



**Universiteit
Leiden**
The Netherlands

Transglycosidase activity of chitotriosidase - Improved enzymatic assay for the human macrophage chitinase

Aguilera, B.; Ghauharali-Van der Vlugt, K.; Helmond, M.T.J.; Out, J.M.M.; Donker-Koopman, W.E.; Groener, J.E.M.; ... ; Aerts, J.M.F.G.

Citation

Aguilera, B., Ghauharali-Van der Vlugt, K., Helmond, M. T. J., Out, J. M. M., Donker-Koopman, W. E., Groener, J. E. M., ... Aerts, J. M. F. G. (2003). Transglycosidase activity of chitotriosidase - Improved enzymatic assay for the human macrophage chitinase. *Journal Of Biological Chemistry*, 278(42), 40911-40916. doi:10.1074/jbc.M301804200

Version: Not Applicable (or Unknown)

License: [Leiden University Non-exclusive license](#)

Downloaded from: <https://hdl.handle.net/1887/50675>

Note: To cite this publication please use the final published version (if applicable).

Transglycosidase Activity of Chitotriosidase

IMPROVED ENZYMATIC ASSAY FOR THE HUMAN MACROPHAGE CHITINASE*

Received for publication, February 20, 2003, and in revised form, July 21, 2003
Published, JBC Papers in Press, July 30, 2003, DOI 10.1074/jbc.M301804200

Begoña Aguilera^{‡§}, Karen Ghauharali-van der Vlugt^{¶§}, Mariette T. J. Helmond[¶],
Jos M. M. Out[¶], Wilma E. Donker-Koopman[¶], Johanna E. M. Groener[¶], Rolf G. Boot[¶],
G. Herma Renkema[¶], Gijs A. van der Marel[‡], Jacques H. van Boom[‡],
Hermen S. Overkleef[‡], and Johannes M. F. G. Aerts^{¶**}

From the [¶]Department of Biochemistry, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands, the [‡]Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P. O. Box 9502, 2300 RA Leiden, The Netherlands, and the [¶]Institute of Medical Technology, University of Tampere, FIN-33014 Tampere, Finland

Chitotriosidase is a chitinase that is massively expressed by lipid-laden tissue macrophages in man. Its enzymatic activity is markedly elevated in serum of patients suffering from lysosomal lipid storage disorders, sarcoidosis, thalassemia, and visceral Leishmaniasis. Monitoring of serum chitotriosidase activity in Gaucher disease patients during progression and therapeutic correction of their disease is useful to obtain insight in changes in body burden on pathological macrophages. However, accurate quantification of chitotriosidase levels by enzyme assay is complicated by apparent substrate inhibition, which prohibits the use of saturating substrate concentrations. We have therefore studied the catalytic features of chitotriosidase in more detail. It is demonstrated that the inhibition of enzyme activity at excess substrate concentration can be fully explained by transglycosylation of substrate molecules. The potential physiological consequences of the ability of chitotriosidase to hydrolyze as well as transglycosylate are discussed. The novel insight in transglycosidase activity of chitotriosidase has led to the design of a new substrate molecule, 4-methylumbelliferyl-(4-deoxy)chitobiose. With this substrate, which is no acceptor for transglycosylation, chitotriosidase shows normal Michaelis-Menten kinetics, resulting in major improvements in sensitivity and reproducibility of enzymatic activity measurements. The novel convenient chitotriosidase enzyme assay should facilitate the accurate monitoring of Gaucher disease patients receiving costly enzyme replacement therapy.

Chitin, the linear polymer of β 1,4-linked *N*-acetylglucosamine, is a structural component of cell walls and coatings of many organisms. For a long time it was thought that mammals are unable to produce endoglucosaminidases that fragment chitin. Investigations on Gaucher disease led to the serendipitous discovery of a functional endogenous chitinase in man (1–3). It was observed that serum samples from Gaucher

patients show a 1000-fold elevated capacity to hydrolyze 4-methylumbelliferyl- β -D-chitotriose. The responsible enzyme, named chitotriosidase, was shown to be able to cleave natural chitin and a variety of artificial chitin-like substrates such as 4-methylumbelliferyl and *p*-nitrophenyl chitoooligosaccharides. Chitotriosidase belongs to the family 18 of glycosylhydrolases and is highly homologous to chitinases from lower organisms. The enzyme consists of a N-terminal TIM-barrel structure containing the catalytic groove and a C-terminal chitin-binding domain connected by a short hinge region. The crystal structure of the 39-kDa catalytic domain and its complexes with a chitoooligosaccharide and allosamidin has been described recently (4). The structures reveal an elongated active site cleft, compatible with binding long chitin polymers. The catalytic center of chitotriosidase closely resembles that of chitinases from lower organisms like chitinase A/B from *Serratia marcescens*. The catalytic reaction of family chitinases takes place through a retaining mechanism, resulting in β -anomers by hydrolysis of β 1,4-glycosidic linkages. The reaction is initiated by distortion of the pyranose ring of the (–1) sugar moiety in the active site and protonation of the glycosidic oxygen by a protonated acidic residue forming a covalent oxazolinium ion intermediate. The subsequent nucleophilic attack differs from classical reaction mechanisms of retaining enzymes such as lysozyme (5) in that it involves the *N*-acetyl group of the (–1) sugar, rather than a carboxylate side chain on the protein, a so-called substrate-assisted mechanism (6, 7).

Chitotriosidase is selectively expressed in chronically activated tissue macrophages, like the lipid-laden storage cells that accumulate in large quantities in various tissues of Gaucher patients (8). Tissue macrophages largely secrete newly synthesized 50-kDa chitotriosidase, but about one-third is directly routed to lysosomes and proteolytically processed to the 39-kDa unit that remains catalytically active (9). Intriguingly, one in every three individuals from various ethnic groups carries one abnormal chitotriosidase gene with a 24-bp duplication that prevents production of enzyme (10). About 6% of the population is homozygous for this mutant allele and consequently completely lacks chitotriosidase activity. In analogy to the function of homologous chitinases in plants, the physiological role of chitotriosidase is most likely in innate immunity toward chitin containing pathogens. An increased risk for nematode infection has indeed been described for chitotriosidase-deficient individuals (11).

Pathological tissue macrophages in specific disease conditions massively express chitotriosidase. A shared feature of such cells is accumulation of lipid material in the lysosomal

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ These two authors should be considered equal first authors of this paper.

** To whom correspondence should be addressed: Dept. of Biochemistry, Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands. Tel.: 31-20-5665156, Fax: 31-20-6915519; E-mail: j.m.aerts@amc.uva.nl.

apparatus. The molecular mechanism that underlies the tightly controlled expression of chitotriosidase is not yet known. In serum of patients suffering from lysosomal lipid storage disorders (12), thalassemia (13), sarcoidosis, and visceral Leishmaniasis (1), as well as in cerebral spine fluid of patients with multiple sclerosis, significantly elevated chitotriosidase activities are detectable using artificial chromogenic and fluorogenic substrates. The abnormality is exploited for diagnostic and monitoring purposes. For example, the efficacy of the extremely costly enzyme replacement therapy of Gaucher patients is monitored by following corrections in plasma chitotriosidase activity levels (8, 14). However, accurate quantification of chitotriosidase levels by enzyme assay is prohibited by apparent substrate inhibition (1). The inevitable use of subsaturating substrate concentrations reduces the reproducibility and sensitivity of the assay and has hampered its widespread application outside expert laboratories.

We have studied the molecular basis for the apparent substrate inhibition of chitotriosidase in more detail. The information obtained allowed us to design a novel artificial substrate that can be used for a substantially improved enzyme activity assay. The results of the study are here reported and discussed.

MATERIALS AND METHODS

Human Recombinant 50-kDa Chitotriosidase—Enzyme was purified from the medium of stably transfected baby hamster kidneys cells using the full-length human chitotriosidase cDNA cloned in the pNUT vector (4). The enzyme was purified by sequential chromatofocusing, isoelectrofocusing, and gel filtration (2). Chitin oligosaccharides were purchased from Seikagyo Kogyo. All other chemicals, including 4-methylumbelliferone, *p*-nitrophenol, and their conjugates with chitooligosaccharides, were purchased from Sigma, Aldrich, or Baker.

Enzyme Activity Measurements—The conventional enzyme assays with commercial artificial fluorogenic or chromogenic substrates were performed exactly as described before (1). Briefly, enzyme preparations were incubated at 37 °C with 22 μ M 4-MU¹-chitooligosaccharides or with 220 μ M PNP-chitooligosaccharides in McIlvain buffer (100 mM citric acid, 200 mM sodium phosphate, pH 5.6) containing 1 mg/ml bovine serum albumin. The enzyme assay was stopped by addition of excess 0.3 M glycine-NaOH, pH 10.3. Formed 4-methylumbelliferone was detected fluorometrically (excitation at 445 nm; emission at 366 nm); formed PNP was detected spectrophotometrically at 405 nm.

The optimal assay conditions with the newly synthesized 4-MU-(4-deoxy)chitobiose were at 114 μ M in the same buffer. A 35 mM stock solution of the novel substrate was prepared in Me₂SO.

Chitooligosaccharide Analysis—Isocratic HPLC chromatography with a Prevail carbohydrate ES column (Alltech), and UV detection at 214 nm was used to analyze chitooligosaccharides. The injection volume was 10 μ l, the flow rate of the eluent (62:28 (w/w) acetonitrile:H₂O) was 1 ml/min.

Synthesis of the Novel Substrate 4-MU-(4-deoxy)chitobiose—Octaacetyl chitobioside (1), prepared by acetylation of chitin (15), was transformed into the partially protected disaccharide (2) according to a previously reported procedure (16). Hydrolysis of the acetate groups followed by regioselective introduction of the 4'-6'-benzylidene group, acetylation and final acidic removal of the benzylidene moiety, rendered 2 with a yield of 41%. Regioselective benzylation of the 6'-hydroxyl function in 2 with 1-(benzyloxy)benzotriazole (17) and subsequent esterification with 1,1'-thiocarbonyldiimidazole rendered key intermediate 3 with a 53% yield. Barton deoxygenation (18) of 3 was accomplished by treatment with tributyltin hydride and a catalytic amount of azoisobutyronitrile in toluene, yielding 4'-deoxyheptaacetyl chitobioside (4) with a 72% yield. Subsequent chlorination to 5, followed by condensation with sodium 4-methylumbelliferonate, and final de-O-esterification (19) rendered homogeneous 4-MU-(4'-deoxy)chitobiose, compound 6, with a 20% yield.

RESULTS

Transglycosylation—The complex relationship of increasing concentrations of 4-methylumbelliferyl chitobiose, triose, and

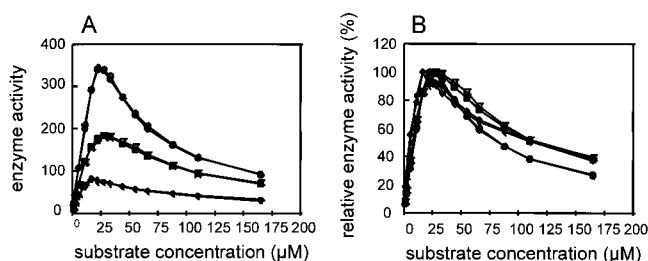


FIG. 1. Comparison of chitotriosidase activity with different 4-MU-(GlcNAc) substrates. The hydrolysis of 4-MU-chitobiose (\circ), 4-MU-(GlcNAc)-chitotriose (\square), and 4-MU-(GlcNAc)-tetraose (\diamond) substrates by 39- and 50-kDa isoforms of recombinant chitotriosidase was compared at different substrate concentrations. A, absolute values for enzyme activity; B, relative activities defined as percentage of maximal activity (closed symbols, 39-kDa chitotriosidase; open symbols, 50-kDa chitotriosidase).

tetraose substrates with the rate of fluorescent (4-MU; 4-methylumbelliferone) product formation is depicted for pure recombinant chitotriosidase (Fig. 1). It can be seen that at higher substrate concentrations less product is formed, nearly independent of the chemical nature of the substrate. Identically shaped curves were obtained for the corresponding *p*-nitrophenyl substrates and the rate of colored *p*-nitrophenol formation (not shown). This suggests that the concentration of *N*-acetylglucosamine moieties at the non-reducing end of the substrate molecules is somehow critical. The finding prompted us to examine the possibility of some transglycosylation event involving these moieties. For this purpose, pure recombinant chitotriosidase was incubated with 4-methylumbelliferyl- β -*N*-acetylglucosamine, *p*-nitrophenyl chitobiose, or a combination of both compounds. Fig. 2 shows that, as expected, no fluorescent 4-MU is formed upon incubation of enzyme with both compounds separately. In sharp contrast, incubation with the mixture of both compounds results in formation of fluorescent 4-MU after a lag phase. Transglycosylation offers an attractive explanation for the outcome of the experiment, as schematically presented in Fig. 2 (right side). As an initial step, chitotriosidase removes a chitobiose moiety from the PNP-chitobiose substrate. Next, the oxazolinium transition state intermediate may either use H₂O or the hydroxyl at the C-4 position in 4-MU-*N*-acetylglucosamine as acceptor. The latter reaction results in formation of 4-MU-chitotriose, which after some time is cleaved by chitotriosidase to yield fluorescent 4-MU.

To investigate the ability of chitotriosidase to transglycosylate natural chitooligosaccharides, pure recombinant enzyme was incubated with 4-methylumbelliferyl- β -*N*-acetylglucosamine and chitopentaose of *p*-nitrophenyl chitobiose. 10-fold larger amounts of fluorescent 4-MU are formed in the presence of chitopentaose as compared with *p*-nitrophenyl chitobiose, indicating that the natural oligosaccharide can act as donor in the transglycosylation reaction.

Fig. 3 shows that upon incubation of 5 mM chitopentaose with recombinant chitotriosidase, not only chitobiose and chitotriose, but also chitotetraose, and even chitohexaose and chitoheptaose, are formed. Since chitotriosidase is unable to remove or add a single *N*-acetylglucosamine moiety, formation of chitotetraose demonstrates that chitotriosidase can exert transglycosidase activity toward chitooligosaccharides. The formation of small amounts of chitoheptaose points in the same direction. The total recovery of glucosamine units in the experiment is virtually 100%. It was noticed that meanwhile unequal amounts of chitobiose and chitotriose are formed. Apparently, the transglycosidase activity of chitotriosidase is very considerable, and predominantly chitobiose is donated to accepting chitooligosaccharides.

¹ The abbreviations used are: 4-MU, 4-methylumbelliferyl; PNP, *p*-nitrophenyl; HPLC, high performance liquid chromatography; AMCCase, acidic mammalian chitinase.

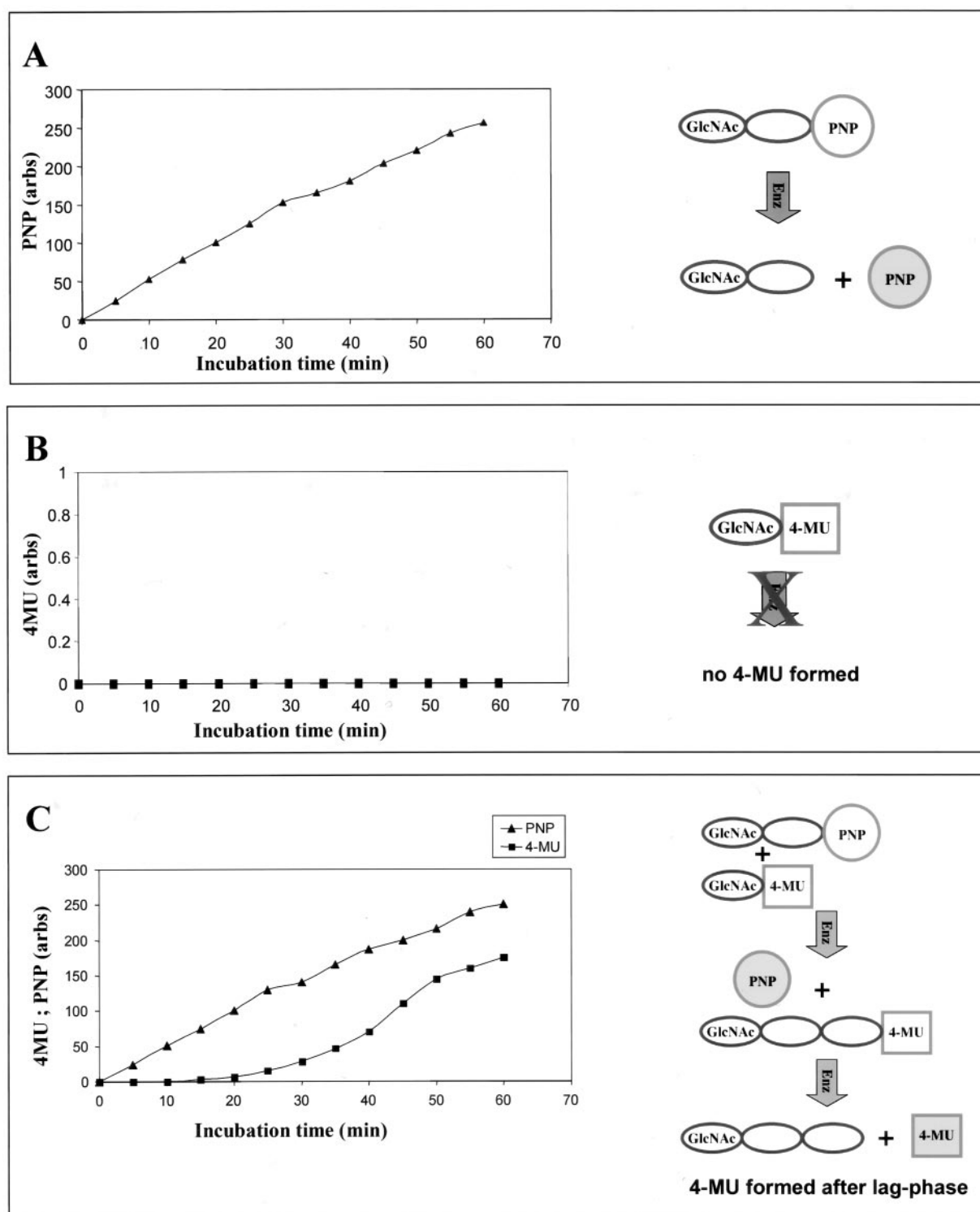


FIG. 2. **Demonstration of transglycosylation with artificial chitooligosaccharides.** *Left-hand side*, recombinant 50-kDa chitotriosidase was incubated for different time periods with 2.7 mM PNP-chitobiose (A), with 1.6 mM 4-MU-*N*-acetylglucosamine (B), or with the mixture of both substrates (C). Released 4-MU (■) and PNP (▲) were detected as described under "Materials and Methods." *arbs*, arbitrary units. *Right-hand side*, explanation of result.

The apparent substrate inhibition with 4-MU-chitooligosaccharide substrates can be fully explained by the transglycosidase capacity of chitotriosidase (see Scheme 1). With increasing concentration of 4-MU-chitobiose there is increasing formation of 4-MU-chitotetraose by transglycosidase activity. Chitobiose units can be subsequently transferred from 4-MU-chitotetraose to 4-MU-chitobiose molecules, a futile cycle generating no new products and not releasing fluorescent 4-MU. The experi-

menter observes this process as inhibition of enzyme activity, since that is monitored by formation of fluorescent 4-MU.

Design and Synthesis of Novel Substrate—The realization that ongoing transglycosylation is the cause of the problems encountered with the measurement of enzymatic activity of chitotriosidase using artificial chitooligosaccharide substrates stimulated us to design a novel substrate molecule that is lacking a hydroxyl at the C-4 position of the non-reducing

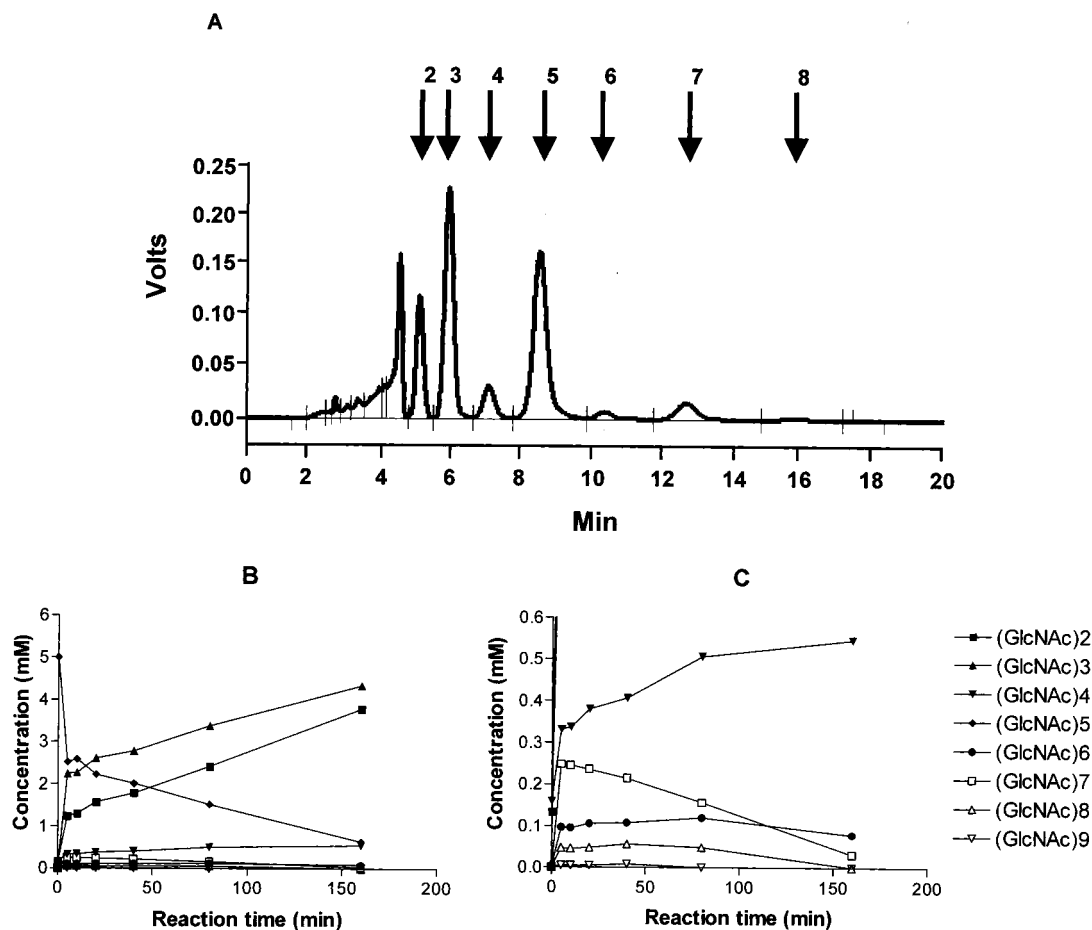
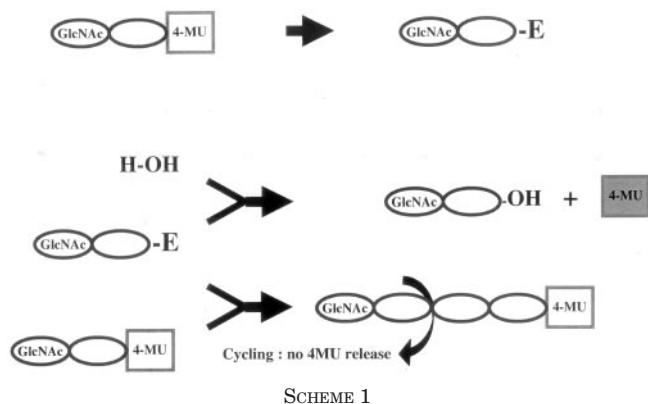


FIG. 3. Demonstration of natural chitooligosaccharide as donor and acceptor for transglycosylation process. Recombinant 50-kDa chitotriosidase was incubated for different time periods with 5 mM chitopentaose in 50 mM acetate buffer, pH 5.2. Reactions were stopped by placing samples in boiling water. A, example of HPLC profile (40-min time incubation). B, Overview of concentrations of all chitooligosaccharides. C, magnification for less abundant chitooligosaccharides.



SCHEME 1

N-acetylglucosamine. A novel compound, 4-methylumbelliferyl-*N*-acetylglucosamine-4,1-(4-deoxy)-*N*-acetylglucosamine (4-MU-(4-deoxy)chitobiose) was synthesized, exactly as described under "Materials and Methods" (see Fig. 4 for its chemical structure and route of synthesis). We envisioned that this compound should only be able to act as substrate for hydrolysis but not as acceptor for transglycosylation. Fig. 5 shows for pure recombinant chitotriosidase the relationship between the rate of fluorescent 4-MU formation and the concentration of 4-MU-chitobiose and 4-MU-(4-deoxy)chitobiose, respectively. Normal Michaelis-Menten kinetics occurs with 4-MU-deoxychitobiose as substrate, sharply contrasting to the marked substrate inhibition with 4-MU-chitobiose. This finding is in accordance

with the fact that the novel substrate 4-MU-deoxychitobiose cannot undergo transglycosylation.

Application of Novel Chitotriosidase Assay—The optimal enzyme activity assay conditions were determined for the novel deoxy-substrate. Maximal activity of recombinant chitotriosidase toward the substrate occurs at pH 5.6 with an apparent K_m of about 50 micromolar. An assay mixture composed of 114 μM of the deoxy-substrate in 0.1/0.2 M McIlvaine buffer, pH 5.6, allows the measurement of enzyme activity at maximal rate. Production of 4-MU is directly proportional to enzyme input and linear in time over a broad range. The standard assay mixture described above also allows reliable detection of the endogenous chitotriosidase in biological samples like plasma, serum, cerebral spine fluid, urine and extracts of blood cells, cultured macrophages, and tissues (not shown). This was further substantiated by the spiking of increasing amounts of recombinant chitotriosidase in the various biological samples. Linearity with time and proportionality to sample input were also examined.

For reason of maximal sensitivity, subsaturating concentrations of 4-MU-chitobiose or 4-MU-chitotriose had to be used in enzymatic assays (1). Although the intra-assay reproducibility with the published method is satisfactory, the inter-assay reproducibility is intrinsically poor due to unavoidable differences in precise substrate concentration. Moreover, the proportionality with enzyme input of such assays is relatively limited. In comparison with enzyme assays with the conventional 4-MU-chitobiose or 4-MU-chitotriose substrate, the enzyme as-

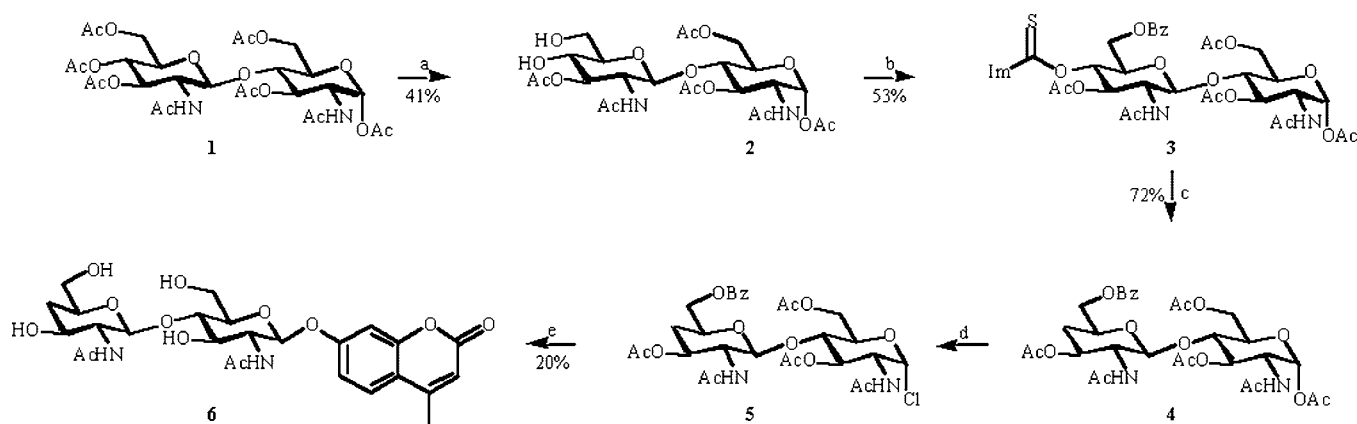


FIG. 4. **Synthesis of 4-MU-(4'-deoxy)-chitobiose.** Structure formulas of starting compound (octa-acetylchitobioside) and intermediates (see "Materials and Methods" for details).

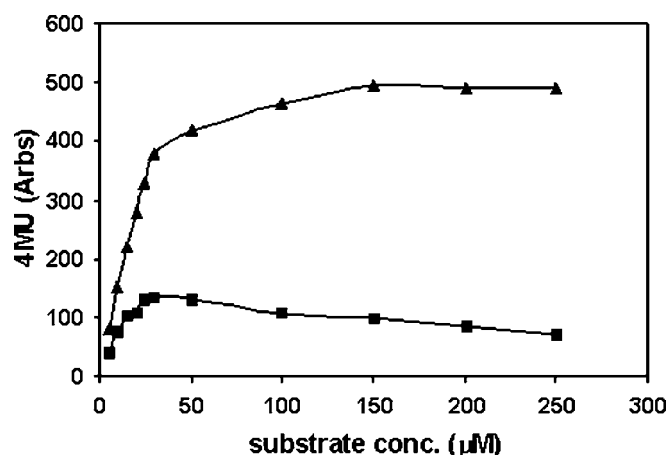


FIG. 5. **Chitotriosidase activity toward 4-MU-chitobiose and its deoxy variant.** Human recombinant chitotriosidase was incubated for 15 min with increasing concentrations of 4-MU substrates, and released 4-MU was detected fluorometrically as described under "Materials and Methods." Activity toward 4-MU-chitobiose (■), activity toward 4-MU-deoxy-chitobiose (▲) expressed in arbitrary units (*Arbs*).

say with the novel deoxy-substrate is far more sensitive, linear in time, and directly proportional to enzyme input over a far larger range (see Fig. 6 for an illustration).

DISCUSSION

Nowadays plasma chitotriosidase activity levels are determined to assist in the clinical management of Gaucher patients. Plasma chitotriosidase has evolved as an important tool in decision making in the clinic. For example, initiation of costly enzyme replacement therapy for Gaucher patients is considered in many centers when the plasma chitotriosidase level exceeds a critical threshold level and changes in activity level serve as guideline for optimization of dosing regimens during enzyme replacement therapy of Gaucher patients (8, 14). Obviously, the application of plasma chitotriosidase measurements for these purposes requires highly reliable and reproducible data while monitoring patients over a large period of time. The intrinsic limitation of the current chitotriosidase activity measurement with non-constant and non-saturating concentrations of 4-MU-chitobiose or 4-MU-chitotriose as substrates is the difficulty to ensure that the measured reaction rate is reproducibly proportional to enzyme concentration (1). In our experience, the only way this can be (nearly) accomplished when using the conventional 4-MU-chitooligosaccharide substrates is to vary enzyme input meticulously to obtain a fixed amount of product formation in a defined assay time period. This laborious approach implies that a sample has to be

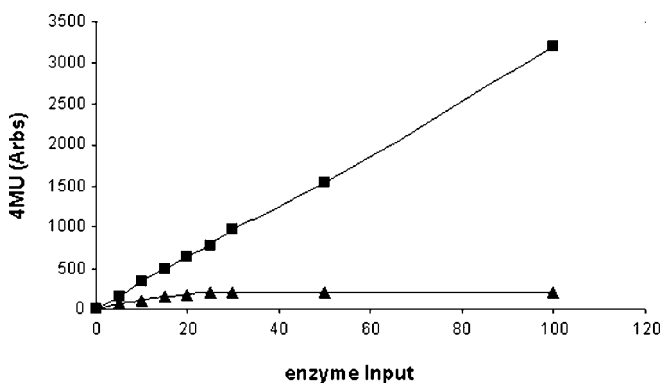
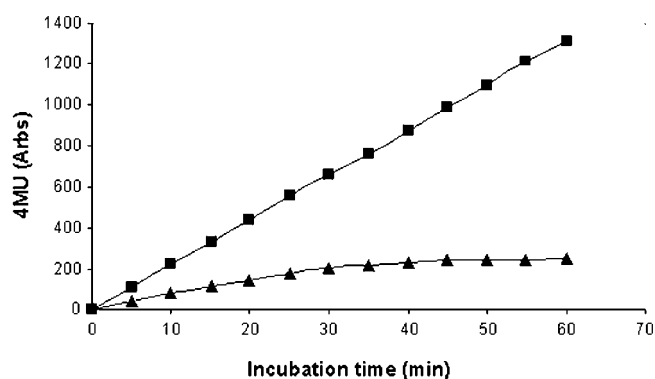


FIG. 6. **Linearity of enzyme activity with time and enzyme input.** Human recombinant chitotriosidase was incubated with 4-MU-chitobiose or 4-MU-deoxychitobiose. Incubation time (*upper panel*) and enzyme input (*lower panel*) were varied (indicated as μ l of recombinant chitotriosidase stock solution). Activity was measured by fluorometrical detection of released 4-MU. *Arbs*, arbitrary units.

measured in duplicate at various dilutions. In sharp contrast, the assay with the novel substrate allows measurement at maximal reaction rate that is continuously proportional to enzyme concentration. The novel assay is therefore far more robust and does not require the tedious precautions associated with the current assay. A significant additional advantage of the assay with the novel substrate is the \sim 5-fold increase in sensitivity of detection. This is extremely helpful for diagnostic applications in relation to a variety of disease conditions with relatively modest (2–10-fold) plasma chitotriosidase elevations like sarcoidosis, thalassemia, and lysosomal lipid storage disorders.

Our demonstration of a marked transglycosidase capacity of chitotriosidase is not entirely unexpected based on some very recent literature reports. During the course of our investigation it was described that family 18 chitinases from plants and microbes are to a variable extent able to catalyze transglycosylation reactions. A comparative study revealed that plant family 18 chitinases have a (-4)(-3)(-2)(-1)(+1)(+2)-type binding cleft, while the microbial family 18 chitinases show a (-2)(-1)(+1)(+2)(+3)(+4)-type binding cleft. Transglycosylation is found in the latter, but not in the former. Soaking of human chitotriosidase crystals with chitooligosaccharides has so far not been informative in this respect. Despite carrying out the experiments at pH 10.6 hydrolysis occurred, rendering electron density maps where only an ordered *N*-acetylglucosamine dimer could be observed at subsites -2 and -1 (4). The production of recombinant human chitotriosidase with one key active site residue, Glu-140, mutated to Asp or Leu has been earlier described by us (20). The inactive mutant protein has retained its ability to bind chitooligosaccharides in the catalytic cleft. The soaking of crystals of inactive mutant chitotriosidase with chitooligosaccharides should render a better insight in reaction mechanism and structural factors controlling enzyme function.

It will be of interest to study in greater detail the prerequisites in the catalytic center of chitotriosidase to act as a transglycosidase. Informative should also be a comparison of chitotriosidase with a highly related, human gastrointestinal chitinase (21). The enzyme was very recently identified and characterized by us and named acidic mammalian chitinase (AMCase) (21). Despite the high degree of homology with the macrophage chitotriosidase, AMCase has a number of distinct features. Interestingly, AMCase uniquely shows a dual pH optimum for hydrolysis. A second optimum of hydrolytic activity occurs at very low pH (about pH 2.0). AMCase shows a different ratio of rates of hydrolysis and transglycosylation of chitooligosaccharides. Particularly at low pH the enzyme seems less able to act as transglycosylase. This observation is consistent with the present assumption that AMCase predominantly fulfils a role as food-processing hydrolase in the stomach.

The results of our investigation raise intriguing questions about the evolutionary origin and physiological significance of the transglycosidase capacity of chitotriosidase. The human chitotriosidase gene has clearly evolved from a chitinase gene in primitive organisms, and many elements have been conserved among species during evolution (10). It is conceivable, although highly speculative, that transglycosidase activity of the primitive chitinase of the ancestral unicellular organisms allowed biosynthetic formation of increasingly larger chitooligosaccharide polymers near their surface. Prior to the evolution of the more complex, biosynthetic oligosaccharide transferases using activated sugars, the enzyme could thus have played a role in the formation of the first generations of protective coats.

An interesting aspect of the transglycosidase activity of chitotriosidase is the ongoing co-formation of chitotetraose while chitin is degraded. Chitotetraose is a structure that recently receives considerable attention. Studies in plants have revealed that chitotetraose may act as signaling molecule that plays important regulatory roles (see for example Ref. 22 for a recent review). Moreover there are reports that chitotetraose may influence embryogenesis in zebra fish (23, 24). In view of

this it might be speculated that also in man a challenge with some chitin-containing organism could result in local generation of chitotetraose and subsequently elicit further responses of local cells and the immune system. In this connection it will be of interest to examine more closely in animals the effects of administration of chitotetraose.

At present it is generally believed that man completely lacks endogenous chitin and also endogenous substrates for chitinases (see for example Ref. 25). Our study, however, raises the question as to whether endogenous compounds act as (temporary) acceptors for chitobiose moieties transferred by chitotriosidase from chitin-containing infectious organisms. In this manner endogenous materials and cells could become decorated with chitobiose motives. Such motives could act as a marker and play a role in local remodeling events that often occur in sites of infections. In connection with this it will be of great importance to investigate in more detail the potential existence of endogenous acceptors of chitobiose moieties. Such studies should help to reveal whether the transglycosidase activity of chitotriosidase is just an evolutionary remnant or still has a physiological function in man.

Acknowledgments—We thank Daan van Aalten, Sonja van Weely, and Carla Hollak for stimulating discussions and Gabor Linthorst, Marri Verhoek, and Anneke Strijland for technical assistance.

REFERENCES

- Hollak, C. E. M., van Weely, S., van Oers, M. H. J., and Aerts, J. M. F. G. (1994) *J. Clin. Invest.* **93**, 1288–1292
- Renkema, G. H., Boot, R. G., Muijsers, A. O., Donker-Koopman, W. E., and Aerts, J. M. F. G. (1995) *J. Biol. Chem.* **270**, 2198–2202
- Boot, R. G., Renkema, G. H., Strijland, A., van Zonneveld, A. J., and Aerts, J. M. F. G. (1995) *J. Biol. Chem.* **270**, 26252–26256
- Fusetti, F., von Moeller, H., Houston, D., Rozeboom, H. J., Dijkstra, B. W., Boot, R. G., Aerts, J. M., and van Aalten, D. M. (2002) *J. Biol. Chem.* **277**, 25537–25544
- Davies, G. J., Mackenzie, L., Varrot, A., Dauter, M., Brzozowski, A. M., Schulein, M., and Withers, S. G. (1998) *Biochemistry* **37**, 11707–11713
- Terwisscha van Scheltinga, A. C., Armand, S., Kalk, K. H., Isogai, A., Henrissat, B., and Dijkstra, B. W. (1995) *Biochemistry* **34**, 15619–15623
- van Aalten, D. M., Komander, D., Synstad, B., Gaseidnes, S., Peter, M. G., and Eijssink, V. G. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 8979–8984
- Aerts, J. M., and Hollak, C. E. (1997) *Baillieres Clin. Haematol.* **10**, 691–709
- Renkema, G. H., Boot, R. G., Strijland, A., Donker-koopman, W. E., van den Berg, M., Muijsers, A. O., and Aerts, J. M. F. G. (1997) *Eur. J. Biochem.* **244**, 279–285
- Boot, R. G., Renkema, G. H., Verhoek, M., Strijland, A., Blik, J., de Meulemeester, T. M., Mannens, M. M., and Aerts, J. M. (1998) *J. Biol. Chem.* **273**, 25680–25685
- Choi, E. H., Zimmerman, P. A., Foster, C. B., Zhu, S., Kumaraswami, V., Nutman, T. B., and Chanock, S. J. (2001) *Genes Immun.* **2**, 248–253
- Guo, Y., He, W., Boer, A. M., Wevers, R. A., de Bruijn, A. M., Groener, J. E., Hollak, C. E., Aerts, J. M., Galjaard, H., and van Diggelen, O. P. (1995) *J. Inher. Metab. Dis.* **18**, 717–722
- Barone, R., Di Gregorio, F., Romeo, M. A., Schiliro, G., and Pavone, L. (1999) *Blood Cells Mol. Dis.* **25**, 1–8
- Mistry, P. K., and Abrahamov, A. (1997) *Baillieres Clin. Haematol.* **10**, 817–838
- Inaba, T., Ohgushi, T., Iga, T., and Hasegawa, E. (1984) *Chem. Pharm. Bull.* **32**, 1597–1603
- Shaban, M., and Jeanloz, R. W. (1971) *Carbohydr. Res.* **19**, 311–318
- Kim, S., Chang, H., and Kim, W. J. (1985) *J. Org. Chem.* **50**, 1751–1752
- Barton, D. H. R., and McCombie, S. W. (1975) *J. Chem. Soc. Perkin Trans. I*, 1574–1585
- Delmotte, F. M., Privat, J.-P. D. J., and Monsigny, M. L. (1975) *Carbohydr. Res.* **40**, 353–364
- Renkema, G. H., Boot, R. G., Au, F. L., Donker-Koopman, W. E., Strijland, A., Muijsers, A. O., Hrebicek, M., and Aerts, J. M. (1998) *Eur. J. Biochem.* **251**, 504–509
- Boot, R. G., Blommaart, E. F., Swart, E., Ghauharali-van der Vlugt, K., Bijl, N., Moe, C., Place, A., and Aerts, J. M. (2001) *J. Biol. Chem.* **276**, 6770–6778
- Bakkers, J., Kijne, J. W., and Spaink, H. P. (1999) *EXS (Basel)* **87**, 71–83
- Bakkers, J., Semino, C. E., Stroband, H., Kijne, J. W., Robbins, P. W., and Spaink, H. P. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 7982–7986
- Semino, C. E., and Allende, M. L. (2000) *Int. J. Dev. Biol.* **44**, 183–193
- Shahabuddin, M., and Kaslow, D. C. (1993) *Parasitol. Today* **9**, 252–255

**Transglycosidase Activity of Chitotriosidase: IMPROVED ENZYMATIC ASSAY
FOR THE HUMAN MACROPHAGE CHITINASE**

Begoña Aguilera, Karen Ghauharali-van der Vlugt, Mariette T. J. Helmond, Jos M. M. Out, Wilma E. Donker-Koopman, Johanna E. M. Groener, Rolf G. Boot, G. Herma Renkema, Gijs A. van der Marel, Jacques H. van Boom, Hermen S. Overkleeft and Johannes M. F. G. Aerts

J. Biol. Chem. 2003, 278:40911-40916.

doi: 10.1074/jbc.M301804200 originally published online July 30, 2003

Access the most updated version of this article at doi: [10.1074/jbc.M301804200](https://doi.org/10.1074/jbc.M301804200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 23 references, 7 of which can be accessed free at <http://www.jbc.org/content/278/42/40911.full.html#ref-list-1>