Biochem. J. (1973) **136**, 721–729
Printed in Great Britain

Identity of 'Acid' β-Glucosidase and Glucocerebrosidase in Human Spleen

By MAE WAN HO
Department of Biochemistry, Queen Elizabeth College, University of London, London W8 7AH, U.K.

(*Received* 30 *May* 1973)

1. Glucocerebrosidase, in association with a membrane-bound 'acid' β-glucosidase, was separated from a soluble 'neutral' β-glucosidase that had no activity towards glucocerebroside as substrate. 2. Glucocerebrosidase, as well as 'acid' β-glucosidase activity depended upon the association of factor P (a heat-stable, soluble, acidic glycoprotein) with factor C (a heat-labile membrane-bound protein). 3. Factor C was solubilized under certain conditions. 4. Solubilized factor C, as well as membrane-bound factor C, could be alternatively stimulated by sodium taurocholate to give both glucocerebrosidase and 'acid' β-glucosidase activities. 5. Membrane-bound factor C reacted optimally with factor P whereas solubilized factor C was preferentially stimulated by taurocholate. 6. Factor P-dependent glucocerebrosidase activity differed in kinetic properties from the taurocholate-stimulated enzyme activity. 7. The results are discussed in the light of (*a*) identity of glucocerebrosidase and 'acid' β-glucosidase, (*b*) application in clinical diagnosis, (*c*) physiological significance of the enzyme system, and (*d*) polygenic inheritance in adult Gaucher's disease.

Glucocerebrosidase hydrolyses ceramide glucoside (Glcβ1-1Cer) to glucose and ceramide (Brady *et al.*, 1965*b*; Gatt, 1966). Ceramide glucoside accumulates in Gaucher's disease (Halliday *et al.*, 1940; Rosenberg & Chargaff, 1958), a metabolic disorder involving a deficiency of glucocerebrosidase (Brady *et al.*, 1965*a*; Patrick, 1965).

Two lines of evidence suggested that glucocerebrosidase was at least in part identical with β glucosidase (EC 3.2.1.21). Patrick (1965) noted that the deficiency of glucocerebrosidase in adult Gaucher's disease was accompanied by a decrease in β-glucosidase activity measured with *p*-nitrophenyl β-glucoside, and observed that the synthetic substrate could be used in clinical diagnosis. This proved to be the case (Öckerman & Köhlin, 1968; Beutler & Kuhl, 1970; Ho *et al.*, 1972). Gatt & Rapport (1966) isolated a *p*-nitrophenyl-β-glucosidase from ox brain and showed that it hydrolysed ceramide glucoside at a rate which was 40% that for the synthetic substrate (Gatt, 1966).

Beutler & Kuhl (1970) found that leucocytes from patients with adult Gaucher's disease were selectively deficient in 4-methylumbelliferyl-β-glucosidase activity measured at pH 4. This finding was confirmed in cultured skin fibroblasts (Ho *et al.*, 1972) and spleen (Ho & O'Brien, 1971). Two β-glucosidases were separated on gel filtration in human spleen: a high-molecular-weight 'acid' isoenzyme with optimum activity at about pH 4, and a low-molecular-weight 'neutral' isoenzyme with optimum activity between pH 5 and 6.5. The spleen from a patient with adult Gaucher's disease was deficient in the 'acid' isoenzyme but the concentration of the 'neutral' isoenzyme was unchanged (Ho & O'Brien, 1971).

'Acid' β-glucosidase was reconstituted *in vitro* from two inactive macromolecular factors, P and C. Factor P was a soluble heat-stable glycoprotein isolated from the spleen of a patient with adult Gaucher's disease. Factor C was a crude particulate preparation from a normal spleen; it was heat-labile and insoluble. The concentration of soluble factor P activity in the patient's spleen was 10 to 15 times normal whereas factor C activity was nearly absent (Ho & O'Brien, 1971).

The identity between 'acid' β-glucosidase and glucocerebrosidase was indicated by the simultaneous reconstitution of both enzyme activities from factors P and C (Ho *et al.*, 1973*a*).

The present paper describes in detail the separation of glucocerebrosidase from the bulk of methylumbelliferyl-β-glucosidase activity, and establishes its identity with 'acid' β-glucosidase.

Experimental

Materials

Human spleens were obtained at autopsy and stored at −20°C for up to 3 years. They were kindly

supplied by Dr. Jules G. Leroy, Dept. of Genetics, Middelheimlaan 1, B-2020, Antwerp, Belgium, and Dr. John S. O'Brien, Dept. of Neurosciences, Univ. of Calif., San Diego, La Jolla, Calif. 92037, U.S.A.

Spleens from patients with adult Gaucher's disease were provided by Dr. A. D. Patrick, Institute of Child Health, Univ. of London, 30 Guildford Street, London W.C.1, U.K.

Radioactive glucocerebroside, made by *N*-acylation of glucosyl sphingosine with [^{14}C]stearic acid (Kopaczyk & Radin, 1965), was a generous gift from Dr. Norman S. Radin, Neuroscience Laboratory, Univ. of Michigan, 1103E Huron St., Ann Arbor, Mich. 48104, U.S.A.

The following materials were from commercial sources as indicated: Triton X-100 (B grade) and sodium taurocholate (A grade) from Calbiochem, Los Angeles, Calif., U.S.A.; 4-methylumbelliferyl β-D-glucopyranoside, 2,5-diphenyloxazole and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.; 1,4-dioxan and naphthalene from BDH Chemicals Ltd., Poole, Dorset, U.K.; Sephadex G-150 from Pharmacia, Uppsala, Sweden.

Methods

Ultracentrifugation. A 10% homogenate of spleen was made in 0.25M-sucrose, 10mM-sodium phosphate buffer, pH 7.0. This was fractionated by centrifugation by the following sequence: 500*g* for 20min; 5000*g* for 30min; 100000*g* for 45min. The residue sedimented at each step and the final clear supernatant were assayed for glucocerebrosidase and methylumbelliferyl-β-glucosidase activities.

Enzyme assays. Glucocerebrosidase and methylumbelliferyl-β-glucosidase activities were assayed in the same buffer systems. This distinguished between effects of detergents on interactions of protein with lipid substrate, and that of detergents on the enzyme system itself. The first buffer consists of 0.05M-sodium acetate, pH 4.5, with 0.05% (v/v) Triton X-100 as previously described (Ho *et al.*, 1973*a*), and was used in reconstitution experiments involving factors P and C. The second buffer resembles the first except for the addition of 0.1% (w/v) sodium taurocholate. Sodium taurocholate stimulated factor C to give active enzyme in the absence of factor P (see the Results section); thus taurocholate-stimulated enzyme activity is roughly equivalent to factor C activity. The processing of the reaction mixture for glucocerebrosidase assay to isolate radioactive ceramide was described in detail by Ho *et al.* (1973*a*).

Preparation of factor P. Purified factor P was prepared from a spleen of a patient with adult Gaucher's disease (Ho & O'Brien, 1971). One unit of factor P activity gives an increase in 1 unit of enzyme activity (1nmol/h) when assayed in an excess of factor C.

Preparation of factor C. A 10% (w/v) homogenate of normal spleen in 0.25M-sucrose, 10mM-sodium phosphate buffer, pH 7.0, was centrifuged at 500*g* for 20min. The residue was discarded, and the supernatant centrifuged at 100000*g* for 45min. The residue was resuspended in 8vol. of water and spun down once more. After one washing with water, the membranous fraction was suspended in 3vol. of distilled water and stored at 4°C. This preparation of membrane-bound factor C was stable for 1 week. The activity of one unit of factor C is that which gives an increase in 1 unit of enzyme activity when assayed in an excess of factor P (Ho & O'Brien, 1971).

Solubilization of factor C. The suspension of membrane-bound factor C was mixed with an equal volume of citric acid–sodium phosphate buffer, pH 4.05 (0.032M-citrate and 0.036M-phosphate), containing 0.04% (v/v) Triton X-100, and incubated at 37°C for 1h. At the end of the incubation, a clear supernatant was separated from the sediment by centrifugation (1000*g*, 15min). The supernatant contained solubilized factor C activity.

Gel filtration. Gel filtration was carried out with Sephadex G-150 in 5mM-sodium phosphate buffer, pH 7.0, containing 5mM-NaCl (Ho & O'Brien, 1971; Ho, 1973).

Determination or kinetic constants. Kinetic constants and the standard error of the estimates were obtained by the FORTRAN computer program of Cleland (1963). Data from at least two experiments were curve-fitted to the following equation:

$$v = \frac{V_{max.}[S]}{K_m + [S]}$$

A substrate concentration range of 6–160M was used for glucocerebroside, and 0.1–2mM for methylumbelliferyl β-glucoside.

Results

Separation of glucocerebrosidase from the bulk of methylumbelliferyl-β-glucosidase activity by ultracentrifugation

The distribution of glucocerebrosidase and methylumbelliferyl-β-glucosidase activities is summarized in Table 1. Activities for the synthetic and natural substrates fractionated differently. Most notable is the total absence of glucocerebrosidase activity in the supernatant, and its association with the particulate fractions sedimented at 5000*g* and 100000*g*. This indicated that the soluble β-glucosidase was without glucocerebrosidase activity. When the particulate fractions were subjected to hypo-osmotic conditions (by 8-fold dilution with water and standing at 4°C for 30min), over 95% of the glucocerebrosidase activity remained associated with the 100000*g* residue on

Table 1. *Distribution of glucocerebrosidase and methylumbelliferyl-β-glucosidase activities*

All assays were performed in the presence of sodium taurocholate (0.1%, w/v). Specific activities are expressed as nmol of substrate cleaved/h per mg of protein. Protein was measured by the method of Lowry *et al.* (1951). Each value represents an average of two determinations which agreed within 5%.

	Glucocerebrosidase (*A*)			Methylumbelliferyl-β-glucosidase (*B*)			
Fraction	Sp. activity	Total activity	% of total	Sp. activity	Total activity	% of total	Ratio of *A*/*B*
Homogenate	6.2	942	100	19.8	2970	100	3.2
Residue (500*g*)	7.5	267	28	40.2	1470	50	5.5
Residue (5000*g*)	57.5	274	29	86.3	408	14	1.5
Residue (100000*g*)	32.7	136	14	58.9	245	8	1.8
Supernatant (100000*g*)	0	0	0	15.0	450	15	—

re-centrifugation. This indicated that glucocerebrosidase activity is tightly membrane-bound.

Effect of pH on activity of particulate and soluble β-glucosidases

Methylumbelliferyl-β-glucosidase in the 5000*g* residue was compared with that in the 100000*g* supernatant. The particulate fraction had optimum activity between pH4.5 and 5.0 whereas the supernatant fraction gave optimum activity between pH5.0 and 6.5. Glucocerebrosidase activity showed a somewhat sharper optimum at about pH4.8 in the particulate fraction, and no activity was demonstrated in the supernatant over the entire range of pH values examined (Fig. 1).

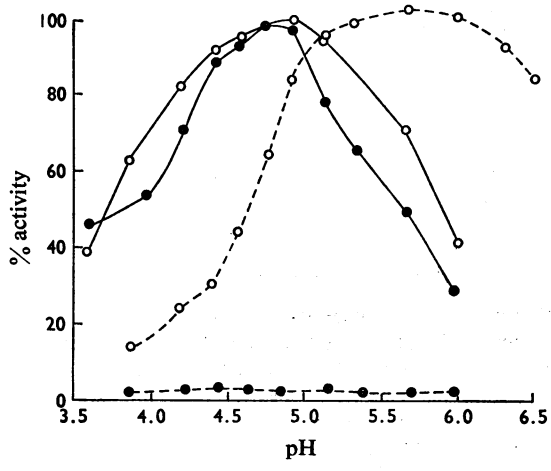

Fig. 1. *pH–activity curves of particulate and soluble β-glucosidases*

All assays were performed in the presence of sodium taurocholate. Sodium acetate (0.1 M) and acetic acid (0.1 M) each containing 0.1% (v/v) Triton X-100 and 0.2% (w/v) sodium taurocholate were mixed in various proportions for a range of pH3.5–6.5. Glucocerebrosidase (●); methylumbelliferyl-β-glucosidase (○). ——, Particulate enzyme from 5000*g* residue; ----, soluble enzyme from 100000*g* supernatant.

Effect of factor P on particulate and soluble β-glucosidases

To distinguish further between the particulate and the soluble glucosidases, factor P was mixed with the fractions, which were then assayed directly for enzyme activities. The expected increase in enzyme activity for both the synthetic and natural substrates was obtained in the particulate fractions as previously described (Ho & O'Brien, 1971; Ho *et al.*, 1973*a*). Soluble β-glucosidase did not respond to factor P at all (Table 2).

Gel filtration of soluble and particulate β-glucosidases

The two β-glucosidases were also distinguishable by molecular size. Gel filtration of the 100000*g* supernatant of the sucrose homogenate gave a single peak of methylumbelliferyl-β-glucosidase activity (Fig. 2*a*).

When the homogenate was made in distilled water, left to stand at 24°C for 1 h and then centrifuged to obtain the 100000*g* supernatant, the gel-filtration pattern was quite different. An additional peak of methylumbelliferyl-β-glucosidase activity was obtained near the void volume (peak I, Fig. 2*b*), which coincided with the elution of the glucocerebrosidase activity.

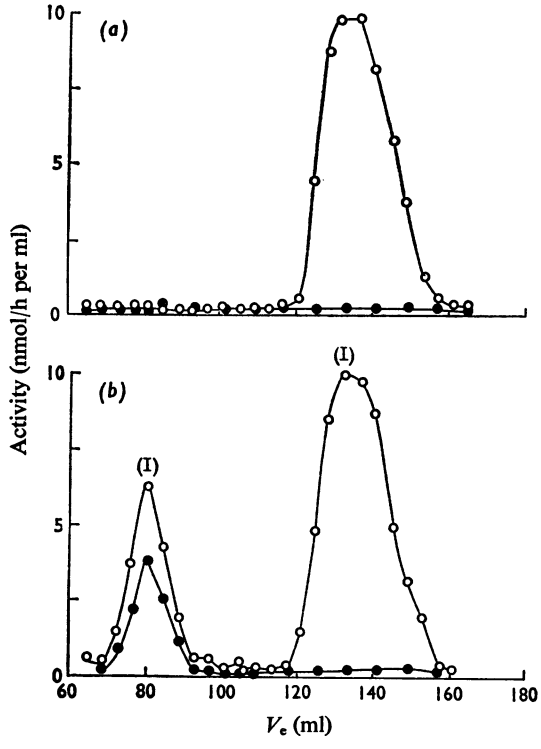

Fig. 2. *Gel filtration of β-glucosidases*

Samples (1.5ml) were applied to a column (2.5cm × 45cm) and eluted at a flow rate of 20ml/h. Fractions (2ml) were collected and assayed for glucocerebrosidase (●) and methylumbelliferyl-β-glucosidase (○) activities in the presence of 0.1% sodium taurocholate (see under 'Methods'). (*a*) A 100000*g* supernatant from a 10% (w/v) homogenate of spleen in 0.25M-sucrose, 10mM-sodium phosphate buffer, pH7.0. (*b*) A 100000*g* supernatant from a 10% homogenate of spleen in distilled water, which was incubated for 1h at 24°C. For further details of peaks see the text.

The amount of enzyme activity in peak (I) depended upon the variable solubilization of the particulate enzyme. About 20% of the particulate enzyme was solubilized by homogenization in water as described above. Homogenization in 0.1% Triton X-100 resulted in 80% solubilization of the particulate enzyme in normal spleens. The same procedures applied to spleens from patients with adult Gaucher's disease failed to produce detectable amounts of the particulate enzyme in solution. Despite the deficiency of the particulate enzyme with 'acid' pH optimum, the activity of the soluble enzyme with 'neutral' pH optimum was not diminished in adult Gaucher's

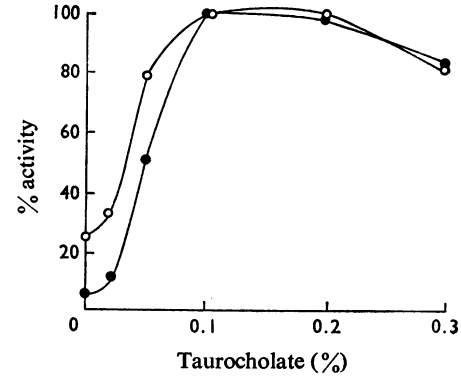

Fig. 3. *Effect of sodium taurocholate on particulate β-glucosidase activities*

Glucocerebrosidase (●) and methylumbelliferyl-β-glucosidase (○) activities were assayed in 0.05M-sodium acetate buffer, pH4.5, containing 0.05% (v/v) Triton X-100 and increasing amounts of sodium taurocholate as indicated. One hundred per cent indicates the maximum activity obtained in each case. Each point is an average of two determinations which agreed within 5%.

Table 2. *Effect of factor P on glucocerebrosidase and methylumbelliferyl-β-glucosidase activities in subcellular fractions*

All assays were performed in the absence of taurocholate. Factor P was present in an excess. Activities are expressed as nmol of substrate cleaved/h per mg of protein. Each value is an average of two determinations which agreed within 10%.

	Glucocerebrosidase		Methylumbelliferyl-β-glucosidase	
Fraction	Control	+Factor P	Control	+Factor P
Residue (500*g*)	0.8	24	10.5	51
Residue (5000*g*)	5.2	104	26.9	162
Residue (100000*g*)	3.0	141	23.0	192
Supernatant (100000*g*)	0	0	18.0	18

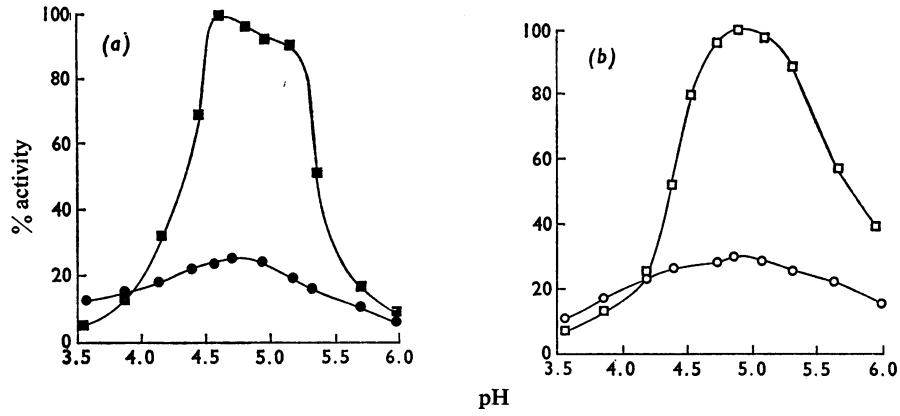

Fig. 4. *Effect of factor P and sodium taurocholate on particulate β-glucosidase activities*

Membrane-bound factor C (see under 'Methods') was diluted with factor P (in experimentally determined excess) and assayed in 0.05M-sodium acetate buffer (pH 3.5–6.0) containing 0.05% (v/v) Triton X-100; this gave factor P-dependent enzyme activities (■, □). A duplicate preparation of membrane-bound factor C diluted with water was assayed in the same buffer with 0.1% (w/v) taurocholate; this gave taurocholate-dependent enzyme activities (●, ○). (*a*) Glucocerebrosidase; (*b*) methylumbelliferyl-β-glucosidase.

disease (Ho & O'Brien, 1971), contrary to some claims (Öckerman, 1968).

The particulate nature of human glucocerebrosidase and 'acid' β-glucosidase is in line with the general membrane-bound nature of the enzymes in other mammalian tissues (Gatt & Rapport, 1966; Beck & Tappel, 1968). This is not consistent with earlier claims that glucocerebrosidase is a soluble enzyme (Brady *et al.*, 1965*a*) in human spleen. This discrepancy is easily understood since the particulate enzyme can be solubilized under conditions which disrupt membrane structure, e.g. hypo-osmoticity and presence of detergents. Thus no conclusion as to the solubility of the enzyme is warranted under the conditions used (Brady *et al.*, 1965*b*).

Properties of glucocerebrosidase and 'acid' β-glucosidase

In the membrane-bound form, glucocerebrosidase activity, as well as 'acid' β-glucosidase activity, depended on the association of soluble factor P with factor C in the particulate fraction (Ho *et al.*, 1973*a*). The kinetics of this association have been described (Ho & O'Brien, 1971; Ho *et al.*, 1973*a*). In the course of further investigations, sodium taurocholate was found to be an alternative activator of factor C to give both glucocerebrosidase and 'acid' β-glucosidase activities. Factor C, though normally membrane-bound, could be solubilized by various treatments (Ho & O'Brien, 1971). The effects of factor P and taurocholate on membrane-bound and solubilized factor C are compared.

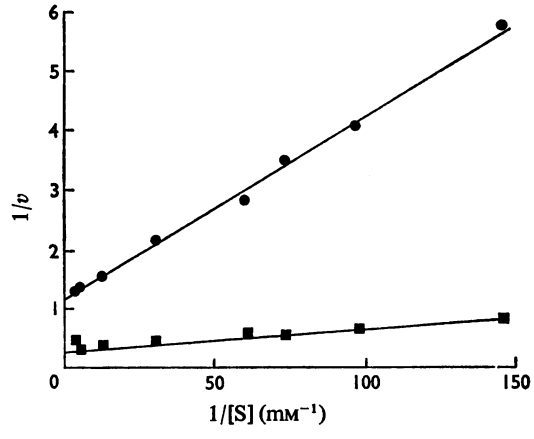

Fig. 5. *Lineweaver–Burk plots for glucocerebroside hydrolysis*

Factor P-dependent (■) and taurocholate-dependent (●) glucocerebrosidase were assayed at pH 4.5 as described in Fig. 5. $1/v$ is expressed as 10^3 c.p.m./h. Each point is the average of two determinations.

Effect of factor P and sodium taurocholate on membrane-bound factor C

Membrane-bound factor C was stimulated by the association with factor P to give both glucocerebrosidase and 'acid' β-glucosidase activities; the maximal activities obtained depended on concentrations of both factors P and C (Ho *et al.*, 1973*a*). Stimulation

of factor C by sodium taurocholate also resulted in a parallel increase in enzyme activity for both substrates. However, the maximal activity achieved was at a detergent concentration of 0.1–0.2% (w/v), and did not depend on the concentration of factor C (Fig. 3).

The pH–activity curve of glucocerebrosidase and 'acid' β-glucosidase in the presence of excess of factor P showed a slightly lower pH optimum than that obtained in the presence of 0.1% taurocholate. The magnitude of enzyme activities attained was greater in the presence of factor P at all pH values between 4.0 and 6.0 (Fig. 4).

Linear Lineweaver–Burk plots were obtained for both substrates in the presence of factor P or of taurocholate. Plots for the natural substrate are shown in Fig. 5. Taurocholate-stimulated enzyme activity gave K_m values of 25±2.6 μM for glucocerebroside and 3.1±0.27 mM for 4-methylumbelliferyl β-glucoside; the corresponding values for factor P-stimulated enzyme activity were 9.0±0.8 μM and 0.83±0.07 mM respectively. The $V_{max.}$ of the factor P-stimulated enzyme was about four times that of the taurocholate-stimulated enzyme.

The results so far strongly indicate that factor C, normally stimulated by factor P, is alternatively stimulated by taurocholate. The two act in competition: taurocholate inhibited factor P-stimulated enzyme activity by about 50% (Table 3). Moreover, the solubilization of factor C activity was accompanied by the solubilization of taurocholate-stimulated enzyme activity (see below).

Solubilization of factor C

When the particulate preparation of factor C was incubated alone under the same conditions which favoured association with factor P, up to 50% of

Table 3. *Effect of factor P on membrane-bound factor C in the presence and absence of sodium taurocholate*

Controls were assayed without taurocholate (see under 'Methods'). Factor P was present in an excess. Sodium taurocholate, where indicated, was present at a concentration of 0.1% (w/v). Activity is expressed as nmol/h per mg of protein. Each value represents an average of three determinations which agreed within 10%.

	Glucocerebrosidase		Methylumbelliferyl-β-glucosidase	
Item	Control	+Taurocholate	Control	+Taurocholate
Factor C	1.4	25.0	13.9	51.4
Factors C and P	101.1	53.4	154.6	84.2

Table 4. *Solubilization of factor C in the presence and absence of factor P*

Controls were assayed without taurocholate (see under 'Methods'). Factor P was present in an excess. Sodium taurocholate, where indicated, was present at a concentration of 0.1% (w/v). Total activity is expressed as nmol/h. Each value represents an average of two determinations which agreed within 10%.

	Glucocerebrosidase		Methylumbelliferyl-β-glucosidase	
Item	Control	+Taurocholate	Control	+Taurocholate
(1) Factor C	1.2	24.7	13.8	51.0
(2) As (1)+factor P	100.6	—	152.4	—
(3) Preincubated* factor C	1.2	23.0	12.9	51.1
(4) As (3)+factor P	92.4	—	140.2	—
(5) Residue from (3)	0.9	12.0	6.0	20.4
(6) As (5)+factor P	47.1	—	77.2	—
(7) Supernatant from (3)	0	16.6	0.4	28.2
(8) As (7)+factor P	11.6	—	20.1	—
(9) Factor C+factor P	100.6	52.2	152.4	79.1
(10) Preincubated* factor C+factor P	115.3	52.3	179.9	80.2
(11) Residue from (10)	99.8	36.4	147.2	69.9
(12) Supernatant from (10)	1.1	5.9	2.2	10.7

* Preincubation was carried out for 1 h at 37°C in citrate–phosphate buffer, pH 4.05 (0.016 M-citrate–0.018 M-phosphate) containing 0.02% (v/v) Triton X-100. The residue and supernatant fractions were then obtained by centrifugation at 1000*g* for 15 min.

factor C activity was progressively lost from the particles, only 12% of which was found in the supernatant when tested with factor P (Table 4, compare items 6 and 8 with item 2 in the 'Control' column). This was thought to be an inactivation of factor C *in situ* or on solubilization. However, when the same preparations were tested with taurocholate before and after incubation, essentially all of the activity lost from the particles was recovered in the supernatant (Table 4, compare items 5 and 7 with item 1 in the '+Taurocholate' column). This suggested that solubilized factor C did not respond optimally to factor P, and that an additional requirement was involved, perhaps in the membranous fraction from which it was derived. Indeed when solubilized factor C was added back to the membranous fraction, then tested with factor P, 100% of the total factor C activity was restored. Of note is that solubilized factor C on its own is devoid of enzyme activity for both the synthetic and natural substrates.

Solubilized factor C remained distinguishable in its alternative stimulation by factor P and taurocholate. Stimulation by factor P gave an enzyme with a low K_m value for glucocerebroside (8.4±0.7 μM) compared with the taurocholate-stimulated enzyme (24.6± 2.5 μM). The $V_{max.}$ ratio was reversed, however, and was about 1.5:1 in favour of the taurocholate-stimulated activity.

Solubilized factor C was eluted in approximately the same position on gel filtration as 'acid' β-glucosidase. Attempts to purify it have so far been unsuccessful as it was extremely labile. All activity was lost on vacuum dialysis at 4°C, as well as on freezing and thawing. Crude solubilized factor C was stable at 4°C for 2–3 days, whereas after gel filtration, all activity was lost overnight. The loss of activity responsive to taurocholate occurred simultaneously with the loss in factor C activity.

The identification of taurocholate-stimulated activity with factor C is strengthened by the total lack of such stimulation in 'acid' β-glucosidase activity in the spleen of two patients with adult Gaucher's disease. A particulate preparation from a spleen homogenate from one of the patients was treated to solubilize factor C. The supernatant after incubation was tested for factor C activity with factor P or taurocholate with both synthetic and natural substrates. The results were negative in all cases, confirming the deficiency of factor C activity in adult Gaucher's disease (Ho & O'Brien, 1971).

Discussion

It is useful at this juncture to review the salient observations which led to the identification of 'acid' β-glucosidase with glucocerebrosidase in human spleen. (*a*) Both activities were associated with the particulate fraction and were absent from the 100000*g* supernatant. (*b*) Both activities were eluted in the same position on gel filtration. (*c*) A parallel reconstitution of enzyme activities occurred on mixing factor P (a soluble heat-stable glycoprotein) and factor C (a membrane-associated heat-labile protein). (*d*) Factor C was stimulated by sodium taurocholate in the absence of factor P to give both 'acid' β-glucosidase and glucocerebrosidase activities. (*e*) Factor P or taurocholate stimulated solubilized factor C to give both 'acid' β-glucosidase and glucocerebrosidase activities. (*f*) Deficiency of factor C in adult Gaucher's disease was accompanied by a drastic decrease in both 'acid' β-glucosidase and glucocerebrosidase activities.

These observations are consistent among themselves and present a coherent genetic and biochemical picture. The practical implications are obvious: the synthetic substrate can (with appropriate discretion) be used in clinical diagnosis. In particular, the inclusion of taurocholate in the assay buffer gives a system which is independent of the concentration of factor C (or of endogenous concentrations of factor P). This means that an accurate determination of factor C is possible irrespective of the protein concentration. In the absence of taurocholate, enzyme activity depended on endogenous concentrations of both factor P and C, and therefore decreased exponentially with dilution of the homogenate protein as previously observed (Ho *et al.*, 1972). The application of taurocholate in clinical diagnosis is illustrated in cultured skin fibroblasts (Fig. 6). In the presence of taurocholate, enzyme activity increased linearly over a 20-fold difference in protein concentrations, whereas in its absence activity decreased so rapidly with dilution that, at sufficiently low protein concentrations, it is easy to see where the activity in controls would seem indistinguishable from that of affected homozygotes, leading to erroneous conclusions that the synthetic substrate cannot be used in diagnosis (see, e.g., Kanfer *et al.*, 1972).

Since the completion of this work, Pentchev & Brady (1973) reported a reconstitution of 'acid' β-glucosidase but not of glucocerebrosidase from factors P and C, and concluded that factor P (and 'acid' β-glucosidase) was not important in glucocerebroside catabolism. This discrepancy is easily understood. The activity towards the synthetic substrate was measured in a buffer system favouring reconstitution (low salt concentrations, low pH and absence of interfering detergents like taurocholate); whereas exactly opposing conditions were used in the assay of glucocerebrosidase.

The effect of taurocholate on factor C appears to be the maintenance of an active soluble conformation of the enzyme protein (perhaps in simulation of the membrane matrix) which is independent of factor P and of the membrane matrix. This contrasts with the

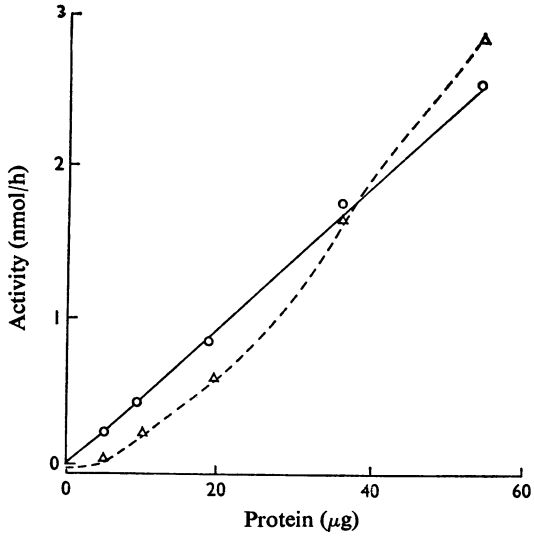

Fig. 6. *Activity of 'acid' β-glucosidase as a function of a homogenate of protein from cultured skin fibroblasts*

A homogenate of cultured skin fibroblasts from a normal individual was assayed in 0.05 M-sodium acetate buffer, pH 4.5, containing 0.05% Triton X-100 with (o) and without (△) 0.1% (w/v) sodium taurocholate. Activity is plotted against protein used in each assay. Each point represents an average of two determinations which agreed within 10%.

membrane-bound factor C, which requires factor P and an additional membrane component for full activity. The requirement for additional membrane component is suggested by the inefficiency with which solubilized factor C responded to factor P, and by the restoration of full efficiency when it was added back to the membranous fraction. The difference in K_m values between the taurocholate-dependent and factor P-dependent enzyme activities reflects that between a soluble and a membrane-bound form of the enzyme, and again raises the possibility that membrane-binding is itself a process whereby enzymes become activated (Ho *et al.*, 1973*b*).

This enzyme system is of considerable interest. The involvement of multiple macromolecular components increases the sensitivity of metabolic control, since the endogenous enzyme activity depends on the concentration of both factors P and C. Factor P activity has been demonstrated in normal spleen and liver, and factor C activity has been demonstrated in normal spleen, liver, brain, leucocytes and skin fibroblasts (M. W. Ho, unpublished work). It remains to be investigated whether preformed complexes of factors P and C already exist in the tissues examined. The following considerations suggest that they do. Since factor C may be prepared in a soluble state devoid of enzyme activity, it may be assumed that it is a totally inactive component of the system unless stimulated by taurocholate or factor P. Low activities are nonetheless observed in the particulate preparations of spleen in the absence of taurocholate or of added factor P (see Table 2). From this it may be inferred that low concentrations of factor P are already bound to the membranous fractions in some form. To prove this, the complexes will have to be isolated and re-dissociated into the component factors.

Hsia *et al.* (1962) predicted the possible polygenic nature of adult Gaucher's disease while commenting on the isolated reports of dominant inheritance in some kindreds. This may now be interpreted in the light of the present findings. If factors P and C are coded by different loci, a mutation involving either locus could result in a deficiency of enzyme activity observed in adult Gaucher's disease. This could account for apparent dominant inheritance as well as for the variation in clinical progression of the disease. Affected individuals could include homozygotes for either factor P or factor C, or both, or the double heterozygote; or individuals may be homozygous for one factor in addition to being heterozygous for the other factor. Assuming that the association between factors P and C involves one molecule of each, then half the concentration of both factors in the double heterozygote, for instance, could decrease the enzyme to one-quarter of the normal value. This could give rise to a protracted form of adult Gaucher's disease. It seems worthwhile to re-examine kindreds with more than one afflicted generation to identify the exact genetic lesions. This is of value in genetic counselling as well as in the development of a coherent enzyme replacement therapy.

This work was done in the laboratories of Dr. John S. O'Brien and Dr. D. Robinson during the tenure of a National Genetics Foundation Fellowship; I am grateful for their interest and support. Special thanks to Dr. Jules G. Leroy, Dr. John S. O'Brien, Dr. A. D. Patrick and Dr. Norman S. Radin for providing valuable materials for study as detailed in the text.

References

Beck, C. & Tappel, A. L. (1968) *Biochim. Biophys. Acta* **151**, 159–164
Beutler, E. & Kuhl, W. (1970) *Lancet* **i**, 612–613
Brady, R. O., Kanfer, J. N. & Shapiro, D. (1965*a*) *Biochem. Biophys. Res. Commun.* **18**, 221–225
Brady, R. O., Kanfer, J. N. & Shapiro, D. (1965*b*) *J. Biol. Chem.* **240**, 39–43
Cleland, W. W. (1963) *Nature (London)* **198**, 463–466
Gatt, S. (1966) *Biochem. J.* **101**, 687–691
Gatt, S. & Rapport, M. (1966) *Biochim. Biophys. Acta* **113**, 567–576

Halliday, N., Deuel, H. J., Jr., Tragerman, L. J. & Ward, W. E. (1940) *J. Biol. Chem.* **132**, 171–180

Ho, M. W. (1973) *Biochem. J.* **133**, 1–10

Ho, M. W. & O'Brien, J. S. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 2810–2813

Ho, M. W., Seck, J., Schmidt, D., Veath, M. L., Johnson, W., Brady, R. O. & O'Brien, J. S. (1972) *Amer. J. Hum. Genet.* **24**, 37–45

Ho, M. W., O'Brien, J. S., Radin, N. S. & Erickson, J. S. (1973*a*) *Biochem. J.* **131**, 173–176

Ho, M. W., Cheetham, P. & Robinson, D. (1973*b*) *Biochem. J.* **136**, 351–359

Hsia, D. Y.-Y., Naylor, J. & Bigler, J. A. (1962) *Cerebr. Sphingolipidoses, Symp. Tay–Sachs Dis. Allied Disord.* pp. 327–342, Academic Press, New York

Kanfer, J. N., Stein, M. & Spielvogal, C. (1972) in *Sphingolipids, Sphingolipidoses and Allied Disorders* (Volk, B. W. & Aronson, S. M., eds.), pp. 225–236, Plenum Press, New York

Kopaczyk, K. C. & Radin, N. S. (1965) *J. Lipid Res.* **6**, 140–145

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Öckerman, P. A. (1968) *Biochim. Biophys. Acta* **165**, 59–62

Öckerman, P. A. & Kohlin, P. (1968) *Scand. J. Clin. Lab. Invest.* **22**, 62–64

Patrick, A. D. (1965) *Biochem. J.* **97**, 17C–18C

Pentchev, P. G. & Brady, R. O. (1973) *Biochim. Biophys. Acta* **297**, 491–496

Rosenberg, A. & Chargaff, E. (1958) *J. Biol. Chem.* **233**, 1323–1326