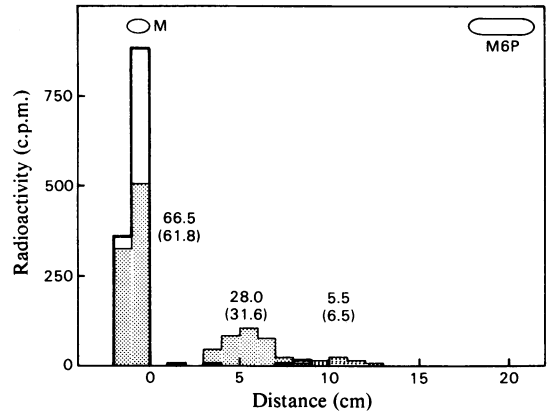

Fig. 6. *Distribution of radioactivity in neutral and acidic oligosaccharides in cathepsin D and β-hexosaminidase in control and cyanate-treated cells*

The cells were labelled with 0.28mCi of [2-^{3}H]mannose for 6h, and cell extracts were prepared and subjected to immunoprecipitation with antisera against cathepsin D and β-hexosaminidase added simultaneously. Oligosaccharides were released from the immunoprecipitates with endo-β-*N*-acetylglucosaminidase H. After mild acid hydrolysis a portion was treated with alkaline phosphatase and the samples were subjected to paper electrophoresis. The stippled area with the thin line and the open area with the bold line correspond to oligosaccharides untreated and treated with alkaline phosphatase, respectively (shown for control). The numbers in the Figure indicate the distribution of the radioactivity in oligosaccharides from control cells: 66.5, 28 and 5.5% respectively in the region of neutral, monophosphorylated and diphosphorylated oligosaccharides. The oligosaccharides from cyanate-treated cells show normal distribution in the electrophoretogram. For simplicity this distribution is indicated only by numbers in parentheses (as % of total). As standards mannose (M) and mannose 6-phosphate (M6P) were used.

previously (Hasilik *et al.*, 1980) for oligosaccharides from lysosomal enzymes secreted by fibroblasts in the presence of NH_4Cl. The charge is nearly completely removed by treating the oligosaccharides with mild acid and alkaline phosphatase. This holds true for the oligosaccharides from both the control and cyanate-treated cells (only the control is shown in Fig. 6).

The oligosaccharides in the immature cathepsin D accumulating in the cyanate-treated cells are largely sensitive to endo-β-*N*-acetylglucosaminidase H (Fig. 7). In cathepsin D of the control cells, most of the oligosaccharides are released by the treatment with endo-β-*N*-acetylglucosaminidase H. Only the secreted precursor contains a larger proportion of uncleavable oligosaccharides. In the fluorogram nearly no radioactive material is detected in the region around M_r 13000, where the small polypeptides of the mature cathepsin D would be expected. The absence of labelling may be explained by a low content of methionine or a loss during the isolation. The precursor cathepsin D present in cyanate-treated cells or in their secretions can nearly completely be converted into M_r-49000 polypeptides, which are carbohydrate-free (Hasilik & von Figura, 1981).

Discussion

Cyanate has been suggested to inhibit the turnover of the glucose-transport system in Nil hamster fibroblasts by inhibiting the degradative functions of

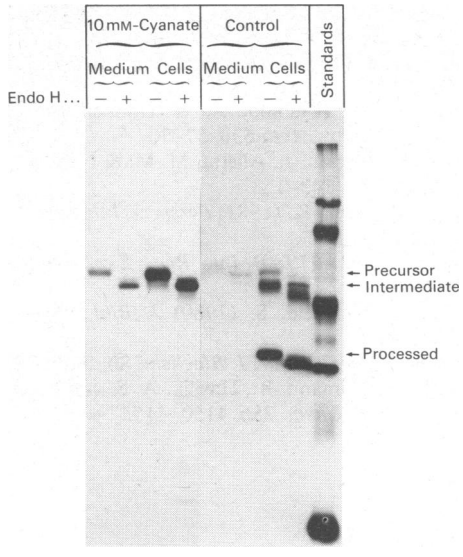

Fig. 7. *Cleavage of high-mannose oligosaccharides in cathespin D from control and cyanate-treated cells and their secretions*

The cells were subjected to pulse–chase labelling with [^{35}S]methionine for 4 and 3h, respectively. In the treated culture 10mM-cyanate was present from 4h before the start of the labelling until the cells were harvested. Samples of cell homogenates and of the concentrated secretions were incubated with or without endo-β-*N*-acetylglucosaminidase H (Endo H). Cathepsin D was immunoprecipitated, and the radioactive polypeptides were separated by gel electrophoresis and detected by flurorography.

In control cells the precursor (M_r 53000), the intermediate (M_r 47000–49000) and the mature (M_r 31000) cathepsin D polypeptides all became nearly completely deglycosylated after treatment with endo-β-*N*-acetylglucosaminidase H. In normal secretions the precursor with one sensitive and one resistant oligosaccharide prevailed, since after the digestion the partially deglycosylated product (M_r 51000) was the most prominent product. In cyanate-treated cells and the corresponding media, precursor of a slightly smaller size than in the control was observed; it was nearly completely converted into the deglycosylated (M_r 49000) form by treatment with endo-β-*N*-acetylglucosaminidase H.

lysosomes. The observed inhibition by cyanate of the turnover of the glucose-transport system (Christopher & Morgan, 1981) can also be secondary to an inhibition of a membrane transport. Our results show that in human fibroblasts treated with 10mM-cyanate the maturation of lysosomal enzymes is abolished. However, the observed effect is secondary to a block in the transport of the lysosomal-enzyme precursors. In inhibited cells the precursors accumulate in a compartment, which can be well separated from lysosomes.

In a Percoll gradient the accumulated precursors are found in the low-buoyant-density membrane fraction, which in our experiments separates well from the bulk of the lysosome marker β-hexosaminidase. Rome *et al.* (1979) have found nearly half of the lysosomal-enzyme activities in the low-buoyant-density fractions. The different distribution in our experiment may be due to different method of cell disruption and materials used.

Cyanate does not significantly interfere with the phosphorylation of oligosaccharides in lysosomal enzymes. This reaction is associated with the Golgi apparatus (Varki & Kornfeld, 1980; Waheed *et al.*, 1981) and is supposed to occur in its *cis* cisternae (Waheed *et al.*, 1981; Goldberg & Kornfeld, 1981). The extensive sensitivity of the accumulated precursor of cathepsin D to endo-β-*N*-acetylglucosaminidase H may indicate an inhibition of the transfer to the distal elements of the Golgi apparatus. In this respect the effect of cyanate resembles that of monensin on the secretory pathway (reviewed by Tartakoff, 1980). More recently we have observed that in monensin-treated fibroblasts the phosphorylation of oligosaccharide side chains in lysosomal enzymes is little changed, whereas formation of the complex type is inhibited (R. Pohlmann, S. Krüger, A. Hasilik & K. von Figura, unpublished work). Curiously, in both monensin- and cyanate-treated fibroblasts there is a stimulation of secretion of the cathepsin D precursor and an inhibition of the secretion of the β-hexosaminidase precursors. The differential effects on the two lysosomal enzymes may indicate a difference in the mode of their normal intracellular transport. Cyanate appears to affect transport and segregation of lysosomal enzymes in the Golgi area.

The accumulation of lysosomal-enzyme precursors in cyanate-treated cells is relieved on removal of cyanate from the culture. However, the precursors mainly become secreted rather than transported into the lysosomes. Thus cyanate may inhibit the transport of lysosomal enzymes distal to the site of segregation, or recovery of the secretory pathway is faster than that of the lysosomal pathway.

This study was supported by the Deutsche Forschungsgemeinschaft (SFB 104).

References

Bainton, D. F. & Farquhar, M. G. (1970) *J. Cell Biol.* **45**, 54–73

Bentfeld-Barker, M. E. & Bainton, D. E. (1982) *Blood* **59**, 472–481

Cantz, M., Kresse, H., Barton, R. W. & Neufeld, E. F. (1972) *Methods Enzymol.* **28**, 884–897

Christopher, C. W. & Morgan, R. A. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4416–4420

Frisch, A. & Neufeld, E. F. (1981) *J. Biol. Chem.* **256**, 8242–8246
Goldberg, D. & Kornfeld, S. (1981) *J. Biol. Chem.* **256**, 13060–13067
Hasilik, A. (1980) *Trends Biochem. Sci.* **5**, 237–240
Hasilik, A. & Neufeld, E. F. (1980) *J. Biol. Chem.* **255**, 4937–4945
Hasilik, A. & von Figura, K. (1981) *Eur. J. Biochem.* **121**, 125–129
Hasilik, A., Klein, U., Waheed, A., Strecker, G. & von Figura, K. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1074–1078
Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341
Neufeld, E. F. (1981) in *Lysosomes and Lysosomal Storage Diseases* (Callahan, J. W. & Lowden, J. A., eds.), pp. 115–129, Raven Press, Toronto
Novikoff, A. B. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2781–2787
Remacle, J. A., Houbion, A. & Houben, A. (1980) *Biochim. Biophys. Acta* **630**, 57–70
Rome, L. H., Garvin, J., Alietta, M. M. & Neufeld, E. F. (1979) *Cell* **17**, 143–153
Sly, W. A. & Creek, K. (1981) *Perspect. Inherited Metab. Dis.* **4**, 453–462
Tartakoff, A. M. (1980) *Int. Rev. Exp. Pathol.* **22**, 227–250
Varki, A. & Kornfeld, S. (1980) *J. Biol. Chem.* **255**, 8398–8401
von Figura, K. (1977) *Eur. J. Biochem.* **80**, 525–533
Waheed, A., Pohlmann, R., Hasilik, A. & von Figura, K. (1981) *J. Biol. Chem.* **256**, 4150–4152