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HYDROLYTIC AND TRANSGLUCOSYLATION ACTIVITIES OF A PURIFIED CALF SPLEEN β-GLUCOSIDASE

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Summary

Certain properties of the transglucosylitic activity and the hydrolytic activity of a purified calf spleen β-glucosidase (β-D-glucoside glucohydrolase EC 3.2.1.21) were investigated. There was a stimulation of both activities by sodium taurocholate and "Gaucher's factor". The K_m values for 4-methylumbelliferyl-β-D-glucoside and glucosylceramide as donors in the transglucosylation reaction were 2 mM and 0.075 mM, respectively. The K_m for ceramide as acceptor was 0.149 mM with both of these compounds. The ability of several glucoside to act as donors was examined. The capacity to catalyze this "transglucosylation" reaction is greatly diminished in spleen tissue samples from Gaucher's patients.

The enzyme possesses the capacity to hyrolyze 4-methylumbelliferyl-β-D-glucoside, *p*-nitrophenyl-β-D-glucoside, glucosylsphingosine, glucosylceramide and deoxycorticosterol-β-D-glucoside. It is postulated that a single enzyme protein may be responsible for both the hydrolytic and the transglucosylitic activities.

Introduction

The hydrolysis of the β-glucosidic linkage present in glucosylceramide to yield glucose and ceramide is catalyzed by the enzyme glucosylceramide: β-glucoside. This enzymatic activity is present in a variety of mammalian tissues [1—3]. Patients with Gaucher's disease are characterized by increased tissue levels of glucosylceramide [4—6] and decreased glucosylceramide: β-glucosidase [7,8]. This enzyme has been purified from rat intestinal mucosa [2], human [1] and bovine [9] spleen tissue and extensively purified from human placenta [10]. A highly purified β-glucosidase from calf spleen has been iso-

lated by affinity column chromatography on a "Sepharose-gluconate" gel [11,12]. This preparation catalyzes the cleavage of the β-glucosidic bond present in 4-methylumbelliferone (Me-Umb)-β-D-glucoside, glucosylceramide and glucosylsphingosine. The enzyme was also shown to catalyze a "transglucosylation" reaction which results in the production of glucosylceramide [13]. This report presents certain characteristics and properties of both the hydrolytic as well as the "transglucosylation" activities of this enzyme preparation.

Materials and Methods

4-Methylumbelliferone (Me-Umb)-β-D-glucoside and *p*-nitrophenyl (Nph)-β-D-glucoside were obtained from Pierce Chemical Co., Rockford, Ill., deoxycorticosterone-β-D-glucoside was obtained from Sigma Chemical Co., St. Louis, Mo.; Sodium taurocholate routinely utilized was from Pfanstiehl Chemical Co. as were the various β-D-glucosides employed as substrate. Nojirimycin and gluconolactam were provided by Dr Inouye. The *N*-[1-^{14}C]stearoyl sphingosine (ceramide) with a specific activity of 628 cpm/nmol was prepared [1] and purified by silica gel chromatography [13] as described earlier. Glucosylsphingosine was prepared from the glucosylceramide by a slight modification [14] of the alkaline degradation technique of Taketomi and Yamakawa [15]. The various plasma glycoproteins (Fraction VI) from various species and the concanavalin A employed were purchased from Miles Research Corp., Ill. The human pathological spleen samples were derived from patients which fulfilled both the clinical and biochemical criteria for the individual diseases.

The calf spleen β-glucosidase preparation, employed in these studies, is eluted from the affinity column in a solution containing 1% Triton X-100 [12]. The detergent concentration was reduced by treating 10 ml of the enzyme-containing solution with 3 g of methanol-washed Bio-beads SM-2 (Bio-Rad, Calif.) in the cold with gentle shaking for 1 h. The mixture was filtered in the cold, the volume of filtrate reduced with an Amicon concentrator with a UM-10 membrane and subsequently employed as the enzyme source. The content of Triton X-100 was determined by measuring the optical density at 280 nm [16]. The "Gaucher's factor" was prepared as described previously [17,18] and where employed is expressed as nmol of total carbohydrate added.

"Transglucosylation" assay

The ceramide was added as a sonicated solution which was prepared by adding 1 ml of water to a mixture of 4.4 μmol ^{14}C-labeled ceramide and either 30 mg of sodium taurocholate (Pfanstiehl) or 600 μg Triton X-100 (New England Nuclear, Boston, Mass.). The mixture was sonicated for several minutes until an opalescent solution was obtained. The routine incubation mixtures contained 90 nmol *N*-[1-^{14}C]stearoyl sphingosine, 1 mg sodium taurocholate, 50 μmol citrate/phosphate buffer, pH 5.0, 1 μmol Me-Umb-β-D-glucoside, 3—10 units of β-glucosidase in a volume of 0.2 ml and was incubated at 37° C for 3 h in air. A unit of enzyme activity is defined as that required to hydrolyse 1 nmol of Me-Umb-β-D-glucoside per h. Each incubation was carried out in at least duplicate and the experimental values were routinely corrected for boiled enzyme controls which were routinely less than 10% of the

experimental values. The reaction was terminated by the addition of 4 ml chloroform/methanol (2 : 1) and this solution subjected to the Folch partitioning procedure [19]. Suitable aliquots, usually 2 ml, of the final lower phase were spotted on Silica Gel G plates (Analtech) as well as authentic standards and developed with chloroform/methanol/acetic acid (90 : 2 : 8) as the solvent system [13]. The areas cochromatogramming with glucosylceramide standard were scraped into counting vials and the radioactivity present determined with Aquasol (New England Nuclear) in a liquid scintillation counter.

Hydrolytic assays

The incubation mixtures and general methodology for quantitating the hydrolysis of the Me-Umb-β-D-glucoside, [^{14}C]glucosylceramide have been described [11]. A published fluorimetric procedure was utilized for the measurement of free glucose liberated from the other glycosides examined as substrates [20]. Each experimental value is an average of at least duplicates and have been corrected for boiled enzyme and zero time control values which were usually less than 10% of the experimental values.

Results

The pH optimum for the transglucosylation reaction was found to be about 5.0 in the presence of citrate/phosphate buffer (Fig. 1). This is similar to that previously reported for the hydrolytic activity [11].

Stimulation by detergents and "Gaucher's factors"

The hydrolysis of both the natural substrate, glucosylceramide, and the synthetic substrates, Me-Umb- and Nph-β-D-glucosides by β-glucosidase requires detergents. In order to evaluate this stimulatory activity it was necessary to remove the Triton X-100 employed for the elution of the enzyme recovered

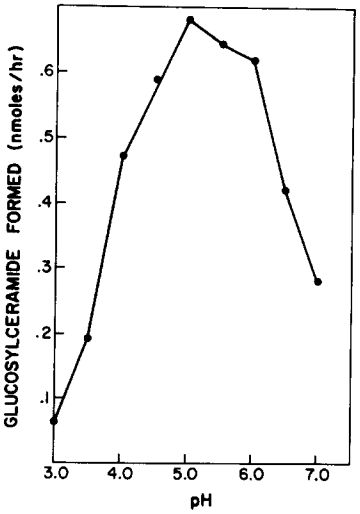


Fig. 1. The effect of varying the pH of the incubation mixture on the trans glucosylation reaction with Me-Umb-β-D-glucoside as the donor and ^{14}C-labeled ceramide as the acceptor.

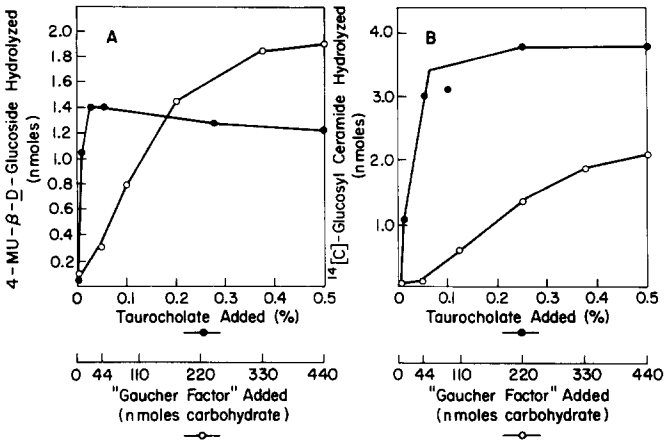


Fig. 2. The effect of varying sodium taurocholate (closed circles) and "Gaucher's factor" (open circles) upon the hydrolysis of Me-Umb-β-D-glucoside (A) and [^{14}C]glucosylceramide (B) by purified calf spleen β-glucosidase.

from the affinity column. More than 90% of this detergent is removed by Bio-Bead SM2 in 20—30 min of treatment. The ability of both taurocholate or "Gaucher factor" to stimulate the hydrolysis of both [^{14}C]glucosylceramide and 4-methyl-umbelliferyl-β-D-glucoside is shown in Fig. 2. Maximal detergent activation occurs between 0.025 and 0.05% for both substrates. This particular value was obtained only with the product purchased from Pfanstiehl while sodium taurocholate purchased from either Calbiochem or Schwartz-Mann were ineffective at these concentrations requiring 4—6-fold larger amounts. Triton X-100 was less effective than taurocholate at all quantities utilized.

Sodium taurocholate was effective in stimulating the hydrolysis of the cerebroside and the Me-Umb-β-D-glucoside. The glycan obtained from

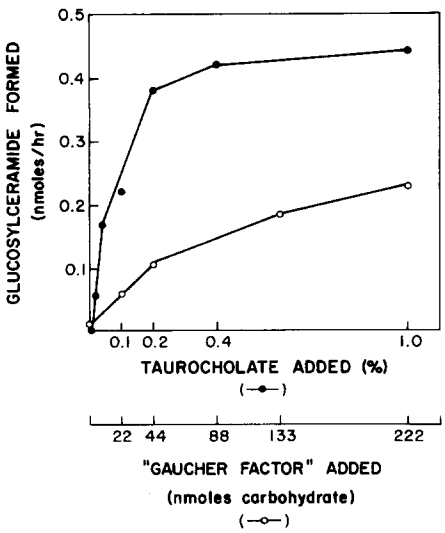


Fig. 3. The effect of varying sodium taurocholate (closed circles) and "Gaucher's factor" (open circles) on the transglucosylation reaction by purified calf spleen β-glucosidase.

Gaucher's spleen was equally effective in stimulating the hydrolysis of both the unnatural substrate and the sphingolipid. Plasma glycoprotein fractions from sheep, rabbit, horse, dog or human had no effect on these hydrolyses.

The presence of sodium taurocholate stimulated the "transglucosylation" reaction for the formation of glucosylceramide with a plateau at about 0.2%. The heteroglycan obtained from Gaucher's spleen also enhanced this "transglycosylation" activity. These studies indicated increased glucosylceramide formation over a 10-fold range of the "Gaucher's factor" (Fig. 3).

Proportionality with time and enzyme concentration

There is increasing enzymatic hydrolysis with both time and protein concentration for Me-Umb-β-D-glucoside and Nph-β-D-glucoside for up to 26 h of incubation. There is a proportional increase in glucosylceramide formation with increasing enzyme addition employing either Me-Umb-β-D-glucoside or glucosylceramide as the carbohydrate donor.

Kinetics of the "transglucosylation" reaction

Studies were undertaken to demonstrate the dependence of the "transglucosylation" reaction upon varying ceramide concentration, as the acceptor, and with either Me-Umb-β-D-glucoside or glucosylceramide, as the glucose donor. A Lineweaver-Burke plot of the data obtained is presented in Fig. 4. The reciprocal studies in which the quantity of ceramide added was kept constant and the concentration of the glucose donor varied is presented as a Lineweaver-Burke plot in Fig. 5. The kinetic data obtained from these curves are summarized in Table I. It is apparent that the K_m for ceramide is identical with either Me-Umb-β-D-glucoside or glucosylceramide as the donor. Although the fluorogenic substrate has a lower affinity than the sphingoglycolipid, it is "transglucosylated" more actively.

A comparative study was undertaken in order to obtain information about glucose donor specificity. The ability of 11 glucosides differing either in the structure of the aglycone or the anomeric configuration of the glucosidic linkage and free glucose to act as donor is presented in Table II. It appears that the

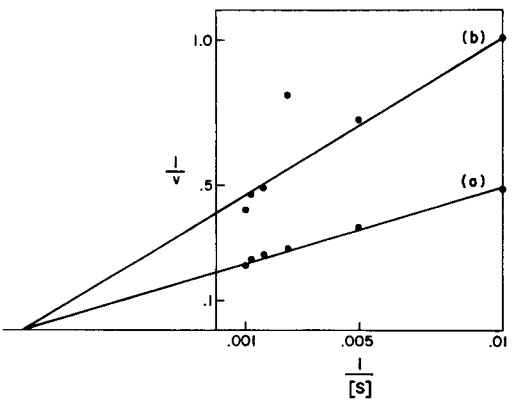


Fig. 4. Lineweaver-Burke plot of the effect of varying ^{14}C-labeled ceramide concentration in the presence of 1 μmol Me-Umb-β-D-glucoside (curve a) or 1 μmol glucosylceramide (curve b) with the purified calf spleen β-glucosidase.

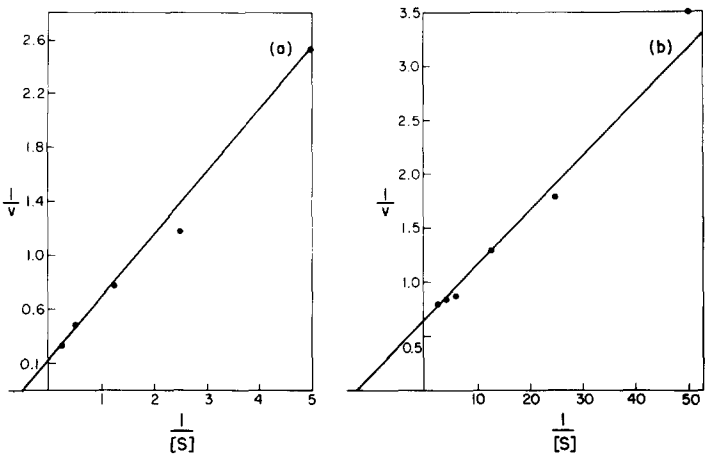


Fig. 5. Lineweaver-Burke plot of the effect of varying either the concentration of Me-Umb-β-D-glucoside (curve a) or glucosylceramide (curve b) in the presence of 90 nmol of ^{14}C-labeled ceramide with the purified calf spleen β-glucosidase.

Me-Umb- and *p*-nitrophenyl-β-D-glucosides are more effective than the other compounds employed. Deoxycortisterone-β-D-glucoside, at the same concentration, was approximately one third as effective.

Inhibitors of the hydrolytic reaction containing a β-D-glucosidic linkage

Several compounds were examined for their ability to inhibit the hydrolysis of both Me-Umb-β-D-glucoside and [^{14}C]glucosylceramide. The ability of these two compounds to mutually inhibit the hydrolysis of one another is presented in Fig. 6 as a Dixon plot. In both instances they appear to be competitive inhibitors for the hydrolysis of one another. Glucosylsphingosine was found to be cleaved by the enzyme and this material was also a competitive inhibitor for the hydrolysis of both glucosylceramide and Me-Umb-β-D-glucoside. An enzyme which cleaves steroid β-D-glucoside has been purified from rabbit liver [21]. Therefore, the effect of deoxycorticosterone-β-D-glucoside was investigated and here also competitive inhibition was observed.

Inhibition of the hydrolytic reaction by structurally related compounds

The inhibitors utilized in the previous section have in common a β-D-glucosidic linkage. An antibiotic, nojirimycin, has been reported to inhibit

TABLE I

K_m VALUES FOR THE TRANSGLUCOSYLATION REACTION WITH ^{14}C-LABELED CERAMIDE AS THE ACCEPTOR AND EITHER Me-Umb-β-D-GLUCOSIDE OR GLUCOSYLCERAMIDE AS THE GLUCOSE DONOR WITH PURIFIED CALF SPLEEN β-GLUCOSIDASE

The values were obtained from Lineweaver-Burke plots of Figs 4 and 5.

	K_m (mM)	*V* with varying ceramide (nmol formed/unit of enzyme)	*V* with constant ceramide (nmol formed/unit of enzyme)
^{14}C-labeled ceramide	0.149	—	—
Me-Umb-β-D-glucoside	2.0	1.81	1.72
Glucosylceramide	0.075	0.89	0.575

TABLE II

THE ABILITY OF VARIOYS GLUCOSIDES TO ACT AS DONORS FOR THE TRANSGLUCOSYLATION REACTION WITH ^{14}C-LABELED CERAMIDE AS THE ACCEPTOR

The final concentration of all donors was 1 mM.

Donor	% Activity
Me-Umb-β-D-glucoside	100
Maltose	3
Gentiobiose	0
Cellobiose	5
Esculin	8
Salicin	3
Phlorizin	5
UDP-glucose	0.5
Glucose 1-phosphate	5
p-Nitrophenyl-β-D-glucoside	90
Deoxycorticosterone-β-D-glucoside	27
Glucose	0

general β-D-glucosidase activity of plant and bacterial sources [22]. This material was found to be a competitive inhibitor for the hydrolysis of both Me-Umb-β-D-glucoside and glucosylceramide. A derivative of this compound, gluconolactam was also found to be a competitive inhibitor for the hydrolysis of these 2 substrates [22]. Neither concanavalin nor its cobalt, zinc and manganese salts had any appreciable effect on these hydrolyses.

K_m values and K_i values for the hydrolytic activity

The calculated value for the K_m values for the hydrolysis of Me-Umb-β-D-glucoside and [^{14}C]glucosylceramide were $2.0 \cdot 10^{-3}$ M and $2.8 \cdot 10^{-4}$ M, respectively. The K_i values for the various inhibitors employed in this series of experiments are found in Table III. It is apparent that both nojirimycin, which is not a substrate, and glucosylsphingosine, which is a substrate, are the most potent inhibitors of the hydrolytic activity.

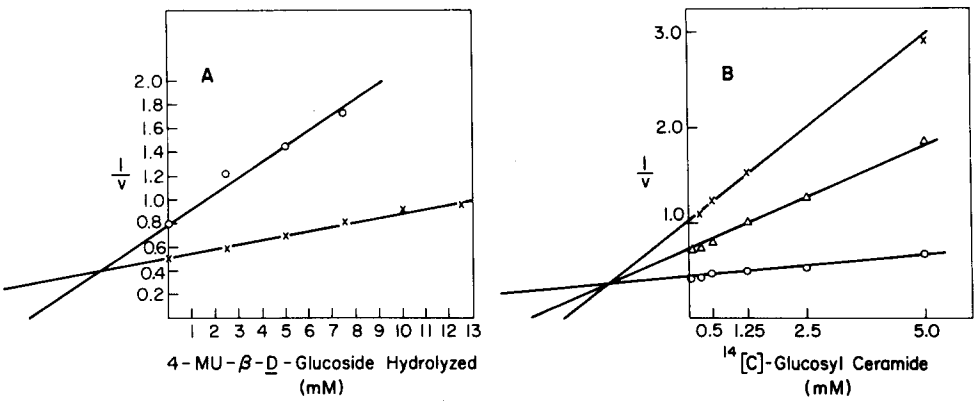


Fig. 6. Effect of Me-Umb-β-D-glucoside (B) on the hydrolysis of [^{14}C]glucosylceramide (X = 0.255 mM; circles = 0.09 mM) and glucosylceramide (A) on the hydrolysis of Me-Umb-β-D-glucoside (X = 1 mM; circles = 5 mM; triangles = 2.5 mM) by the purified calf spleen β-glucosidase.

TABLE III

K_i VALUES FOR VARIOUS INHIBITORS ON THE HYDROLYSIS OF Me-Umb-β-D-GLUCOSIDE AND [^{14}C] GLUCOSYLCERAMIDE BY PURIFIED CALF SPLEEN β-GLUCOSIDASE

Inhibitors	Substrate	
	Me-Umb-β-D-glucoside	Glucosycleramide
Me-Umb-β-D-glucoside	—	$2.8 \cdot 10^{-3}$ M
Glucosylceramide	$1.8 \cdot 10^{-3}$ M	—
Glucosylsphingosine	$6.9 \cdot 10^{-5}$ M	$7.5 \cdot 10^{-5}$ M
Deoxycorticosterone-β-D-glucoside	$4.0 \cdot 10^{-4}$ M	$4.3 \cdot 10^{-4}$ M
Nojirimycin	$1.0 \cdot 10^{-6}$ M	$1.5 \cdot 10^{-6}$ M
Gluconolactam	$4.0 \cdot 10^{-4}$ M	$2.0 \cdot 10^{-4}$ M

Hydrolysis of other β-glucosides

Several other compounds possessing a β-glucoside linkage were investigated as possible substrates for hydrolysis by the purified enzyme and these data are presented in Table IV. This enzyme apparently has only slight activity for the hydrolysis of disaccharides, methyl glucoside and salicin. The steroidal compound deoxycorticosterone-β-D-glucoside was cleaved nearly as actively as glucosylceramide. The procedure utilized for quantitating the free glucose liberated is based upon a coupled reaction with its initial conversion to glucose 6-phosphate by hexokinase, this product being oxidized by glucose-6-phosphate dehydrogenase in the presence of NADP. The resulting NADPH was then measured fluorometrically [20]. Other potential substrates of interest such as Nph-β-D-glucoside, Me-Umb-β-D-glucoside, esculin and phlorizin possessed intrinsic intense fluorescence. Therefore, they could not be examined as substrates with this technique.

"Transglucosylation" activity of Gaucher's tissues

Since Gaucher's disease is characterized by a β-glucosidase deficiency, it was of interest to examine the capacity of pathological tissues to carry out the transglucosylation reaction. Tissue extracts of frozen human spleens from individuals with Gaucher's disease, Niemann-Picks disease, metachromatic leukodystrophy and two controls were examined for both the "transglucosylation" and β-D-glucosidase activities (Table V). Of the samples assayed, only the Gaucher's materials were reduced for both activities.

TABLE IV

THE ABILITY OF VARIOUS GLUCOSIDES TO ACT AS SUBSTRATES FOR THE PURIFIED CALF SPLEEN β-GLUCOSIDASE

The glucose liberated was measured flurometrically as described in the text.

	% Activity
Glucosylceramide	100
Deoxycorticosterone-β-D-glucoside	80
Cellobiose	28
Gentiobiose	28
Methyl-β-D-glucoside	26
Salicin	28

TABLE V

TRANSGLUCOSYLATION AND Me-Umb-β-D-GLUCOSIDE HYDROLYTIC ACTIVITY OF VARIOUS HUMAN SPLEEN TISSUES*

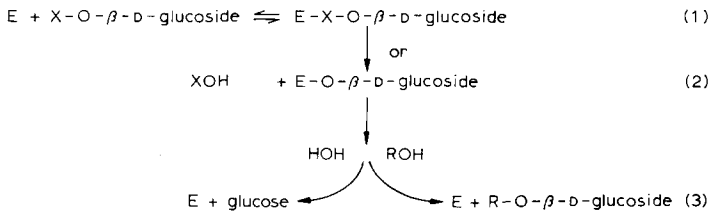
	Transglucosylation activity**	Me-Umb-β-D-glucosidase***
Gaucher's, adult	0.2	0.7
Gaucher's infantile	0.085	0.2
Gaucher's, adult	0.85	2.2
Gaucher's, adult	0.18	0.3
Gaucher's, adult	1.71	5.5
Gaucher's, infantile	0.36	0.6
Niemann-Picks	5.88	73.0
Niemann-Picks	1.66	13.5
Metachromatic leukodystrophy	3.79	44.6
Congestive heart failure	3.50	29.9
Congestive heart failure	1.28	9.7

* A 20% homogenate in 0.1 M citrate pH 4.75/1% Triton X-100 solution was centrifuged at 3000 rev/min for 10 min. Aliquots of the supernatant were employed as the enzyme source for these studies.
** Values expressed as nmol glucosylceramide formed per mg protein.
*** Values expressed as nmol Me-Umb-β-D-glucoside hydrolyzed/mg protein.

Discussion

The β-glucosidase employed in these studies has been shown to actively catalyze the hydrolysis of Nph-β-D-glucoside, Me-Umb-β-D-glucoside, glucosylceramide, glucosylsphingosine and deoxycorticosterone-β-D-glucoside. Previous studies from this laboratory demonstrated the ability of the enzyme preparation to catalyze the formation of glucosylceramide, which was unequivocally characterized by several procedures, from Me-Umb-β-D-glucoside as the glucose donor and ceramide as the acceptor [13]. It is postulated that its synthesis was due to the "transglucosylation" activity of this enzyme preparation. A possible mechanism which could be formulated is: where X could be either 4-methyl-

SCHEME I



umbelliferyl-, *p*-nitrophenyl-, deoxycorticosteroyl- or ceramide, E = purified calf spleen β-glucosidase

$$R = CH_3\text{-}(CH_2)_{12}\text{ - CH = CH - CH(OH) - CH(NHC(=O)R) - } CH_2$$

Line 1 is merely a representation of the formation of the classical

Michaelis-Menten complex. The subsequent conversion of this postulated product involves the release of the aglycone and the generation of a glucosylated intermediate. This presumably is not merely due to an electrostatic or hydrophilic interaction but rather to the formation of a covalent bond [23]. Whether the conversion of intermediate 1 or 2 is reversible is not known. The formation of such glucosyl intermediates has recently been suggested from studies with an almond β-glucosidase and *p*-nitrophenyl-β-D-glucoside [24].

It is suggested from the *V* values presented in Table I that the transglucosylation activity with Me-Umb-β-D-glucoside, as donor, is nearly efficient as its hydrolysis. Similarly, glucosylceramide is 60—70% as effective as a glucose donor as it is for the hydrolytic activity. It could therefore be speculated that ceramide at 0.88 mM is relatively more effective in the reaction on line 3 than water which is 55 M.

Gaucher's disease is characterized by the inability to cleave the β-glucosidic linkage in Me-Umb-β-D-glucoside, glucosylceramide and glucosylsphingosine, suggesting an absence of a common enzyme which is responsible for the cleavage of the β-glucosidic bond. The reduced capacity of the spleen samples for catalyzing the "transglucosylation" reaction is in accord with this generalized deficiency (Table V). The Gaucher patient has reduced cleavage capacity and therefore would not be expected to catalyze the "transglucosylation" reaction.

The isolation and partial purification of a glycopeptide from the spleen of Gaucher's patient has been reported [17,18]. This material, in a manner similar to taurocholate, stimulates the hydrolysis of Me-Umb-β-D-glucoside and glucosylceramide (Fig. 2). The purified material did not effect the rate of heat inactivation of enzyme activity. It was not effective in stimulating the hydrolysis of Me-Umb-β-*N*-acetylglucosaminide by purified human serum β-*N*-acetylhexosaminidase. Both taurocholate and the Gaucher's factor stimulated the "transglucosylation" activity (Fig. 3). Recently a material was purified which stimulated the hydrolysis of dihexosylceramide and $G_{M\,1}$ by β-D-galactosidase, trihexosylceramide by α-galactosidase and $G_{M\,2}$ by β-*N*-acetyl hexosaminidase [25].

The absolute substrate specificity of mammalian β-glucosidase is a matter of conjecture. The preparation employed in these studies hydrolyzes at least five different β-glucosides. The principal naturally occurring β-glucosidic bond found in mammalian tissue occurs in glucosylceramide. There are few reports of this linkage occurring in glycoproteins and none in the glycosaminoglycan. Glycogen is composed of α-glucosidic bonds. The transglucosylation experiments suggest that this calf spleen enzyme preparation will utilize Me-Umb-β-D-glucoside *p*-nitrophenyl-β-D-glucoside and deoxycorticosterone-β-D-glucoside as glucosyl donors (Table III). Previous studies indicated that free glucose, methyl-β-D-glucoside and Me-Umb-α-D-glucoside were ineffective in this regard [13]. These results suggest that there is a degree of substrate specificity for this β-glucosidase. Other workers have suggested an identity of human spleen "acid" β-glucosidase and glucocerebrosidase [26]. The linkage must be of the β-configuration and the aglycone probably should be lipoidal since gentiobiose, possessing a β-1 → 6 and cellulose, possessing a β-1 → 4 glycosyl linkage are inactive. The inability to utilize esculin and salicin is difficult to explain. The

aglycone portion of these β-glucosides are structurally similar to that of 4-methylumbelliferone and *p*-nitrophenol, respectively.

It seems reasonable therefore to propose that the enzyme preparation employed in these studies, possess a single catalytic protein which binds and utilizes these 5 compounds as substrates. This would explain the competitive nature of the inhibitors observed, as well as, their participation as donors in the transglucosylation reaction. Whether these dual characteristics will prove generally applicable and useful for resolving the problem of single or multiple enzymes will await further studies.

A steroid β-glucosidase has been extensively purified from rabbit liver. This enzyme appears to utilize a variety of steroidal aglycones containing a glucosyl β-linkage [21]. A preparation has been isolated from intestinal tissue which hydrolyzes the β-glucosidic bond in phlorizin, galactosylceramide and glucosylceramide [27].

Several hydrolytic enzymes have been shown to catalyze a reverse or transglycosylation reaction. These include α-galactosidase (28), α-glucosidase [29,30], β-*N*-acetylglucosaminidase [31], β-glucuronidase [32], β-galactosidase [33—35] and hyaluronidase [36]. It could be useful to establish the substrate specificities both for the hydrolytic and for the transglycolytic activities of any given "hydrolytic" enzyme.

Nojirimycin and gluconolactam have been shown to be competitive inhibitors of plant and bacterial β-glucosidases [22]. This appears to be similar to the well documented observations on the general inhibitory role of sugar acid lactones on acid glycosidases.

Deoxycorticosterone β-D-glucoside was shown to be a competitive inhibitor of both Me-Umb-β-D-glucoside and glucosylceramide hydrolysis. It also served as a substrate and was cleaved to yield free glucose and also served as a donor in the transglucosylation reaction. This might suggest that Gaucher patients may not possess the capacity to hydrolyze steroid β-D-glucosides. Such compounds have been isolated from rabbit urine [37].

Acknowledgements

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References

1 Brady, R.O., Kanfer, J.N. and Shapiro, D. (1965) J. Biol. Chem. 240, 39—43
2 Brady, R.O., Gal, A.E., Kanfer, J.N. and Bradley, R.M. (1965) J. Biol. Chem. 240, 3766—3770
3 Gatt, S. (1966) Biochem. J. 101, 687—691
4 Swami, E.D. and Agranoff, B.W. (1965) J. Lipid Res. 6, 211—219
5 Kennoway, N.G. and Woolf, L. (1968) J. Lipid Res. 9, 755—765
6 Kuske, T.T. and Rosenberg, A. (1972) J. Lab. Clin. Med. 80, 523—529
7 Brady, R.O., Kanfer, J.N., Bradley, R.M. and Shapiro, D. (1966) J. Clin. Invest. 45, 1112—1115
8 Patrick, A.D. (1965) Biochem. J. 97, 176
9 Weinreb, N.J. and Brady, R.O. (1972) Method. Enzymol. 28, 830—834
10 Pentchev, P.G., Brady, R.O., Hibbert, S.R., Gal, A.E. and Shapiro, D. (1973) J. Biol. Chem. 248, 5256—5261
11 Kanfer, J.N., Mumford, R.A., Raghavan, S.S. and Byrd, J. (1974) Anal. Biochem. 60, 200—205
12 Kanfer, J.N., Mumford, R.A., Raghavan, S.S., Friedman, R. and Byrd, J. (1974) Fed. Proc. 33, 1299

13 Raghavan, S.S., Mumford, R.A. and Kanfer, J.N. (1974) Biochem. Biophys. Res. Commun. 58, 99—106
14 Raghavan, S.S., Mumford, R.A. and Kanfer, J.N. (1973) Biochem. Biophys. Res. Commun. 54, 256—263
15 Taketomi, T. and Yamakawa, T. (1963) J. Biochem. (Tokyo) 54, 444—450
16 Holloway, P.W. (1973) Anal. Biochem. 53, 304
17 Kanfer, J.N., Stein, M. and Spielvogel, C. (1972) Sphingolipids, Sphingolipidoses and Allied Disorders (Volk, B.W. and Aronson, S.M. eds), pp. 225—236, Plenum, N.Y.
18 Friedman, R.B. and Kanfer, J.N. (1974) Biochem. Med. 9, 327—333
19 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497—509
20 Lowry, O.H. and Passanneau, J.V. (1972) A Flexible System of Enzymatic Analysis, pp. 174—175, Academic Press, N.Y.
21 Mellor, J.D. and Layne, D.S. (1974) J. Biol. Chem. 249, 361—365
22 Niwa, T., Inouye, S., Tsuruoka, T., Koaze, K. and Niida, T. (1970) Agr. Biol. Chem. 34, 966—968
23 Hehre, E.J., Okada, G. and Genghof, D.S. (1973) Adv. Chem. Series No. 117, 309—333
24 Fink, A.L. and Good, N.E. (1974) Biochem. Biophys. Res. Commun. 58, 126—131
25 Li, S-C., Wan, C-C., Mazzatta, M.Y. and Li, Y-T. (1974) Carbohyd. Res. 34, 189—193
26 Ho, M.W. (1973) Biochem. J. 136, 721—729
27 Leese, H.J. and Semeza, G. (1973) J. Biol. Chem. 248, 8170—8173
28 Pridham, J.B. (1972) Adv. Enzymol. 36, 91—130
29 Kawai, F., Yamada, H. and Ogata, K. (1973) Agr. Biol. Chem. 37, 1963—1965
30 DeBarsy, T., Jacquemin, P., Devas, P. and Hers, H.G. (1972) Eur. J. Biochem. 31, 156—165
31 Mega, R., Ikenaka, T. and Matsushima, Y. (1972) J. Biochem. 72, 1391—1396
32 Fishman, W.H. and Green, S. (1957) J. Biol. Chem. 225, 435—446
33 Wierzbicki, L.E. and Kosikowski, F.V. (1973) J. Dairy Sci. 56, 1400—1404
34 Wallenfels, K. and Fischer, J. (1960) Hoppe-Seyler's Z. Physiol. Chem. 321, 223—228
35 Distler, J.J. and Jourdian, G.W. (1973) J. Biol. Chem. 248, 6772—6783
36 Aronson, N.N. and Davidson, E.A. (1968) J. Biol. Chem. 243, 4494—4501
37 Williamson, D.G., Collins, D.C., Layne, D.S., Conrow, R.B. and Bernstein, S. (1969) Biochemistry 8, 4299—4304