Endocytosis of β -N-Acetylglucosaminidase from Secretions of Mucopolidosis-II and -III Fibroblasts by Non-Parenchymal Rat Liver Cells

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β -N-Acetylglucosaminidase isolated from the secretions of fibroblasts of mucopolidosis-II and -III patients is internalized by cultured non-parenchymal rat liver cells. The rate of endocytosis compared with that of β -N-acetylglucosaminidase from control fibroblasts was 11 and 19% for the enzyme from mucopolidosis-II and -III patients respectively. The inhibition of endocytosis by mannan indicates that the β -N-acetylglucosaminidase from mucopolidosis-II and -III patients is recognized by cell-surface receptors specific for mannose.

Mucopolidosis II (I-cell disease) and III are two inherited lysosomal storage disorders, which appear to be biochemically closely related. Cultured skin fibroblasts derived from mucopolidosis-II and -III patients have a partial deficiency of multiple lysosomal enzymes (Leroy & DeMars, 1967; Lightbody *et al.*, 1971; Thomas *et al.*, 1973). Correspondingly, a marked elevation of the activity of these lysosomal enzymes is found in the culture medium of fibroblasts (Thomas *et al.*, 1973; Wiesmann *et al.*, 1971a) and in the body fluids of the patients (Wiesmann *et al.*, 1971b). Evidence has been provided that lysosomal enzymes from fibroblasts of mucopolidosis-II and -III patients are deficient in a common recognition marker that is involved in the receptor-mediated endocytosis of lysosomal enzymes by fibroblasts (Hickman & Neufeld, 1972). The mislocation of lysosomal enzymes in mucopolidosis II has been tentatively related to a deficiency in the recognition marker by postulating a secretion-recapture mechanism for transport of lysosomal enzymes into lysosomes of fibroblasts (Hickman & Neufeld, 1972; Neufeld *et al.*, 1977).

Biochemical and histological findings indicate that the deficiency of multiple lysosomal enzymes and the enlargement of lysosomes in mucopolidosis II and III is confined to certain types of cells, such as fibroblasts, chondrocytes and peripheral neurons, whereas others, such as hepatocytes, Kupffer cells and leukocytes, are not or only slightly affected (Tondeur *et al.*, 1971; Gilbert *et al.*, 1973; Berman *et al.*, 1974; for review see McKusick *et al.*, 1978). It therefore seems likely that transport of lysosomal enzymes into lysosomes does not follow a common mechanism in all types of cells. For example, in non-affected cells transport of lysosomal enzymes could proceed independently of a recognition marker or could

involve recognition markers differing from that required in fibroblasts. Recent studies have provided evidence that mannose 6-phosphate residues function as recognition markers for lysosomal-enzyme endocytosis by fibroblasts (Kaplan *et al.*, 1977a,b; Sando & Neufeld, 1977; Ullrich *et al.*, 1978a), whereas endocytosis by non-parenchymal rat liver cells involves the recognition of mannose residues by lysosomal enzymes (Achord *et al.*, 1978; Ullrich *et al.*, 1979b).

The present study provides evidence that β -N-acetylglucosaminidase derived from fibroblasts of mucopolidosis-II and -III patients is internalized by non-parenchymal rat liver cells via recognition of mannose residues. Thus lysosomal enzymes that are secreted by the types of cells affected in mucopolidosis II and III may contribute in other types of cells to their complement of lysosomal enzymes and to an adequate lysosomal catabolism.

Materials and Methods

Mannose 6-phosphate was obtained from Sigma (St. Louis, MO, U.S.A.); the phosphate-poor mannan I was that previously described (Ullrich *et al.*, 1978b). Human skin fibroblasts from healthy individuals and from patients with mucopolidosis II and III or Sandhoff's disease were maintained in culture as described by Cantz *et al.* (1972). The mucopolidosis-II and -III cell strains were kindly provided by Dr. M. Cantz, of this Institute.

Liver perfusion was done as described by Seglen (1976); non-parenchymal rat liver cells were isolated and cultured as previously described (Ullrich *et al.*, 1979b). The cultures of non-parenchymal cells were free of hepatocytes and contained about 40–70% Kupffer cells, as estimated by phagocytosis of ink

Table 1. Uptake of β -N-acetylglucosaminidase derived from control fibroblasts and from those of mucopolipidosis-II and -III patients by non-parenchymal rat liver cells and human skin fibroblasts

The values of duplicates are given for the internalized enzymes. For calculation of internalized enzyme the endogenous activity was subtracted. The clearance was calculated from the mean of duplicates. The experiments were repeated once with a different set of lysosomal enzymes and recipient cells; essentially the same results were obtained. As inhibitors for endocytosis of lysosomal enzyme by non-parenchymal rat liver cells mannan I (0.01 mg/ml), and for that by human skin fibroblasts 0.5 mM-mannose 6-phosphate, were used. n.d., Not detectable.

Cell type	β -N-Acetylglucosaminidase derived from	Mannan I (0.1 mg/ml)	Mannose 6-phosphate (0.5 mM)	Internalized enzyme activity (munits/mg of cell protein)	Clearance (μ l/h per mg of cell protein)
Non-parenchymal rat liver cells	Human skin fibroblasts (200munits/ml)	-	-	1114; 1141	470
	Mucopolipidosis II (172munits/ml)	+	-	157; 169	69
	Mucopolipidosis III (270munits/ml)	+	-	106; 109	52
		+	-	21; 25	11
Human skin fibroblasts		+	-	259; 302	87
		+	-	48; 60	17
	Human skin fibroblasts (53munits/ml)	-	-	36.2; 36.2	30.1
	Mucopolipidosis II (109munits/ml)	-	+	10.6; 11.4	8.7
	Mucopolipidosis III (109munits/ml)	-	+	0.5; 1.6	0.5
		-	+	n.d.	0
		-	-	n.d.	0
		-	+	n.d.	0

or of fluorescein isothiocyanate-conjugated immunoglobulins.

Enzyme preparation

β -N-Acetylglucosaminidase from human skin fibroblasts was partially purified by affinity chromatography (Ullrich *et al.*, 1979b). β -N-Acetylglucosaminidase was prepared from the secretions of fibroblasts of mucopolipidosis-II and -III patients (Hickman & Neufeld, 1972) and subjected to affinity chromatography (Ullrich *et al.*, 1979b).

Endocytosis of β -N-acetylglucosaminidase by human skin fibroblasts deficient in that enzyme (Sandhoff's disease) and by non-parenchymal rat liver cells was studied exactly as described (Ullrich *et al.*, 1978a, 1979b).

Results and Discussion

β -N-Acetylglucosaminidase partially purified from the secretions of fibroblasts of mucopolipidosis-II and -III patients and from control fibroblasts was assayed for endocytosis by non-parenchymal rat liver cells and human skin fibroblasts (Table 1). As previously reported, endocytosis of β -N-acetylglucosaminidase from mucopolipidosis II and III by human fibroblasts approaches zero (Hickman & Neufeld, 1972; Hickman *et al.*, 1973), whereas enzyme prepared from control fibroblasts is endocytosed. Non-parenchymal rat liver cells internalized the β -N-acetylglucosaminidase regardless of the source, though the rate of endocytosis for β -N-acetylglucosaminidase from fibroblasts of mucopolipidosis-II and -III patients was only 11 and 18% respectively of that found for the enzyme derived from control fibroblasts. Mannan I inhibited endocytosis of the three β -N-acetylglucosaminidases to a similar extent. This mannan preparation was previously shown to inhibit only lysosomal-enzyme endocytosis mediated by mannose-specific receptors, but not that mediated by receptors specific for mannose 6-phosphate (Ullrich *et al.*, 1978b). Thus endocytosis of β -N-acetylglucosaminidase of mucopolipidosis-II and -III patients appears to be mediated by the same mannose/N-acetylglucosamine-specific cell-surface receptors that are known to mediate endocytosis of β -glucuronidase (Achord *et al.*, 1978), α -N-acetylglucosaminidase, α -mannosidase and β -N-acetylglucosaminidase (Ullrich *et al.*, 1979b) by non-parenchymal rat liver cells. The structures recognized by the cell-surface receptors specific for either mannose 6-phosphate or mannose/N-acetylglucosamine are both located on carbohydrate side chains of the high-mannose type, which are susceptible to cleavage by endoglucosaminidase H (Ullrich *et al.*, 1979a,b; von Figura & Klein, 1979). Thus oligosaccharides of the high-mannose type seem to be present on β -N-acetylglucosaminidase of mucopolipidosis-II and -III patients, but seem to lack

mannose 6-phosphate residues, which could be recognized by cell-surface receptors on fibroblasts. The fact that the endocytosis rate is less than 20% of that found for β -N-acetylglucosaminidase of control fibroblasts may indicate that the side chains of the high-mannose type on β -N-acetylglucosaminidase of mucopolipidosis-II and -III patients are structurally altered or diminished in number.

The observation that lysosomal enzymes deficient in the recognition marker for fibroblasts (and related types of cells) may become recognized by other types of cells has obvious implications for the plasma clearance of lysosomal enzymes in patients with mucopolipidosis II or III.

It is not clear whether the intralysosomal catabolism in those cells from mucopolipidosis-II and -III patients that are not affected depends on the uptake of circulating enzymes that are deficient in the recognition marker for fibroblasts. It is likewise feasible that in mucopolipidosis II and III transport of lysosomal enzymes into lysosomes is not affected in cells that recognize carbohydrate structures other than mannose 6-phosphate and that intralysosomal catabolism in such cells does not depend on uptake of circulating lysosomal enzymes.

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References

- Achord, D. T., Brot, R. E., Bell, C. E. & Sly, W. (1978) *Cell* **15**, 269–274
- Berman, E. R., Kohn, G., Yatziv, S. & Stein, H. (1974) *Clin. Chim. Acta* **52**, 115–124
- Cantz, M., Kresse, H., Barton, R. W. & Neufeld, E. F. (1972) *Methods Enzymol.* **28**, 884–897
- Gilbert, E. F., Dawson, G., ZuRhein, G. M., Opitz, J. M. & Spranger, J. W. (1973) *Z. Kinderheilkd.* **114**, 259–292
- Hickman, S. & Neufeld, E. F. (1972) *Biochem. Biophys. Res. Commun.* **49**, 992–999
- Hickman, S., Shapiro, L. J. & Neufeld, E. F. (1973) *Biochem. Biophys. Res. Commun.* **57**, 55–61
- Kaplan, A., Achord, D. T. & Sly, W. S. (1977a) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2026–2030
- Kaplan, A., Fischer, D., Achord, D. & Sly, W. S. (1977b) *J. Clin. Invest.* **60**, 1088–1093
- Leroy, J. G. & DeMars, R. J. (1967) *Science* **157**, 804–806
- Lightbody, J., Wiesmann, U., Hadorn, B. & Herschkowitz, N. (1971) *Lancet* **i**, 451
- McKusick, V. A., Neufeld, E. F. & Kelly, T. E. (1978) in *The Metabolic Basis of Inherited Disease* (Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S., eds.), 4th edn., pp. 1282–1307, McGraw-Hill, New York
- Neufeld, E. F., Sando, G. N., Garvin, A. J. & Rome, L. H. (1977) *J. Supramol. Struct.* **6**, 95–101
- Sando, G. N. & Neufeld, E. F. (1977) *Cell* **12**, 619–627
- Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83
- Thomas, G. H., Taylor, H. A., Reynolds, L. W. & Miller, C. S. (1973) *Pediatr. Res.* **7**, 751–756
- Tondeur, M., Vamos-Hurwitz, E., Modal-Pohl, S., Déréume, J. P., Cremer, N. & Loeb, H. (1971) *J. Pediatr.* **79**, 366–378
- Ullrich, K., Mersmann, G., Weber, E. & von Figura, K. (1978a) *Biochem. J.* **170**, 643–650
- Ullrich, K., Mersmann, G., Fleischer, M. & von Figura, K. (1978b) *Hoppe-Seyler's Z. Physiol. Chem.* **359**, 1591–1598
- Ullrich, K., Basner, R., Gieselmann, V. & von Figura, K. (1979a) *Biochem. J.* **180**, 413–419
- Ullrich, K., Gieselmann, V., Mersmann, G. & von Figura, K. (1979b) *Biochem. J.* in the press
- von Figura, K. & Klein, U. (1979) *Eur. J. Biochem.* **94**, 347–354
- Wiesmann, U. N., Lightbody, J., Vasella, F. & Herschkowitz, N. (1971a) *N. Engl. J. Med.* **284**, 109–110
- Wiesmann, U., Vasella, F. & Herschkowitz, N. (1971b) *N. Engl. J. Med.* **285**, 1090