Biochem. J. (1979) **180**, 413–419
Printed in Great Britain

Recognition of Human Urine α-*N*-Acetylglucosaminidase by Rat Hepatocytes

INVOLVEMENT OF RECEPTORS SPECIFIC FOR GALACTOSE, MANNOSE 6-PHOSPHATE AND MANNOSE

By KURT ULLRICH, REINHARD BASNER, VOLKMAR GIESELMANN and KURT VON FIGURA
Physiologisch-Chemisches Institut der Westfälischen Wilhelms-Universität Münster, Waldeyerstrasse 15, *D* 4400 *Münster, West Germany*

(*Received* 6 *October* 1978)

Adsorptive endocytosis of α-*N*-acetylglucosaminidase from human urine by isolated rat hepatocytes is inhibited by glycoproteins, polysaccharides and sugars that are known to bind to cell-surface receptors specific for either terminal galactose/*N*-acetylgalactosamine residues, terminal mannose residues or mannose 6-phosphate residues. Recognition of α-*N*-acetylglucosaminidase by a cell-surface receptor specific for terminal galactose/*N*-acetylgalactosamine residues is supported by the observations (*a*) that neuraminidase pretreatment of the enzyme enhances endocytosis, (*b*) that β-galactosidase treatment decreases endocytosis and (*c*) that neuraminidase pretreatment of hepatocytes decreases α-*N*-acetylglucosaminidase endocytosis. Recognition of α-*N*-acetylglucosaminidase via receptors recognizing mannose 6-phosphate residues is lost after treatment of the enzyme with alkaline phosphatase and endoglucosaminidase H. The effect of endoglucosaminidase H supports the view that the mannose 6-phosphate residues reside in *N*-glycosidically linked oligosaccharide side chains of the high-mannose type. The weak inhibition of endocytosis produced by compounds known to interact with cell-surface receptors specific for mannose residues suggests that this recognition system plays only a minor role in the endocytosis of lysosomal α-*N*-acetylglucosaminidase by hepatocytes.

Asialoglycoproteins are cleared from the plasma via receptor-mediated endocytosis. Cell-surface receptors on hepatocytes recognize terminal galactose residues exposed on asialoglycoproteins (for review see Ashwell & Morell, 1974). In addition, pathways for the hepatic recognition of glycoproteins exposing terminal *N*-acetylglucosamine (Stockert *et al.*, 1976; Kawasaki & Ashwell, 1977), mannose (Winkelhake & Nicolson, 1976*a*; Baynes & Wold, 1976; Brown *et al.*, 1978) and L-fucose (Prieels *et al.*, 1978) residues were found. Rat lysosomal enzymes appear to become recognized by receptors that recognize terminal *N*-acetylglucosamine residues (Stahl *et al.*, 1976*a*,*b*), whereas hepatic clearance of β-*N*-acetylglucosaminidase (EC 3.2.1.30) and glucocerebrosidase from human sources seems to be mediated by galactose-specific receptors (Bearpark & Stirling, 1977; Furbish *et al.*, 1978). In contrast, endocytosis of human urine α-*N*-acetylglucosaminidase (EC 3.2.1.50) by two permanent epithelial rat liver cell lines is mediated only by mannose 6-phosphate-recognizing cell-surface receptors (Ullrich *et al.*, 1978*b*).

The present study shows that endocytosis of human urine α-*N*-acetylglucosaminidase by isolated rat hepatocytes is mediated by at least three different cell-surface receptor systems.

Materials

Sugar phosphates and sugars, all sodium salts, were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Boehringer Mannheim (Mannheim, Germany), and *o*-nitrophenyl β-D-galactoside and *p*-nitrophenyl 2-acetamido-2-deoxy-α-D-glucopyranoside were from Koch–Light (Colnbrook, Bucks., U.K.). *Escherichia coli* alkaline phosphatase (EC 3.1.3.1) was purchased from Boehringer Mannheim, *Vibrio cholerae* neuraminidase (EC 3.2.1.18) from Behringwerke (Marburg, Germany), endoglucosaminidase H from Seikagaku (Tokyo, Japan) and collagenase I (EC 3.4.24.3) and yeast mannan from Sigma Chemical Co. Fetuin was from Koch–Light, orosomucoid was a gift from the American Red Cross Research Laboratory (Bethesda, MD, U.S.A.), and desialylated bovine submandibularis mucin was kindly provided by Dr. Schauer (Kiel, Germany). Sepharose 4B and Percoll were obtained from Pharmacia (Uppsala, Sweden). *p*-Aminophenyl 1-thio-β-D-galactopyranoside was prepared as de-

scribed by Iino & Yoshida (1976). Asialo derivatives of fetuin and orosomucoid were prepared by acid hydrolysis (Stockert *et al.*, 1976). Mannan fractions I and VIII, obtained by separation of yeast mannan according to charge, were those described by Ullrich *et al.* (1978*b*), fraction I containing 16mmol and fraction VIII 216mmol of phosphate/mg of mannan. Female Wistar rats were provided by Hannover'sche Versuchstieranstalt (Hannover, Germany).

Methods

Liver perfusion

Perfusion was done under light diethyl ether anaesthesia as described by Seglen (1976). Female Wistar rats (200–250g) were fed with water and Altromin (Lange, Lappe, Germany) *ad libitum*. Perfusion was done *in situ* via the portal vein with 300ml of Ca-free perfusion buffer (Seglen, 1976) containing 20mM-glucose, followed by 300ml of the same buffer without EGTA at 37°C and a flow rate of 50ml/min in a non-recirculating system. The buffer in the reservoir cylinder was oxygenized by bubbling. After surgical removal, the liver was transferred to a dish and collagenase perfusion was done for 15min in a recirculating system with 100ml of the collagenase buffer of Seglen (1976), supplemented with 20mM-glucose. After perfusion, liver capsula was removed and cells were obtained by combing with a dog comb in 100ml of cold EGTA-free perfusion buffer. After passage through a nylon net (250μm mesh) the cells were gently shaken under O_2 for 10min at 37°C followed by a second passage through a 50μm nylon net.

Hepatocytes were pelleted three times by centrifugation at 65*g* for 1min in ice-cold Eagle's minimal essential medium (Eagle, 1959) containing 10% foetal calf serum (LS-Labor Service, München, Germany). Only hepatocyte preparations with more than 90% viable cells were used for differential centrifugation in a Percoll gradient in sucrose as described (Ullrich *et al.*, 1978*b*). Up to 40×10^6 cells were layered on a gradient of 22ml. Hepatocytes banding at a density of 1.06–1.08g/ml were washed twice with cold medium containing 10% foetal calf serum by centrifugation at 65*g* for 3min. The final preparations contained less than 8% non-viable cells.

Cell culture

Rat hepatocytes (2.5×10^6) in 5ml of medium supplemented with 20% foetal calf serum, non-essential amino acids and antibiotics (Cantz *et al.*, 1972) were plated into 60mm Falcon plastic dishes. The cultures were free of macrophages as demonstrated by the lack of phagocytosis of (*a*) ink (Günther Wagner, Hannover, Germany) and (*b*) fluorescein isothiocyanate-conjugated immunoglobulin G (Behringwerke).

Enzyme preparation

Four different preparations of human urine α-*N*-acetylglucosaminidase were used. Preparations A and B were purified to homogeneity as described by von Figura (1977*a*) to specific activities of 2.0 (form A) and 1.75 (form B) units/mg of protein (Lowry *et al.*, 1951). Preparations C and D were obtained after purification step 2 of von Figura (1977*a*) (specific activities: form C 92, and form D 100munits/mg of protein). β-Galactosidase (EC 3.2.1.23) and *N*-acetylglucosaminidase from *Diplococcus pneumoniae* were obtained as described by Hughes & Jeanloz (1964*a*,*b*). One unit of enzyme activity is that amount of enzyme catalysing the breakdown of 1μmol of substrate/min at 37°C.

Endocytosis of α-N-acetylglucosaminidase

Unattached cells were sucked off, and 1.7ml of medium containing 20% foetal calf serum was added 6h after plating. α-*N*-Acetylglucosaminidase dialysed for 24h against 10mM-sodium phosphate (pH6.0)/150mM-NaCl and inhibitors in iso-osmotic solution were added in a volume of 0.3ml. Unless otherwise stated α-*N*-acetylglucosaminidase concentration was 2munits/ml and the period of endocytosis was 14h. Cells were harvested with trypsin. After ten cycles of freezing and thawing, protein (Kaltwasser *et al.*, 1965) and α-*N*-acetylglucosaminidase activity were assayed (von Figura, 1977*a*). Unless otherwise stated each experimental value is the mean of duplicates. Each experiment was repeated at least three times.

Treatment of α-N-acetylglucosaminidase with alkaline phosphatase and glycosidases

Alkaline phosphatase [free of β-galactosidase, β-hexosaminidase and α-mannosidase (EC 3.2.1.24)] treatment of α-*N*-acetylglucosaminidase forms A and D was done exactly as described by Ullrich *et al.* (1978*a*). For treatment with *Vibrio cholerae* neuraminidase (specific activity 4 units/mg of protein), α-*N*-acetylglucosaminidase form A and neuraminidase were dialysed for 16h against 50mM-sodium acetate, (pH5.5)/2mM-$CaCl_2$.α-*N*-Acetylglucosaminidase (85munits/ml) was digested with neuraminidase (90munits/ml) for 65h under toluene in 50mM-sodium acetate (pH5.5)/2mM-$CaCl_2$. After digestion, neuraminidase was completely removed by affinity chromatography on a fetuin–Sepharose 4B column as described by Winkelhage & Nicolson (1976*b*). Treatment of α-*N*-acetylglucosaminidase with β-galactosidase (specific activity 3.4 units/mg of protein), final concentration 1.5 units/ml, or a mixture of β-galactosidase and β-*N*-acetylglucosamini-

dase (specific activity 1.4 units/mg of protein; final concentration of both enzymes 1.5 units/ml) was done as described by von Figura (1977*b*). After digestion, β-galactosidase was removed by affinity chromatography on a Sepharose 4B column substituted with *p*-aminophenyl 1-thio-β-D-galactopyranoside and equilibrated in 10mM-sodium phosphate (pH6.0)/150mM-NaCl.

Digestion of α-*N*-acetylglucosaminidase forms A and D with 10munits of endoglucosaminidase H (specific activity 30 units/mg of protein) for 6h at 37°C was done in 80mM-sodium phosphate buffer (pH5.5)/130mM-NaCl/0.01% bovine serum albumin in a final volume of 0.1ml.

Treatment of hepatocytes with β-galactosidase and neuraminidase

β-Galactosidase from *Diplococcus pneumoniae* was dialysed against 10mM-sodium phosphate (pH6.0)/150mM-NaCl and neuraminidase from *Vibrio cholerae* against 50mM-sodium acetate (pH5.5)/2mM-$CaCl_2$ for 16h. The cultures were incubated, 20h after plating, with 0.7ml of 10mM-sodium phosphate buffer/150mM-NaCl, pH7.0, to which either 0.3ml of β-galactosidase (500munits) or 0.3ml of neuraminidase (65munits) was added. Controls were incubated with the buffer mixtures alone. After incubation for 1h at 37°C the enzymes were removed by washing the plates five times with medium. Endocytosis was assayed at 4munits of α-*N*-acetylglucosaminidase/ml during a 4h incubation period.

Results

Adsorptive endocytosis of α-N-acetylglucosaminidase by hepatocytes

α-*N*-Acetylglucosaminidase showed a saturable uptake by hepatocytes (Fig. 1). At 2munits/ml the α-*N*-acetylglucosaminidase clearance rates varied from 1.2 to 10μl/ml per mg of cell protein for forms A–D (see Table 3). The K_{uptake} (enzyme concentration at half-maximal saturation of uptake) for form A (clearance rate 10μl/ml per mg of cell protein) was calculated to be 4.5munits/ml (approx. 7.5nM-enzyme). A single hepatocyte may internalize up to 1.7×10^5 α-*N*-acetylglucosaminidase molecules/h. When uptake was assayed 6–32h after plating, the clearance rate was not affected by the interval between plating and the endocytosis experiment. The rate of endocytosis was linear from 3 to 24h. The internalized human urine α-*N*-acetylglucosaminidase differs from the endogenous rat hepatocyte α-*N*-acetylglucosaminidase by its greater heat-lability (Fig. 2).

Inhibition of endocytosis by glycoproteins, sugar phosphates, sugars and mannans

Endocytosis of α-*N*-acetylglucosaminidase was inhibited by glycoproteins with terminal galactose residues (asialo derivatives of orosomucoid and fetuin), terminal *N*-acetylgalactosamine residues (desialylated bovine submandibularis mucin) and by mannose 6-phosphate and a mannan rich in phosphorylated mannose residues (mannan VIII). Glycoproteins with terminal neuraminic acid residues and a variety of other sugar phosphates were not inhibitory (Table 1). All monosaccharides tested, at a concentration of 50mM, were found to inhibit

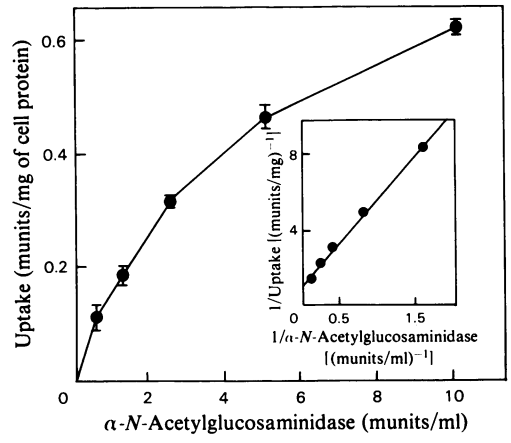

Fig. 1. *Endocytosis of α-N-acetylglucosaminidase as a function of enzyme concentration in the medium*
The range of duplicates is indicated by vertical bars.

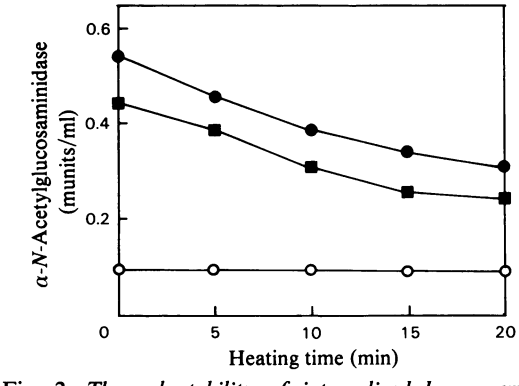

Fig. 2. *Thermal stability of internalized human urine α-N-acetylglucosaminidase*
Homogenates of control hepatocytes (○), and hepatocytes incubated in the presence of human urine α-*N*-acetylglucosaminidase (●) were heated for up to 20min at 56°C. A mixture of heat-inactivated homogenate of hepatocytes (5min at 100°C) and human urine α-*N*-acetylglucosaminidase at a concentration of 0.44munit/mol (■) served as control.

Table 1. *Effect of glycoproteins, mannans and sugar phosphates on endocytosis of α-N-acetylglucosaminidase*
All results represent the means of triplicates. The clearance rates of controls of forms A and D were 10 (9.3–10.4) and 1.2 (1.1–1.3) μl/h per mg of protein respectively (ranges in parentheses). Non-inhibitory sugar phosphates were: fructose 1-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, galactose 1-phosphate, glucose 1-phosphate, mannose 1-phosphate. Abbreviation: n.d., not determined.

Addition	Concentration	α-*N*-Acetylglucosaminidase endocytosis (% of control) Form A	Form D
Orosomucoid	0.1 mg/ml	109	96
Asialo-orosomucoid	0.1 mg/ml	24	81
Fetuin	0.1 mg/ml	91	92
Asialofetuin	0.1 mg/ml	20	63
Bovine asialosubmandibularis mucin	0.1 mg/ml	19	n.d.
Horseradish peroxidase	0.1 mg/ml	82	88
Mannan I	1.0 mg/ml	80	84
Mannan VIII	1.0 mg/ml	75	64
Mannose 6-phosphate	0.5 mM	82	52
Asialo-orosomucoid + mannose 6-phosphate	0.1 mg/ml 0.5 mM	5	27

Table 2. *Effect of monosaccharides on endocytosis of α-N-acetylglucosaminidase*
Range of triplicates are given in parentheses. All sugars were of D-configuration if not otherwise stated.

Inhibitor (50 mM)	α-*N*-Acetylglucosaminidase endocytosis (% of control) Form A
Glucose	65 (56–73)
N-Acetylglucosamine	75 (71–81)
Mannose	73 (71–78)
L-Fucose	72 (61–79)
Galactose	44 (39–48)

α-*N*-acetylglucosaminidase endocytosis to 65–75% of that of controls. Only galactose inhibited endocytosis to a higher extent (Table 2). All compounds inhibiting α-*N*-acetylglucosaminidase endocytosis were assayed for their effect on receptor-mediated endocytosis of sulphated proteoglycans by hepatocytes. None of these compounds inhibited proteoglycan endocytosis, except asialo-orosomucoid, which consistently reduced proteoglycan endocytosis by 20% (R. Prinz, K. Ullrich & K. von Figura, unpublished results). These results exclude a general inhibitory effect of these compounds on the endocytosis system. The extent of inhibition produced by the different inhibitors varied for different α-*N*-acetylglucosaminidase charges (Table 1). However, when the amounts of enzyme cleared by either the galactose-recognizing receptor or the mannose 6-phosphate-recognizing receptor of four different α-*N*-acetylglucosaminidase forms were compared, it became evident that a fairly constant amount of enzyme was cleared via the mannose 6-phosphate-recognizing receptor, whereas a great variation was found for clearance via the galactose-recognizing receptor (Table 3). The purified enzyme forms A and B exhibited high, and the partially purified forms C and D low, clearance rates via the galactose-recognizing receptor. These findings suggest the presence of endogenous inhibitors for the galactose-recognizing receptor in the partially purified forms C and D. This was supported by the observation that a mixture of equal amounts of forms A and D was cleared only at the low rate observed for form D alone. Upon purification of form D to homogeneity, the total clearance rate and that part of the enzyme cleared via the galactose-recognizing receptors increased in a parallel manner (results not shown).

The effects of asialo-orosomucoid and mannose 6-phosphate were additive (Table 1). The low residual clearance, equivalent to 0.3–0.5 μl/h per mg of cell protein, might partly represent non-specific fluid endocytosis. The weak inhibition produced by horseradish peroxidase and mannan I, both of which are known to interact with cell-surface receptors specific for mannose (Achord *et al.*, 1977*b*; Stahl *et al.*, 1978), might suggest that mannose-specific receptors are present on hepatocytes.

Treatment of α-N-acetylglucosaminidase with alkaline phosphatase and glycosidases

Treatment of lysosomal enzymes with alkaline phosphatase and endoglucosaminidase H is known to destroy the recognition of lysosomal enzymes via mannose 6-phosphate-specific receptor (Kaplan *et al.*, 1977*a*; von Figura *et al.*, 1978; von Figura & Klein, 1979). Uptake of α-*N*-acetylglucosaminidase forms A and D was reduced after treatment with both enzymes (Table 4). The degree of inhibition after

Table 3. *Effect of asialo-orosomucoid and mannose 6-phosphate on clearance of four different α-N-acetylglucosaminidase forms*
All values are the means of triplicates (ranges are given for the controls in parentheses). For controls, the clearance rate (μl/h per mg of cell protein) is given, which expresses the volume of medium that is cleared of enzyme/h per mg of cell protein. The difference between the clearance of controls and that in the presence of the inhibitors asialo-orosomucoid and mannose 6-phosphate is given. This difference is a measure of the amount of enzyme cleared via the galactose- or mannose 6-phosphate-specific receptors respectively. Hepatocytes had a mean α-N-acetylglucosaminidase activity of 0.077munit/mg of cell protein. Under our experimental conditions a clearance of 2.8μl/h per mg of cell protein increased the intracellular α-N-acetylglucosaminidase 2-fold.

Enzyme form	Control	Asialo-orosomucoid (0.1mg/ml)	Mannose 6-phosphate (0.5mM)
A	10 (9.3–10.4)	7.6	1.8
B	8 (7.6–8.3)	5.8	0.9
C	2 (2.0–2.1)	1.0	1.0
D	1.2 (1.1–1.3)	0.2	0.6

Table 4. *Effect of treatment with alkaline phosphatase, endoglucosaminidase H, β-galactosidase, β-N-acetylglucosaminidase and neuraminidase on α-N-acetylglucosaminidase endocytosis*
Treatment of α-N-acetylglucosaminidase with enzyme was done as described in the Methods section. To the medium of the controls, α-N-acetylglucosaminidase treated with buffer was added. Alkaline phosphatase, endoglucosaminidase H and β-galactosidase had no effect on the activity of α-N-acetylglucosaminidase and endocytosis of α-N-acetylglucosaminidase, when added to the medium of the controls. α-N-Acetylglucosaminidase was separated from neuraminidase and β-galactosidase by affinity chromatography (see the Methods section) before determination of uptake. The buffer-treated controls of the experiments were likewise subjected to affinity chromatography. Abbreviation: n.d., not determined.

Pretreatment	Inhibitor	α-N-Acetylglucosaminidase endocytosis (% of control) Form A	Form D
Alkaline phosphatase	—	81	50
Endoglucosaminidase H	—	76	43
β-Galactosidase	—	28	n.d.
β-Galactosidase	Asialo-orosomucoid (0.1mg/ml)	18	n.d.
β-Galactosidase	Mannose 6-phosphate (0.5mM)	18	n.d.
β-Galactosidase +β-N-acetylglucosaminidase	—	15	n.d.
Neuraminidase	—	127	n.d.

treatment with these enzymes was comparable with that produced by mannose 6-phosphate (see Table 1).

Treatment of α-N-acetylglucosaminidase form A with β-galactosidase reduced endocytosis to 28% of that of controls. Endocytosis of the β-galactosidase-treated α-N-acetylglucosaminidase was still susceptible to inhibition by asialo-orosomucoid and mannose 6-phosphate, indicating that β-galactosidase treatment (*a*) was not complete and (*b*) did not affect the mannose 6-phosphate-containing structures. Neuraminidase treatment, which is thought to expose additional terminal galactose residues on α-N-acetylglucosaminidase, enhanced endocytosis rate to 127% of that of control.

Treatment of hepatocytes with β-galactosidase and neuraminidase

Pretreatment of hepatocytes with up to 300munits of β-galactosidase from *Diplococcus pneumoniae*/ml had no influence on α-N-acetylglucosaminidase endocytosis. In contrast, treatment with 65munits of *Vibrio cholerae* neuraminidase/ml reduced α-N-acetylglucosaminidase endocytosis to 70% of that of controls pretreated with buffer.

Discussion

Several studies have demonstrated the rapid clearance of lysosomal enzymes from the plasma and predominant uptake by the liver (Thorpe *et al.*, 1974; Marinkovic *et al.*, 1976; Schlesinger *et al.*, 1976; Achord *et al.*, 1977*a*: Fiddler & Desnick, 1977). The low clearance of sialylated human β-N-acetylglucosaminidase was greatly reduced after neuraminidase treatment, suggesting a role for terminal galactose residues in recognition of human β-N-

acetylglucosaminidase (Bearpark & Stirling, 1977). Clearance of human glucocerebrosidase was likewise enhanced after neuraminidase treatment (Furbish *et al.*, 1978). In studies with isolated hepatocytes and non-parenchymal liver cells, Furbish *et al.* (1978) demonstrated a preferential uptake of the desialylated glucocerebrosidase by hepatocytes. Studies with a permanent epithelial rat liver cell line (Ullrich *et al.*, 1978*b*) suggested that hepatocytes may recognize lysosomal enzymes via the same phosphorylated carbohydrate, that is recognized by cell-surface receptors on fibroblasts (Kaplan *et al.*, 1977*a*,*b*; Sando & Neufeld, 1977; Ullrich *et al.*, 1978*a*).

A third recognition system for lysosomal enzymes predominantly located on non-parenchymal cells (Achord *et al.*, 1978), which recognizes both terminal *N*-acetylglucosamine and α-mannose residues (Achord *et al.*, 1977*b*), was shown to be involved in the hepatic clearance of several rat lysosomal enzymes (Stahl *et al.*, 1976*a*,) and of the low-uptake form of human placenta β-glucuronidase (Achord *et al.*, 1978).

The present findings indicate that hepatocytes endocytose native human urine α-*N*-acetylglucosaminidase predominantly by recognition of terminal galactosyl residues. The receptor system involved in α-*N*-acetylglucosaminidase recognition via galactose had similar characteristics to those described by Ashwell & Morell (1974) and Stockert *et al.* (1977), namely inhibition by glycoproteins exposing galactose or *N*-acetylgalactosamine residues and susceptibility to pretreatment with neuraminidase. Minor parts of α-*N*-acetylglucosaminidase were endocytosed by receptors recognizing mannose 6-phosphate and terminal mannose residues. Evidence for participation of mannose 6-phosphate residues in the recognition of α-*N*-acetylglucosaminidase stems from inhibition experiments and the effect of pretreatment with modifying enzymes. These results confirm earlier findings in two epithelial rat liver cell lines (Ullrich *et al.*, 1978*b*). Involvement of terminal mannose residues in the recognition was suggested by the inhibitory effect of horseradish peroxidase and mannan I. In contrast with human glucocerebrosidase of which the agalacto form was more rapidly endocytosed than its sialo and asialo forms by hepatocytes (Furbish *et al.*, 1978; Steer *et al.*, 1978), the agalacto form of α-*N*-acetylglucosaminidase was only poorly endocytosed. Whether the *N*-acetylglucosamine-recognizing receptors on hepatocytes are identical with the well-characterized *N*-acetylglucosamine/mannose-recognizing receptors that are predominantly located on the macrophage system (Achord *et al.*, 1978; Stahl *et al.*, 1978) is not clear. The minor effect of β-*N*-acetylglucosaminidase treatment on endocytosis of agalacto-α-*N*-acetylglucosaminidase would be compatible with the view that receptors are present on hepatocytes that interact with both *N*-acetylglucosamine and mannose as reported for macrophages.

The *N*-glycosidically linked oligosaccharide side chains of glycoproteins fall into two classes, the simpler ones of the high-mannose type, containing only mannose and *N*-acetylglucosamine residues, and the more complex ones of the lactosaminyl type, which in addition contain neuraminic acid, galactose and L-fucose (for review see Kornfeld & Kornfeld, 1976). Recognition of α-*N*-acetylglucosaminidase via mannose 6-phosphate residues is lost after treatment with endoglucosaminidase H, which specifically cleaves *N*-glycosidically linked oligosaccharides of the high-mannose type (von Figura *et al.*, 1978). The oligosaccharides released by endoglucosaminidase H bear phosphate groups on mannose residues (von Figura & Klein, 1979). The present findings confirm that the phosphorylated mannose residues are located on side chains of the high-mannose type, whereas the receptors specific for galactose probably recognize oligosaccharide side chains of the lactosaminyl type. The present results do not allow us to decide whether the different types of side chains are located on the same enzyme molecule or whether they are present only on different enzyme molecules. The additive effect of asialo-orosomucoid and mannose 6-phosphate, however, would fit better with the latter possibility.

We are indebted to Mr. J. Kreusch, Freiburg, West Germany, and Dr. J. H. Geelen, Utrecht, The Netherlands for introducing us to the methodology of isolating hepatocytes. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 104).

References

Achord, D., Brot, F., Gonzales-Noriega, A., Sly, W. & Stahl, P. (1977*a*) *Pediat. Res.* **11**, 816–822

Achord, D. T., Brot, F. E. & Sly, W. (1977*b*) *Biochem. Biophys. Res. Commun.* **77**, 409–415

Achord, D. T., Brot, F. E., Bell, C. E. & Sly, W. (1978) *Cell* **15**, 269–274

Ashwell, G. & Morell, A. G. (1974) *Adv. Enzymol. Relat. Areas Mol. Biol.* **41**, 99–128

Baynes, J. W. & Wold, F. (1976) *J. Biol. Chem.* **251**, 6016–6024

Bearpark, T. & Stirling, J. L. (1977) *Biochem. J.* **168**, 435–439

Brown, T. L., Henderson, L. A., Thorpe, S. & Baynes, J. W. (1978) *Arch. Biochem. Biophys.* **188**, 418–428

Cantz, M., Kresse, H., Barton, R. W. & Neufeld, E. F. (1972) *Methods Enzymol.* **28**, 884–896

Eagle, H. (1959) *Science* **130**, 432–437

Fiddler, M. B. & Desnick, R. J. (1977) *Arch. Biochem. Biophys.* **179**, 397–408

Furbish, F. S., Steer, C. J., Barranger, J. A., Jones, E. A. & Brady, R. O. (1978) *Biochem. Biophys. Res. Commun.* **81**, 1047–1053

Hughes, R. C. & Jeanloz, R. W. (1964*a*) *Biochemistry* **3**, 1535–1543
Hughes, R. C. & Jeanloz, R. W. (1964*b*) *Biochemistry* **3**, 1543–1548
Iino, N. & Yoshida, K. (1976) *Carbohydr. Res.* **51**, 223–228
Kaltwasser, F., Wolters, G. & Pieper, J. (1965) *Clin. Chim. Acta* **15**, 347–351
Kaplan, A., Achord, D. T. & Sly, W. S. (1977*a*) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2026–2030
Kaplan, A., Fischer, D., Achord, D. & Sly, W. (1977*b*) *J. Clin. Invest.* **60**, 1088–1093
Kawasaki, T. & Ashwell, G. (1977) *J. Biol. Chem.* **252**, 6536–6543
Kornfeld, R. & Kornfeld, S. (1976) *Annu. Rev. Biochem.* **45**, 217–237
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Marinkovic, D. V., Petrovic, S. L., Martinovic, J. V. & Marinkovic, J. N. (1976) *Biochem. J.* **159**, 729–736
Prieels, J.-P., Pizzo, S., Glasgow, L. R., Paulson, J. L. & Hill, R. L. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2215–2219
Sando, G. N. & Neufeld, E. F. (1977) *Cell* **12**, 619–628
Schlesinger, P., Rodman, J. S., Frey, M., Lang, S. & Stahl, P. (1976) *Arch. Biochem. Biophys.* **177**, 606–614
Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83
Stahl, P., Schlesinger, P. H., Rodman, J. S. & Doebber, T. (1976*a*) *Nature (London)* **264**, 86–88
Stahl, P., Six, H., Rodman, J. S., Schlesinger, P., Tulsiani, D. R. P. & Touster, D. (1976*b*) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4045–4049
Stahl, P. D., Rodman, J. S., Miller, M. J. & Schlesinger, P. H. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1399–1403
Steer, C. J., Furbish, F. S., Barranger, J. A., Brady, R. O. & Jones, E. A. (1978) *FEBS Lett.* **91**, 202–205
Stockert, R. J., Morell, A. G. & Scheinberg, I. H. (1976) *Biochem. Biophys. Res. Commun.* **68**, 988–994
Stockert, R. J., Morell, A. G. & Scheinberg, I. H. (1977) *Science* **197**, 667–668
Thorpe, S. R., Fiddler, M. B. & Desnick, R. J. (1974) *Biochem. Biophys. Res. Commun.* **61**, 1464–1470
Ullrich, K., Mersmann, G., Weber, E. & von Figura, K. (1978*a*) *Biochem. J.* **170**, 643–650
Ullrich, K., Mersmann, G., Fleischer, M. & von Figura, K. (1978*b*) *Hoppe-Seyler's Z. Physiol. Chem.* **359**, 1591–1598
von Figura, K. (1977*a*) *Eur. J. Biochem.* **80**, 525–533
von Figura, K. (1977*b*) *Eur. J. Biochem.* **80**, 535–542
von Figura, K. & Klein, U. (1979) *Eur. J. Biochem.* in the press
von Figura, K., Klein, U., Mersmann, G. & Ullrich, K. (1978) *Arch. Genet.* **51**, abstr. 6
Winkelhake, J. L. & Nicolson, G. L. (1976*a*) *J. Biol. Chem.* **251**, 1074–1080
Winkelhake, J. L. & Nicolson, G. L. (1976*b*) *Anal. Biochem.* **71**, 281–289