Identification of a Novel Acidic Mammalian Chitinase Distinct from Chitotriosidase*

Rolf G. Boot‡‡§, Edward F. C. Blommaart‡, Erwin Swart‡, Karen Ghauharali-van der Vlugt‡, Nora Bijl‡, Cassandra Moe‡, Allen Place‡, and Johannes M. F. G. Aerts‡

From the ‡Department of Biochemistry, University of Amsterdam, Academic Medical Center, 1105 AZ Amsterdam, The Netherlands and the ¶Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, Maryland 21202

Chitinases are ubiquitous chitin-fragmenting hydro-}

Received for publication, October 30, 2000
Published, JBC Papers in Press, November 20, 2000, DOI 10.1074/jbc.M009886200

Next to cellulose, chitin is the most abundant glycopolyp}
Identification of an Acidic Mammalian Chitinase

Measurements of chitin activity were performed as described under “Experimental Procedures.” Chitinolytic activity was measured using 4MU-chitin substrate. The enzyme activity present in the different isoelectric focusing fractions is expressed as a percentage of the total activity present in all fractions.

Crab shell chitin (Poly- (1–4)-β-D-N-acetylglucosamine, Sigma) was used as a natural substrate to determine chitinase activity as described (10). The chitin fragments were analyzed by fluorophore-assisted carbohydrate electrophoresis as described by Jackson (15).

Degradation of Fungal Cell Wall Chitin—Measurements of chitin formation during regeneration of fungal spheroplasts was performed as described by Hector and Braun (16). Briefly, spheroplasts were prepared from the Candida albicans strain CAi-4 (ura3), grown overnight in YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 28 °C. Cells were concentrated by centrifugation and incubated with 2.5 mg/ml zymolyase (100T, ICN Immuno Biologicals, Costa Mesa, CA) in buffer containing 50 mM sodium phosphate, pH 7.5, 1.2 M sorbitol, and 27 mM β-mercaptoethanol for 60 min at 37 °C. After extensive washing, spheroplasts were allowed to regenerate in 96-well microtiter plates in regeneration buffer (0.25% (w/v) MES buffer, pH 6.7, containing 0.17% (w/v) yeast nitrogen base (without amino acids and ammonium sulfate; Sigma), 0.15% (w/v) ammonium sulfate, 2% (w/v) glucose, 1.2 M sorbitol, 20 μg/ml uridine) at 37 °C. Chitinase enzyme preparations were added to a final concentration of 3 μg/ml. After a 2-h incubation, 50 μl of 300 μg/ml Calcofluor white (Sigma) in 10 mM sodium phosphate buffer, pH 7.5, containing 1.2 M sorbitol was added. After 5 min the plates were washed with buffer only, and fluorescence was determined using a LS 50 Perkin Elmer fluorimeter (excitation, 405 nm; emission, 450 nm).

Purification of the Mouse AMCase—Detergent-free extracts of mouse tissues were prepared by homogenization in 10 volumes of potassium phosphate buffer, pH 6.5, using an Ultra-turrax and centrifugation for 20 min at 15,000 × g. The mouse intestine extract was adjusted to pH 5.0 by the addition of citric acid (0.2 M); NaCl was added to a final concentration of 2 M. A chitin column was prepared by mixing 10 g of colloidal chitin, followed by equilibration with phosphate-buffered saline containing 2 mM NaCl with a flow speed of 0.4 ml/min. After extensive washing, bound chitinase was eluted from the column with 8M urea, which was subsequently removed by dialysis. Protein concentrations were determined according to the method of Lowry et al. (17) using bovine serum albumin as a standard. Fractions containing chitinase activity were subjected to SDS-PAGE and Western blotting as described (8). N-terminal protein sequencing was performed as described using a Procise 494 sequencer (Applied Biosystems Perkin Elmer) (8). Colloidal chitin was prepared as described by Shimahara and Takiguchi (18).

SDS-PAGE and Glycol-Chitin Gel Electrophoresis—SDS-PAGE was performed with an Amersham Pharmacia Biotech phast gel system, according to the instructions of the manufacturer, using 12.5% polyacrylamide gels, followed by silver staining. Glycol-chitin electrophoresis was conducted as described by Escott and Adams (19), except for an extension of the renaturation time to 8 h. Glycol-chitin was prepared from glycol chitosan (Sigma) as described by Trudel and Asselin (20).

Isoelectric Focusing—The native isoelectric point of chitinases was determined by flat bed isoelectric focusing in granulated Ultrogel dex (Amersham Pharmacia Biotech) as described (8).

Northern Blot and RNA Master Blot Analysis—Total RNA was isolated using RNAzol B (Bisolve, Barneveld, The Netherlands) according to the instructions of the manufacturer. Northern blots, using 15 μg of total RNA, were performed as described (9). Human and mouse RNA Master Blots (CLONTECH, Palo Alto, CA) were used to examine the tissue distribution of transcripts according to the instructions of the manufacturer. The following probes were used: the full-length mouse acidic chitinase cDNA, the human EST clone oq35c04.s1 (GenBank accession number AA976830) and glyceraldehyde-3-phosphate dehydrogenase as control. Radiolabeling and hybridization was conducted as described previously (9). Quantification of radioactivity was performed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

cDNA Cloning of the Mouse AMCase—Reverse transcription polymerase chain reaction (PCR) fragments were generated from mouse lung total RNA using degenerate oligonucleotides, as described (9). Obtained fragments were cloned in pGem-T (Promega, Madison, WI), sequenced, and compared with the amino acid sequence established by N-terminal protein sequencing. A comparison with the GenBank™ mouse EST (expressed sequence tag) data base using the Basic local alignment search tool (BLAST) at the National Center for Biotechnology Information showed that several EST clones matched the mouse chitinase cDNA sequence, for example, ms33 h09 y1 (GenBank accession number AI892792). This clone was obtained and sequenced. Antisense primers were generated complementary to the most 3′ region of the EST clone (A tail primer, 5′-TTTTGCGTACCAATTTATTTG-3′) and two internal antisense primers (MAS1, 5′-CGATTAAGCCAGCAGTAC-3′ and MAS2, 5′-TCCGGTATCATGACAGC-3′). The MAS1 and MAS2 primers were used to clone the most 5′ end of the mouse acidic chitinase cDNA using 5′ rapid amplification of cDNA ends and the Marathon-Ready mouse Lung cDNA kit (CLONTECH) according to the instructions of the manufacturer. To obtain the complete coding sequence a 5′ sense primer was generated (MS1, 5′-CGATGCGC-CAAGCTACTTCTCGT-3′). The total cDNA sequence was subsequently

FIG. 1. Isoelectric focusing profile of chitinolytic activity in mouse lung extract. Isoelectric focusing was performed as described under “Experimental Procedures.” Chitinolytic activity was measured using 4MU-chitin substrate. The enzyme activity present in the different isoelectric focusing fractions is expressed as a percentage of the total activity present in all fractions.
Identification of an Acidic Mammalian Chitinase

Fig. 2. Mouse AMCase cDNA sequence and deduced amino acid sequence. The cDNA sequence (GenBank™ accession number AF290003) is indicated by the upper sequence, and the deduced amino acid sequence is depicted below the nucleotide sequence. The characteristic hydrophobic signal peptide (amino acids 1–21) is underlined with a single line. The putative chitin binding domain (amino acids 426–473) is underlined with a double line. The hinge region separating the catalytic domain from the chitin binding domain is underlined with a dashed line. The part of the protein purified from mouse intestine that was determined by Edman sequencing is boxed.

Fig. 3. Degradation products with colloidal chitin as substrate. The fluorophore-assisted carbohydrate electrophoresis technique (described under “Experimental Procedures”) was used to visualize the cleavage products of recombinant human chitotriosidase and recombinant mouse AMCase using colloidal chitin as substrate. Lane 1, no enzyme added. Lane 2, products formed after incubation with 50-kDa recombinant human chitotriosidase and chitin. Lane 3, products formed with recombinant mouse AMCase and chitin. Lane 4, human chitotriosidase incubated without substrate. Lane 5, mouse AMCase incubated without substrate. Marker lane is indicated with M (sugar polymers are indicated on the right-hand side).

Fig. 4. Electrophoretic behavior of chitinases. A, purified recombinant human chitotriosidase and mouse AMCase were separated on a 12.5% SDS-PAGE gel in the presence or absence of a reducing agent and visualized by silver staining as described under “Experimental Procedures.” Lane 1, recombinant mouse AMCase under reducing conditions. Lane 2, recombinant human chitotriosidase under reducing conditions. Lane 3, recombinant mouse AMCase under nonreducing conditions. Lane 4, recombinant human chitotriosidase under nonreducing conditions. M indicates the molecular mass standards (mass (kDa) indicated at the left-hand side). B, the same purified recombinant enzymes as described in A were separated on a 10% SDS-PAGE gel containing glycol-chitin as described under “Experimental Procedures.” Chitinolytic activity was visualized as clearing zones in the gel. Lane 1, recombinant human 39-kDa chitotriosidase. Lane 2, recombinant human 50-kDa chitotriosidase. Lane 3, recombinant mouse AMCase (mass (kDa) indicated at the right-hand side).
Identification of an Acidic Mammalian Chitinase

RESULTS

To obtain more insight into the potential occurrence of multiple mammalian chitinases, tissues of mouse and rat were examined for chitinolytic activity using the chitin-like 4-methylumbelliferyl-β-chito-oligosaccharide substrates. In extracts of stomach and intestine, a high level of activity was detected, whereas extracts of lung, tongue, kidney, and plasma showed significant but lower activities. Isoelectric focusing of a mouse lung extract revealed a major peak of chitinolytic activity with pI 4.5, whereas minor peaks were found with pI levels of 5.5–6.5 (Fig. 1). Extracts of other mouse and rat tissues showed similar profiles of chitinolytic activity upon isoelectric focusing.

The mouse acidic chitinase activity was found to bind to chitin particles with high affinity. Chitin affinity chromatography was used to purify the enzyme, as described under “Experimental Procedures.” The procedure resulted in a 30,000-fold purification of an apparently homogeneous 50-kDa protein.

The observed rodent chitinase with acidic isoelectric point (pI 4.5 form) differs strikingly from human chitotriosidase which has an apparent neutral/basic pI.

The N-terminal amino acid sequence of purified acidic chitinase was determined (Fig. 2) and was found to be almost identical to that of other known members of the chitinase family. This amino acid sequence allowed the cloning of the corresponding full-length mouse acidic chitinase cDNA, as described under “Experimental Procedures.” The full-length cDNA predicts the synthesis of a 50-kDa protein with a characteristic signal peptide (Fig. 2). Expression of this cDNA in COS-1 cells led to the secretion of an 50-kDa active chitinase with a pI of 4.8.

The mouse acidic chitinase protein shows considerable sequence homology to human chitotriosidase. Comparison of the
amino acid sequence of both mature proteins revealed an identity of 52% and a similarity of 60%. Like the human chitotriosidase, the mouse enzyme is predicted to contain an N-terminal catalytic domain of about 39 kDa, a hinge region, and a C-terminal chitin binding domain (Fig. 2). The mouse acidic chitinase, like chitotriosidase, is predicted to lack N-linked oligosaccharides, explaining the observed absence of binding to concanavalin A (data not shown).

Several different assays revealed that the mouse acidic chitinase is able to degrade chitin and therefore has to be considered a true chitinase. Firstly, fluorophore-assisted carbohydrate electrophoresis analysis revealed that recombinant mouse chitinase, like chitotriosidase, releases mainly chitobioside fragments from chitin (Fig. 3). Secondly, like chitotriosidase and some other nonmammalian chitinases, the mouse acidic chitinase is strongly inhibited (IC50 of 0.4 \(\mu\)M) by the competitive chitinase inhibitor allosamidin (21–23). Finally, the mouse acidic chitinase and chitotriosidase were both able to digest chitin in the cell wall of regenerating spheroplasts of \(C.\) \textit{albicans}. The chitin content of the cell wall was determined with the Calcofluor white stain (see "Experimental Procedures"). When regenerating cells were incubated for 2 h with 3 \(\mu\)g/ml recombinant chitotriosidase or 3 \(\mu\)g/ml recombinant mouse acidic chitinase, the chitin content was reduced by 27 and 33%, respectively. Concomitant presence of allosamidin during the incubation completely abolished the effect of both recombinant chitinases.

A further striking difference between human chitotriosidase and the mouse acidic chitinase is their behavior at acidic pH. The mouse acidic chitinase shows a pronounced pH optimum at pH 2.3 and a less pronounced optimum at more neutral pH (pH 4–7). Chitotriosidase, however, shows only a broad pH optimum (Fig. 5A) and is completely inactivated by pre-incubation at low pH (Fig. 5B). In the presence of 0.5% (w/v) trichloroacetic acid 58% of chitotriosidase is precipitated, whereas under similar circumstances the mouse acidic chitinase remains in solution. At 2.5% (w/v) trichloroacetic acid all chitotriosidase precipitates, whereas 26% of mouse acidic chitinase remains unprecipitated (Fig. 5C).

Another major difference between human chitotriosidase and the mouse acidic chitinase is revealed by comparison of RNA expression patterns. Although human chitotriosidase mRNA is mainly found in lymph node, bone marrow, and lung, the mouse acidic chitinase mRNA is predominantly found in stomach, submaxillary gland, and, at a lower level, in the lung (Fig. 6). Surprisingly, no mouse acidic chitinase mRNA can be detected in the small intestine, suggesting that the protein in the intestine is probably derived from the upper parts of the gastrointestinal tract, such as the stomach.

In rat tissues a comparable acidic chitinase was observed. Our findings indicate that the acidic chitinase in rodents is distinct from human chitotriosidase. The discrete enzyme is therefore referred to as acidic mammalian chitinase or AMC-
ase. It was investigated whether such an acidic chitinase is also present in man. Screening the human EST database at the National Center for Biotechnology Information with the mouse acidic chitinase cDNA revealed the presence of a highly homologous human EST clone (oq35c04.s1, GenBank™ accession number AA976830). The tissue distribution of this human mRNA was examined using a human Masterblot (CLONTECH). The expression pattern of this mRNA is similar to the expression pattern of the mouse acidic chitinase (Fig. 7), being highly expressed in the stomach and at a lower level in the lung. Using degenerate oligonucleotides directed against members of the chitinase family, we were able to amplify other regions of the human acidic chitinase, generating enough information to clone the full-length human acidic chitinase cDNA (Fig. 8A). Screening the GenBank™ database using the full-length human cDNA revealed that it was almost identical to TSA1902-L (GenBank™ accession number AB025008) and TSA1902-S (GenBank™ accession number AB025009) from a lung cDNA library described by Saito et al. (24). These two sequences are most probably splice variants of the human acidic chitinase mRNA. Only expression of full-length human AMCase cDNA in COS-1 cells led to the production of a protein with chitinolytic activity (data not shown). Sequence comparison of the human acidic chitinase and the mouse acidic chitinase revealed an 82% identity and a similarity of 86% (Fig. 8B).

The demonstration by Saito et al. (24) that the gene encoding TSA1902 located on chromosome 1p13 indicates that mammals contain indeed at least two discrete genes that encode functional chitinases, being chitotriosidase (locus 1q32) and AMCase (locus 1p13). Definitive proof for the existence of at least two distinct, functional mammalian chitinase genes was recently obtained by the partial cloning of chitotriosidase cDNA from the rat. The cloned rat cDNA (80% of the complete cDNA) encodes a protein that is 80% identical to the human counterpart.

DISCUSSION

For many years the existence of chitinase has been well documented for a large variety of organisms, including bacteria, plants, insects, and fungi (for an overview see Ref. 6). More recently, it has become clear that mammals also contain such enzymes. Chitotriosidase was the first mammalian chitinase that had been cloned and characterized (7–9). Besides this human phagocyte-specific chitinase, several inactive members of the mammalian chitinase protein family have also been identified. These include oviduct-specific glycoprotein from several mammalian species (reviewed in Refs. 25–27), human HC gp39/YKL-40 (28, 29), mouse BRP39 (30), pig gp38K (31), human YKL-39 (32), and mouse YM1/ECFL/MCRP (33, 34). The functions of these proteins, of which some have been shown to express lectin-like properties (35), are at present unknown. It has been speculated that they might have a role in tissue remodelling processes (28) or chemotaxis (33, 36).

To our knowledge chitotriosidase is the only mammalian chitinase that has been cloned and characterized in detail so far. Our present study describes the discovery of a second acidic mammalian chitinase named AMCase. This enzyme is also able to degrade artificial chitin-like substrates as well as chitin from crab shell and chitin as present in the fungal cell wall.

Sequence homology, conservation of intron-exon boundaries and chromosomal location suggest that the genes of members of the mammalian chitinase protein family evolved from a common ancestor by duplication. This is also suggested by their structural similarities, in particular between AMCase and human chitotriosidase. Both are members of family 18 of glycosyl hydrolases, showing an 8-stranded α/β (TIM) barrel catalytic
core structure (37, 38). Like chitotriosidase, AMCase contains a N-terminal catalytic core domain of 39 kDa and a C-terminal chitin binding domain separated by a hinge region (11). An ongoing crystallographic study on the three-dimensional structures of human chitotriosidase and AMCase (collaboration with F. Fusetti and B. Dijkstra from the University of Groningen, The Netherlands) should answer some intriguing questions. For example, the molecular basis for the profound differences in stability and catalytic capacity at low pH between the enzymes has to be resolved. It will also be of interest to establish whether the difference in migration of the two enzymes upon SDS-PAGE at nonreducing conditions is caused by differences in disulfide bonds. All 10 cysteines residues in chitotriosidase are conserved in mouse AMCase. The primary amino acid sequence of mouse AMCase shows the presence of 2 additional cysteines in the catalytic core, which are conserved in the human AMCase. Tjoelker et al. (11) have recently shown that all 6 cysteines in the chitin-binding domain of human chitotriosidase are involved in disulfide bonds within this domain and are essential for lectin activity.

In view of our observation that mouse and human AMCase mRNA is highly expressed in the stomach, the noted acidic pH optimum and profound acid stability of AMCase is not surprising. The extreme environment in these parts of the gastrointestinal tract requires such special features. The fact that no AMCase mRNA was detected in the intestine suggests that the protein present in these lower parts of the gastrointestinal tract may originate from the stomach and submaxillary glands. However, AMCase EST clones have been identified in the mouse caecum, tongue, and pancreas recently, indicating that several additional parts of the gastrointestinal tract are involved in the generation of AMCase. Whether the observed chitinase activity in the saliva of patients with periodontal inflammation described by van Steijn et al. (39) can be ascribed to AMCase remains to be established.

We also observed that AMCase mRNA is expressed in the lung (although to a lesser extent than in the stomach) and that enzyme activity is detectable there. At present the exact cellular sources of AMCase are unknown. Recently Guoping et al. (40) identified a silica-induced bronchoalveolar lavage protein with fibroblast growth promoting activity in the rat. This protein is identical to the AMCase we isolated from the rat. It has been shown that the protein could be identified in alveolar macrophages of silicotic rats (40), suggesting that at least in the rat lung this enzyme could be generated by macrophages. However, we have been unable to demonstrate any chitinolytic activity in rat alveolar macrophages (not shown). This could indicate that alveolar macrophages are only capable of producing AMCase under a specific stimulus. Moreover, we have also not observed any expression of AMCase in human monocyte-derived macrophages, even under conditions when the cells massively produce chitotriosidase. A detailed characterization of the promoter regions of AMCase and chitotriosidase is required to understand the selective expression of these enzymes. In situ hybridization analysis has to reveal which cells in the respiratory and gastrointestinal tract can express AMCase.

For several vertebrates and invertebrates of lower classes the presence of chitinase activity in the gastrointestinal tract has been reported (for an overview see Refs. 6, 41, and 42). This activity has sometimes been ascribed to the microorganisms present in the tract. However, gut chitinases have been cloned from several insect species and are thought to be involved in maintenance of the peritrophic matrix (43–45). The peritrophic matrix is a chitinous extracellular layer that surrounds a food bolus in the guts of most arthropods (46), providing a physical barrier to pathogens, facilitating digestion, and protecting against damage by food particles. Our study shows that, at least in rodents and man, a part of the chitinolytic activity found in the gut should be ascribed to an endogenous source also.

The presence of chitinase activity in vertebrates has actually been described earlier, but little is known about the corresponding proteins (6, 42, 47). Place (48) described the purification of a rainbow trout chitinase, which was isolated from the cardiac portion of the stomach. Comparison of the first 26 amino acids of this fish chitinase showed that it is 54% identical to mouse AMCase. Comparison of the complete sequence should reveal more information regarding the evolutionary relationship between the mammalian and fish stomach chitinases.

At present the physiological function of AMCase is unknown. Our study has revealed a remarkable parallel between chitinases and another group of endo-glucosaminidases, the lysozymes. It is well known that distinct lysozyme isoforms occur in various organisms. Lysozymes produced by phagocytes are basic proteins that fulfill a defense function by virtue of their ability to degrade the cell wall of Gram-negative bacteria. In the gastrointestinal tract of some species, acidic lysozymes are expressed that are acid stable and active at low pH. These enzymes are thought to function as food processors (49). By their action, the cell walls of bacteria that ferment plant materials are degraded, allowing the subsequent release and assimilation of their contents. It is conceivable that AMCase also plays a role in food assimilation as earlier proposed for fish chitinases by Lindsay (50), whereas the phagocyte-specific chitotriosidase is primarily involved in defense. The observation that AMCase is also expressed in the lung may point to a dual function for the enzyme, both in defense and food processing.

In ruminant artiodactyls, leaf-eating monkeys, and the bird hoatzin, lysozyme has been adapted by rapid convergent evolution to allow survival and functioning in the acidic, proteolytic environment of the stomach (51). These adaptations changed the global properties of the enzyme by a reduction of the isoelectric point so that the protein is neutral or acidic rather than basic and by a reduction in the number of acid labile bonds and side chains (51). Similar differences can be observed between chitotriosidase and AMCase, suggesting that the same kind of evolutionary processes played a role in chitinase adaptation.

Because AMCase is a functional chitinase, it is conceivable that the existence of AMCase in man has allowed the high panethic incidence of deficiency in chitotriosidase. It will be of great interest to study also in detail the precise composition of chitinases and their respective functions in lower vertebrates such as fish.

Our demonstration of a novel chitinolytic member of the mammalian chitinase family that might play an important role in defense and/or nutrition warrants further investigation. Re-
search on structural properties, regulation of expression, and the evolutionary relationship of the different members of the mammalian chitinase family could give insights into the physiological role of these interesting proteins.

Acknowledgments—We acknowledge Dr. Anton Muijsers, Marri Verhoek, Wilma Donker-Koopman, and Anneke Strijland for skillful technical assistance. Dr. S. van Weely, Dr. D. Speijer, and Dr. D. Blom are acknowledged for helpful comments and suggestions during the preparation of the manuscript. Dr. F. M. Klis, Dr. J. C. Kapteyn, and Dr. G. W. Gooday are acknowledged for helpful suggestions during the studies regarding the fungal cell wall.

REFERENCES

1. Debono, M., and Gordee, R. S. (1994) Annu. Rev. Microbiol. 48, 471–497
2. Fuhrman, J. A., and Piessens, W. F. (1985) Mol. Biochem. Parasitol. 17, 93–104
3. Araujo, A. C., Souto-Padron, T., and de Souza, W. J. (1993) Histo. Cytol. 41, 571–578
4. Neville, A. C., Parry, D. A., and Woodhead-Galloway, J. (1976) J. Cell Sci. 21, 73–82
5. Shahabuddin, M., and Kaslow, D. C. (1994) Exp. Parasit. 79, 85–88
6. Flach, J., Pilet, P. E., and Jolles, P. (1992) Experientia 48, 701–716
7. 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
8. Renkema, G. H., Boot, R. G., Muijsers, A. O., Donker-Koopman, W. E., and Aerts, J. M. F. G. (1997) J. Biol. Chem. 272, 2198–2202
9. 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
10. Renkema, G. H., Boot, R. G., Strijland, A. Denker-Koopman, W. E., and Aerts, J. M. F. G. (1996) J. Biol. Chem. 270, 2198–2202
11. Tjoelker, L. W., Gosting, L., Frey, S., Hunter, C. L., Trong, H. L., Steiner, B., Berg, M., Muijsers, A. O., and Aerts, J. M. F. G. (1997) Eur. J. Biochem. 244, 279–285
12. Tjepkema, L. W., Gosting, L., Frey, S., Hunter, C. L., Trong, H. L., Steiner, B., Berg, M., Muijsers, A. O., and Aerts, J. M. F. G. (1997) Eur. J. Biochem. 244, 279–285
13. Poulsen, D. D. C., and Gloer, L. E. (1996) FEBS Lett. 354, 1–11
14. van Royen, M. J., and Aerts, J. M. F. G. (1998) Genomics 45, 316–322
15. Semenza, G., and Fournier, S. (1999) Eur. J. Oral Sci. 107, 328–337
16. Guoping, C., Fan, P., Jingxi, S., Xiaoping, L., Shiqin, J., and Yuri, L. (1997) J. Biol. Chem. 272, 25680–25685
17. Jackson, P. (1990) Biochem. J. 270, 705–713
18. Heter, R. F., and Braun, P. (1986) J. Clin. Microbiol. 24, 620–624
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
20. Shimabara, K., and Takiguchi, Y. (1988) Methods Enzymol. 166, 417–423
21. Escofet, G. M., and Adams, D. J. (1995) Infect. Immun. 63, 4770–4773
22. Trudel, J. J., and Asselin, A. (1989) Anal. Biochem. 178, 362–366
23. Milewski, S., O’Donell, R. W., and Gooday, G. W. (1992) J. Gen. Microbiol. 138, 2545–2550
Identification of a Novel Acidic Mammalian Chitinase Distinct from Chitotriosidase
Rolf G. Boot, Edward F. C. Blommaart, Erwin Swart, Karen Ghausarali-van der Vlugt, Nora Bijl, Cassandra Moe, Allen Place and Johannes M. F. G. Aerts

J. Biol. Chem. 2001, 276:6770-6778.
doi: 10.1074/jbc.M009886200 originally published online November 20, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M009886200

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 46 references, 17 of which can be accessed free at http://www.jbc.org/content/276/9/6770.full.html#ref-list-1