Biosynthesis of secreted β -hexosaminidase in *Tetrahymena thermophila*

A comparison of the wild type with a secretory mutant

Peter HÜNSELER,* Arno TIEDTKE* and Kurt VON FIGURA†

*Zoologisches Institut der Westfälischen Wilhelms-Universität, Münster, Schloßplatz 5, D-4400 Münster, and †Institut für Biochemie, Georg-August-Universität, Göttingen, Federal Republic of Germany

The synthesis and secretion of β -hexosaminidase was studied in wild type and secretion-deficient *Tetrahymena thermophila* cells by metabolic labelling and immunoprecipitation. β -Hexosaminidase is synthesized as a M_r 79 000 polypeptide which is within 10 min converted into a M_r 59 000 form. The M_r 59 000 polypeptide is further processed (within 20 min) into at least three major mature forms of M_r 58 000–54 000, which are almost quantitatively secreted into the culture medium within 1–2 h after their synthesis. Both precursor and mature forms contain asparagine-linked oligosaccharide chains which are cleavable by endoglucosaminidase F, but not by endoglucosaminidase H. Neither [32 P]orthophosphate nor [35 S]sulphate are incorporated into immunoprecipitable precursor and mature β -hexosaminidases, suggesting the absence of a phosphorylated recognition marker. Biosynthesis and processing of β -hexosaminidase is apparently unaltered in the secretory mutant MS-1; however the processed polypeptides remain cellular bound in the mutant, indicating that the mutation affects a late event in the secretion pathway of lysosomal enzymes.

INTRODUCTION

Lysosomal enzymes are synthesized on membrane-bound ribosomes, transferred cotranslationally into the lumen of the endoplasmic reticulum and then transported through the Golgi complex before they finally reach the lysosome. In mammalian cells, the asparagine-linked carbohydrate moieties of lysosomal enzymes undergo extensive modification on their way through endoplasmic reticulum and Golgi complex, which facilitate their separation from secretory products of the cells (Creek & Sly, 1984). As a result of these modifications, lysosomal enzymes are predominantly delivered (intracellularly) to the lysosome, with only minor amounts secreted into the culture medium.

However, in a variety of lower eukaryotic cells secretion of large amounts of lysosomal enzymes seems to be a normal physiological property. *Tetrahymena thermophila*, a ciliated protozoon, secretes lysosomal enzymes constitutively into the culture medium (Müller, 1972). Under nutrient conditions the cells replace the secreted enzymes, whereas cells starved for 4 h secrete as much as 80% of the initially present cellular enzyme activities of some lysosomal glycosidases into the culture medium (Müller, 1972; Hünseler *et al.*, 1987).

We have recently isolated a sec⁻ mutant (MS-1) of *Tetrahymena thermophila* which is blocked in secretion of lysosomal enzymes (Hünseler *et al.*, 1987). However, the cellular lysosomal enzyme activities of the mutant are comparable to those of wild type cells. Genetic analysis demonstrated that the block in lysosomal enzyme secretion is caused by a recessive single gene mutation which is located on chromosome 4 of the *Tetrahymena*

thermophila genome (P. Hünseler & A. Tiedtke, unpublished work).

In the present study, we report on the biosynthesis and processing of β -hexosaminidase in the wild type and in the secretory mutant MS-1 of *Tetrahymena thermophila*.

EXPERIMENTAL PROCEDURES

Cells and cell culture

Tetrahymena thermophila strain CU 399, a functional heterokaryon derived from B strain, was provided by Dr. P. Bruns, Cornell University, Ithaca, NY, U.S.A. CU 399 is wild type with respect to lysosomal enzyme release and secretes large amounts of lysosomal enzymes constitutively into the culture medium. MS-1 is a mutant blocked in secretion of all lysosomal enzymes. MS-1 was selected in our laboratory after chemical mutagenesis of strain CU 399 followed by short circuit genomic exclusion (Hünseler *et al.*, 1987). Both strains were cultivated on autoclaved PPYS medium at 30 °C [PPYS medium: 1.0% proteose peptone (PP), supplemented with 0.1% yeast extract (Y) and 0.003% Sequestrene (S) (Tiedtke, 1983)].

Materials

L-[35 S]Methionine (specific activity 53.4 TBq/mol), [32 P]orthophosphate (specific activity 7.4 GBq/mol) and [35 S]sulphate (specific activity 0.9–1.5 TBq/mg) were from Amersham. [14 C]Methylated standards were from New England Nuclear. Endo- β -N-acetylglucosaminidase H (endo H) was obtained from Health Research Inc., endoglycosidase F (endo F) was from Boehringer, and *Staphylococcus aureus* V8 proteinase was from Miles.

Abbreviations used: endo H, endo- β -N-acetylglucosaminidase H (EC 3.2.1.96); endo F, endo- β -N-acetylglucosaminidase F (EC 3.2.1.96); PMSF, phenylmethanesulphonyl fluoride.

Labelling of cells

Pulse-chase labelling was performed as follows: at 45 min prior to pulse labelling cells were harvested by centrifugation, washed twice with chemical defined medium (Rasmussen, 1973) without methionine and then incubated in fresh methionine-free medium for 40 min at 30 °C. Cell density was 0.5×10^6 cells/ml. For pulse labelling the cells were sedimented and transferred to fresh methionine-free defined medium now supplemented with 1.85–5.55 MBq of [35 S]methionine for the times indicated. Cell density was adjusted to 1.0×10^6 cells/ml. After radioactive labelling, cells were harvested by centrifugation, prepared for immunoprecipitation or transferred to chase media (PPYS supplemented with 0.08 mmol of methionine) for the times indicated (cell density now 0.5×10^6 cells/ml). Labelling with [32 P]orthophosphate and [35 S]sulphate was as above using the defined medium without unlabelled phosphate or sulphate but supplemented with 3.7 MBq of [32 P]orthophosphate or 9.25 MBq of [35 S]sulphate. Labelling times were 15 or 45 min for phosphate and 2 h for sulphate, respectively.

Immunoprecipitation

After labelling, the cells were centrifuged into a cushion of 10% Ficoll, the sedimented cells were immediately frozen in liquid nitrogen, and 500 μ l of 50 mM-Tris/HCl, pH 7.4, which contained 1 mM-phenylmethanesulphonyl fluoride (PMSF), 1 mM-EDTA and 5 mM-iodoacetamide, was added. The cell suspension was sonicated for 3×5 s in a sonication bath. Triton X-100 and protamine sulphate were added to a final concentration of 0.08% and 0.12%, respectively. After 10 min on ice the samples were centrifuged (15000 g) and a volume of A-buffer equal to the supernatant was added. A-buffer contained 2% Triton X-100, 1% sodium deoxycholate, 4% bovine serum albumin, 1 mM-EDTA, 1 mM-PMSF, 5 mM-iodoacetamide and 0.15 M-NaCl in 10 mM-sodium phosphate, pH 7.4. The cell-free medium was adjusted to 1 mM-EDTA, 1 mM-EDTA, 1 mM-PMSF, 5 mM-iodoacetamide before an equal volume of A-buffer was added.

The prepared cell homogenates and media were incubated for 30 min with 0.5 vol. or 0.125 vol. of a 10% immunoprecipitin solution (Bethesda Research Laboratories), respectively. The supernatant was collected after centrifugation for 3 min at 15000 g and the adsorption with immunoprecipitin was repeated. The collected supernatant was then centrifuged at 40000 g for 1 h. The 40000 g supernatant was mixed with antiserum and incubated for 15 h at 4 °C. The resulting immune complexes were collected with the aid of immunoprecipitin pretreated as described (von Figura *et al.*, 1985). The immune complexes were washed as described (von Figura *et al.*, 1985), solubilized in the presence of dithiothreitol, separated by polyacrylamide gel electrophoresis in the presence of SDS (Laemmli, 1970) and visualized by fluorography (Bonner & Laskey, 1974).

Treatment with glycosidases

Endoglucosaminidase H (endo H): immune complexes from cell lysates were solubilized and incubated for 16 h with endo H as described previously (Hentze *et al.*, 1984). Endoglucosaminidase F (endo F): immune complexes from cell lysates or culture medium were solubilized by heating at 95 °C for 5 min in 20 μ l of 0.1 M-

Tris/HCl, pH 6.8, containing 1.2% Nonidet P40 and 0.2% SDS. The supernatant was adjusted to 1 mM-PMSF, 5 mM-iodoacetamide, 1 mM-EDTA and divided into two portions. Each portion was incubated for 20 h at 30 °C either with 10 m-units of endo F or with the buffer alone.

Other methods

The peptide patterns of polypeptides separated by gel electrophoresis and digested with staphylococcal V8 proteinase were determined as described (Glössl *et al.*, 1984).

Purification of β -hexosaminidase and preparation of antiserum

N-Acetyl- β -D-hexosaminidase (EC 3.2.1.52) was purified from spent media of wild type cells (CU 399) by affinity chromatography on 2-acetamido-*N*-(ϵ -aminocaproyl)-2-deoxy- β -D-glucopyranoside coupled to Sepharose-4B. Antibodies were raised in rabbits injected intradermally three times with a total of 800 μ g of purified β -hexosaminidase; 1 ml of the antiserum precipitated 66 units, equivalent to 0.5 mg, of purified β -hexosaminidase.

RESULTS

Biosynthesis of β -hexosaminidase

β -Hexosaminidase was immunoprecipitated from lysates of metabolically labelled cells of the wild type (CU 399) of *Tetrahymena thermophila* with an antiserum prepared against β -hexosaminidase purified from spent culture medium of wild type cells. The antiserum precipitated polypeptides with M_r values of >150000, 100000, and 59000–56000 (Fig. 1, lane 3) from cell lysates which had been incubated for 15 min in the presence of [35 S]methionine. All polypeptides were absent from mock immunoprecipitates obtained with preimmune serum (Fig. 1, lane 2). The polypeptides with apparent M_r of 59000–56000 resembled in size the β -hexosaminidase purified from the spent culture medium and used to raise the antiserum (Fig. 1, lane 1).

To clarify the identity of the polypeptides the immunoprecipitation was performed in the presence of an excess of purified β -hexosaminidase (Fig. 1, lane 5). The precipitation of the labelled polypeptides with M_r 59000–56000 and the M_r 100000 polypeptide was almost completely inhibited, while the precipitation of the M_r >150000 polypeptide was not affected. This suggested an immunological identity between purified β -hexosaminidase and the M_r 59000–56000 and M_r 100000 polypeptides, while the M_r >150000 polypeptide was apparently unrelated to β -hexosaminidase.

Processing of β -hexosaminidase

Immunoprecipitated material from the wild type cells which had been metabolically labelled with [35 S]-methionine for 3 min (not shown) and 7 min showed polypeptides with apparent M_r of 100000, 79000 and 59000 (Fig. 2, panel a). Within 10 min of chase the M_r 79000 polypeptide disappeared, and the M_r 59000 band broadened, while the M_r 100000 polypeptide remained unchanged. In a further experiment (Fig. 2, panel b), the cells were labelled for 10 min and chased for up to 40 min. Within 20 min of chase the M_r 100000 poly-

Fig. 1. β -Hexosaminidase polypeptides in *Tetrahymena thermophila* cells

The cells were incubated for 15 min in the presence of [35 S]methionine. Cell extracts were reacted with preimmune serum (lane 2) or antiserum against β -hexosaminidase (lane 3). Lanes 4 and 5 show the immunoprecipitation of aliquots of a cell extract with antiserum (lane 4) or antiserum plus 4 μ g of purified unlabelled β -hexosaminidase (lane 5). The immunoprecipitates were solubilized under reducing conditions and, after polyacrylamide gel electrophoresis in the presence of SDS, the labelled polypeptides were visualized by fluorography. For comparison the β -hexosaminidase preparation from the spent culture medium used for immunization is shown in lane 1. The purified β -hexosaminidase (2 μ g) was visualized by silver staining. The position of M_r standards (Bio-Rad) [myosin (200000), β -galactosidase (116250), phosphorylase *b* (92500), bovine serum albumin (66200), ovalbumin (45000), carbonic anhydrase (31000), soybean trypsin inhibitor (21500) and lysozyme (14400)] are indicated on the left-hand side and that of the [14 C]-methylated M_r standards on the right-hand side. The following [14 C]-methylated M_r standards were used: phosphorylase *b* (97400); bovine serum albumin (69000); ovalbumin (46000); and carbonic anhydrase (30000).

peptide was partially converted into polypeptides with apparent M_r values between 104000 and 110000, appearing as a diffuse band in the fluorogram. Within 40 min of chase the M_r 100000 polypeptide disappeared, while the M_r 104000–110000 polypeptides were still visible. The M_r 79000 polypeptide disappeared completely within 10 min of chase. The M_r 59000–56000 polypeptides were converted within 20 min into at least three polypeptides of M_r 58000–54000 and into polypeptides of M_r 50000–48000 and M_r < 20000. Within 40 min of chase, the M_r 50000–48000 polypeptides were converted into polypeptides with M_r 48000–46000, while the apparent sizes of the other polypeptides (M_r 58000–54000 and M_r < 20000) remained unchanged. During longer chase

Fig. 2. β -Hexosaminidase in pulse-chase-labelled cells

β -Hexosaminidase was immunoprecipitated from cells labelled for 7 min (a) or 10 min (b) and subjected to chase as indicated.

periods (up to 4 h) no further changes of the sizes of the polypeptides were observed. After 2 h of chase the processed polypeptides (M_r 104000–110000, M_r 58000–54000, M_r 48000–46000 and M_r < 20000) were immunoprecipitated from the culture medium of the wild type strain (see below, Fig. 5).

The changes in the molecular masses of the various polypeptides could be due to carbohydrate and/or proteolytic processing. To differentiate between these possibilities the immunoprecipitates were digested with endoglycosidases to liberate *N*-linked carbohydrate structures. Immunoprecipitated polypeptides of cells metabolically labelled for 7 or 15 min in the presence of [35 S]methionine were resistant to endo H digestion (results not shown). Digestion of the early β -hexosaminidase forms (M_r 100000, 79000, 59000) with endo F yielded polypeptides with apparent M_r of 98000, 64000 and 52000 (Fig. 3, panel a). Therefore, the M_r 100000, 79000 and 59000 polypeptides contain *N*-linked carbohydrate structures with apparent M_r of 2000, 15000 and 7000, respectively. In a similar experiment late β -hexosaminidase polypeptides (isolated from cell extracts that had been labelled for 30 min and chased for 1 h) were digested with endo F (Fig. 3, panel b). The M_r 104000–110000 polypeptides (barely visible in Fig. 3b) were not converted into a distinct polypeptide by endo F, suggesting that the diffuse appearance of the M_r 104000–110000 polypeptide does not result from heterogeneity of *N*-linked carbohydrates. Endo F converted the M_r 58000–54000 polypeptides into a M_r 52000 product and the M_r 48000–46000 polypeptides into a M_r 42000 product. Thus the heterogeneity in the M_r 58000–54000 and M_r 48000–46000 polypeptides can be explained entirely by carbohydrate, while the conversion of the M_r 58000–54000 polypeptides into the M_r 48000–46000

Fig. 3. Oligosaccharide chains in β -hexosaminidase

Cells were labelled for 7 min (a) or 30 min (b) and harvested directly after labelling (a) or after a chase of 60 min (b). The immunoprecipitated polypeptides were split and incubated without or with endo F. For comparison equal amounts of purified β -hexosaminidase (2 μ g) were incubated without or with endo F (c). Indicated by the arrowhead is the position of the endo F, which was also visualized by the silver staining.

polypeptides apparently involves a proteolytic step. Endo F digestion of the secreted polypeptides yielded the same products as obtained from the late intracellular β -hexosaminidase polypeptides (results not shown). The β -hexosaminidase polypeptides purified from the culture medium (M_r 58000 and M_r 56000) were converted into a single product with an apparent M_r of 52000, demonstrating that the two forms differ only in their asparagine-linked carbohydrates (Fig. 3, panel c).

Taniguchi *et al.* (1985) have recently shown that purified lysosomal enzymes of *Tetrahymena* contain no phosphorylated residues. To study whether β -hexosaminidase might become phosphorylated during the course of their biosynthesis, wild type cells (CU 399) and mutant cells (MS-1) were also metabolically labelled for 15 and 45 min in the presence of [32 P]orthophosphate and for 2 h in the presence of [35 S]sulphate. No labelled β -hexosaminidase forms were observed, suggesting the absence of phosphate and sulphate groups in the β -hexosaminidase of *Tetrahymena thermophila*.

The loss of the M_r 79000 polypeptide during chase and the simultaneous increase in labelling of the M_r 59000–56000 polypeptides suggested a precursor–

Fig. 4. Peptide patterns of the immunoprecipitated M_r 79000 and M_r 59000 polypeptides

Immunoprecipitated polypeptides from cells which had been pulse labelled for 7 min with [35 S]methionine were incubated with endo F. The deglycosylated polypeptides were separated by gel electrophoresis and visualized by autoradiography. The polypeptides (M_r 64000 and M_r 52000) were removed from the gel and subjected to limited proteolysis with the indicated concentrations of staphylococcal V8 protease. The peptide fragments were separated by gel electrophoresis and visualized by fluorography. Indicated by the arrows are the common peptide fragments of both the M_r 64000 (panel a) and the M_r 52000 (panel b) polypeptides.

product relationship between these polypeptides. To test this hypothesis the immunoprecipitated polypeptides were digested with staphylococcal V8 protease and the peptide fragments produced were separated by electrophoresis. Several peptides of identical size were obtained from the deglycosylated M_r 79000 polypeptide and the deglycosylated M_r 59000 β -hexosaminidase. The common peptide fragments had apparent M_r values of 30000, 28000, 14500, 14000, 11500 and 10500 (indicated by arrows in Fig. 4). These results support the hypothesis that the M_r 59000 polypeptide is generated from the M_r 79000 polypeptide. The reduction in size by endo F (see above) suggests that the glycopeptide(s) removed during processing of the M_r 79000 into the M_r 59000 form are comprised of protein and carbohydrate moieties of 12000 and 8000, respectively.

Secretion of β -hexosaminidase in wild type and secretory mutant cells

Tetrahymena wild type strains secrete lysosomal enzymes constitutively into the culture medium (Müller, 1972). We have recently isolated a mutant (MS-1) of

Fig. 5. Secretion of β -hexosaminidase in the wild type (CU 399) and the mutant strain (MS-1)

Cells were incubated for 20 min in the presence of [35 S]methionine and chased for the times indicated. β -Hexosaminidase was immunoprecipitated from the cell lysate and the culture medium after the times indicated.

Tetrahymena which is blocked in secretion of lysosomal enzymes. The mutant MS-1 was derived from progeny of the chemically mutagenized wild type strain CU 399. MS-1 is characterized by a recessive single gene mutation, which specifically blocks the secretion of lysosomal enzymes. The cellular lysosomal enzyme activities of MS-1 are however similar to those found in wild type cells (Hünsele *et al.*, 1987).

Wild type cells (CU 399) and mutant cells (MS-1) were metabolically labelled for 20 min in the presence of [35 S]methionine and subjected to chase periods of up to 2 h. Under our labelling conditions, the wild type (CU 399) secreted during 2 h 48% of the total β -hexosaminidase activity into the culture medium. The secretory mutant MS-1 released during the same period only background amounts of β -hexosaminidase activity. Immunoprecipitation of cell homogenates and culture medium of the wild type showed that secretion of the M_r 110000 polypeptide starts within 1 h of chase and is completed within 2 h of chase in the wild type (Fig. 5, CU 399).

In this experiment a stepwise processing of the M_r 100000 polypeptide via a M_r 104000 intermediate into the M_r 110000 polypeptide which then is secreted is clearly visible. Secretion of the M_r 58000–54000 polypeptides, the M_r 48000–46000 polypeptides and the M_r < 20000 polypeptides started only after 1 h of chase and was almost completed within 2 h of chase.

Immunoprecipitations of cell lysates and culture medium of the mutant MS-1 demonstrated the deficiency in secretion (Fig. 5, MS-1). No β -hexosaminidase polypeptides appeared in the culture medium within 2 h of chase. Instead the M_r 100000 and the M_r 104000 polypeptide as well as the M_r 58000–54000 and the M_r

48000–46000 polypeptides remained cellularly bound. The processing of the M_r 100000 polypeptide into the M_r 110000 form was not observed in the mutant MS-1. This experiment was repeated with chase periods up to 4 h. The wild type strain (CU 399) showed no further modification of the secreted β -hexosaminidases. The amount of labelled polypeptides immunoprecipitated from the cell lysate of MS-1 decreased within 2 to 4 h of chase. Again, no β -hexosaminidase polypeptides were detectable in the culture medium, suggesting that the decrease in intracellular forms resulted from proteolytic degradation.

DISCUSSION

β -Hexosaminidase purified from spent culture medium of *Tetrahymena thermophila* consists of two polypeptides (M_r 58000 and M_r 56000) which differ only in their asparagine-linked carbohydrate moieties. The present study demonstrated that the β -hexosaminidase is synthesized as a precursor with an apparent M_r of 79000 which is very rapidly converted into a M_r 59000 polypeptide. The M_r 59000 polypeptide is further processed into polypeptides with M_r 58000–54000 and partially into smaller polypeptides with M_r 48000–46000 and M_r < 20000. These polypeptides are secreted almost quantitatively within 1 and 2 h after synthesis.

The precursor-product relationship between the M_r 79000 and the M_r 59000 polypeptides was indicated by the loss of the M_r 79000 polypeptide during the chase and a simultaneous increase in labelling of the M_r 59000 polypeptide and supported by the partial identity of the V8 peptide patterns obtained from both polypeptides.

Several observations indicated that the half-life of the M_r 79000 precursor is less than 10 min.

The M_r 59000 polypeptide is already visible even in the shortest pulse experiments (3 min labelling). Immunoprecipitates obtained after 7 min pulse labelling of cells show a 1:1.7 distribution of radioactivity between the M_r 79000 and the M_r 59000 polypeptides, while in immunoprecipitates obtained after 15 min pulse labelling, the M_r 79000 polypeptide is barely visible. During 10 min of chase the M_r 79000 polypeptide is totally lost.

The short time required for processing of the M_r 79000 precursor may indicate that the processing occurs directly after synthesis and transfer of the polypeptide into the lumen of the endoplasmic reticulum.

Analysis of the molecular masses of the deglycosylated β -hexosaminidase polypeptides indicate that both carbohydrate processing and proteolytic cleavages contribute to the processing of the β -hexosaminidases. The conversion of the M_r 79000 into the M_r 59000 polypeptide is due to proteolytic processing. Further conversion into the M_r 58000–54000 forms involves carbohydrate processing, while the M_r 48000–46000 and M_r < 20000 polypeptides result from additional proteolytic cleavages of the M_r 58000–54000 polypeptides.

In addition to the β -hexosaminidase polypeptides that are related by their molecular weights to the β -hexosaminidases purified from the spent medium of *Tetrahymena*, we observed a polypeptide with M_r 100000. In the wild type this polypeptide was processed via a M_r 104000 intermediate to a M_r 110000 form that was secreted. It is not clear whether these immunologically related polypeptides possess β -hexosaminidase activity. The failure of MS-1 cells to secrete these polypeptides (see below), however, indicates that they share the pathway of lysosomal enzymes.

Taniguchi *et al.* (1985) have analysed *N*-linked oligosaccharides in α -glucosidase and acid phosphatase from spent media of *Tetrahymena pyriformis*. The structures were compatible with the assumption that in *Tetrahymena* *N*-glycosylation occurs via the alternate pathway, where truncated $(\text{Glc})_3(\text{Man})_5\text{GlcNAc}_2$ units are transferred from the lipid donor to the nascent polypeptide. The resistance of early β -hexosaminidase forms to endo H is in accordance with that view. The *N*-linked carbohydrates of α -glucosidase and acid phosphatase contained neither sulphate nor phosphate (Taniguchi *et al.*, 1985). Likewise, neither sulphate nor phosphate was incorporated into β -hexosaminidase. Absence of mannose 6-phosphate residues, a common feature of mammalian lysosomal enzymes, was also indicated by the inability of human skin fibroblasts to internalize β -hexosaminidase from secretions of *Tetrahymena* (our unpublished work).

We also analysed the biosynthesis and processing of β -hexosaminidase in the secretory mutant MS-1 which is blocked in secretion of lysosomal enzymes (Hünseler *et al.*, 1987). The biosynthesis of the M_r 79000 β -hexosaminidase and the processing into its mature

forms (M_r 58000–54000, M_r 48000–46000 and M_r < 20000) did not differ from that of the wild type; however, the processed polypeptides were not secreted by the mutant. Within 2 to 4 h partial degradation of the cellular bound polypeptides was observed in the mutant.

The M_r 104000 intermediate, but not the M_r 110000 processing product of the M_r 100000 polypeptide, could be detected in the cell lysates of MS-1. Because the recovery of the M_r 110000 polypeptide was highly variable even in the wild type strain, we cannot be sure whether this reflects an altered processing of the M_r 100000 polypeptide in the mutant. Neither the M_r 100000 polypeptide nor the M_r 104000 form appeared in the culture medium of the mutant.

The mutant cells grow on bacteria as well as wild type cells. Under these conditions growth of the cells is supported solely by digestion of the phagocytosed bacteria by lysosomal enzymes. MS-1 has intracellular lysosomal enzyme activities comparable with those of the wild type and, as indicated by the growth of the mutant cells on bacteria, its lysosomes appear to be active intracellularly. These observations and the apparently unaltered biosynthesis and processing of the M_r 79000 β -hexosaminidase in the mutant suggest that the block in MS-1 does not affect the transport of β -hexosaminidase to functionally active lysosomes. The mutation appears rather to affect a late event in the secretion of lysosomal enzymes, possibly the fusion of lysosomes with specific sites of the cell membrane.

This work was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

- Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88
- Creek, K. E. & Sly, W. S. (1984) in *Lysosomes in Biology and Pathology* (Dingle, J. T., Dean, R. T. & Sly, W. S., eds.), vol. 7, pp. 63–82, Elsevier, Amsterdam
- Glössl, J., Beck, M. & Kresse, H. (1984) *J. Biol. Chem.* **259**, 14144–14150
- Hasilik, A. & von Figura, K. (1984) in *Lysosomes in Biology and Pathology* (Dingle, J. T., Dean, R. T. & Sly, W. S., eds.), vol. 7, pp. 3–16, Elsevier, Amsterdam
- Hentze, M., Hasilik, A. & von Figura, K. (1984) *Arch. Biochem. Biophys.* **230**, 375–382
- Hünseler, P., Scheidgen-Kleyboldt, G. & Tiedtke, A. (1987) *J. Cell Sci.* **88**, 47–55
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Müller, M. (1972) *J. Cell Biol.* **52**, 478–487
- Rasmussen, L. (1973) *J. Cell Sci.* **12**, 275–286
- Taniguchi, T., Mizuochi, T., Banno, Y., Nozawa, Y. & Kobata, A. (1985) *J. Biol. Chem.* **260**, 13941–13946
- Tiedtke, A. (1983) *Comp. Biochem. Physiol.* **75B**, 239–243
- von Figura, K., Gieselmann, V. & Hasilik, A. (1985) *Biochem. J.* **225**, 543–547