REVIEW

Hyaluronidases – A group of neglected enzymes

GUNTHER KREIL

Institute of Molecular Biology, Austrian Academy of Sciences, Billrothstrasse 11, A-5020 Salzburg, Austria

(RECEIVED February 28, 1995; ACCEPTED July 3, 1995)

Abstract

Hyaluronan is an important constituent of the extracellular matrix. This polysaccharide can be hydrolyzed by various hyaluronidases that are widely distributed in nature. The structure of some bacterial and animal enzymes of this type has recently been elucidated. It could be shown that the hyaluronidases from bee and hornet venom and the PH-20 hyaluronidase present on mammalian spermatozoa are homologous proteins.

Keywords: hyaluronan; hyaluronidase; PH-20 protein

Hyaluronan (hyaluronic acid, HA) is a ubiquitous component of the extracellular matrix (ECM) of vertebrates. This linear polysaccharide is composed of a repeating unit with the structure [D-glucuronic acid(1- β -3)N-acetyl-D-glucosamine(1- β -4)]. HA can form highly viscous solutions and thereby influence the properties of this matrix. It has been implicated in many biological processes including fertilization, embryonic development, cell migration and differentiation, wound healing, inflammation, and growth and metastasis of tumor cells (Laurent & Frazer, 1992). HA interacts with a variety of receptors and binding proteins on the surface of cells, of which CD-44 and RHAMM have been characterized most extensively (Aruffo et al., 1990; Hardwick et al., 1992; Yang et al., 1994). It therefore seems likely that the formation and degradation of HA and the concomitant modulation of the physicochemical properties of the ECM have to be tightly controlled. However, until recently, none of the enzymes involved in HA synthesis and degradation have been studied in any detail. In the latter case, this is all the more surprising because partially purified preparations of hyaluronidase from mammalian testis and from other sources have been available from several companies.

This situation seems to be changing. Recently, the first HA synthase gene from a prokaryote has been sequenced (DeAngelis et al., 1993). This gene shows substantial similarity to a number of proteins in the sequence database (DeAngelis et al., 1994), including chitin synthases from yeast and DG42, a gene that is expressed early in the development of *Xenopus laevis* (Rosa et al., 1988). Recent evidence suggests that DG42 codes for an enzyme that catalyzes the synthesis of chitin-oligosaccharides (Semino & Robbins, 1995). The HA synthases of animal cells

have been only partially characterized (Klewes et al., 1993). These enzymes are probably located on the cytoplasmic side of the plasma membrane and the newly synthesized polysaccharide is transported directly to the extracellular space. As opposed to other components of connective tissue, HA does not traverse the different compartments of the secretory pathway. It seems likely that the results of DeAngelis et al. (1993, 1994) will soon be extended to HA synthases from animal cells.

In the past years, the first structures of prokaryotic and animal hyaluronidases have also been presented and it can thus be expected that our knowledge on the mechanism and control of HA turnover will increase rapidly in the near future. In this brief review, I am trying to summarize both the early work as well as recent findings on hyaluronidases (hyases) from vertebrates and insects.

Historical overview

Two independent lines of investigation led to the discovery of hyases. One area of research started with the observation by Duran-Reynals about 70 years ago that extracts from mammalian testes and other tissues contained a "spreading factor," a substance that facilitated the diffusion of antiviral vaccines, dyes, toxins, etc., injected subcutaneously (Duran-Reynals, 1928; see review by Meyer, 1971). The other area was the isolation and characterization of HA from vitreous humor (Meyer & Palmer, 1934). HA was subsequently isolated from many other sources and the structure, physicochemical properties, and biological role of this polysaccharide were studied in numerous laboratories. This work has been summarized in a Ciba Foundation Symposium (Laurent, 1989) and a recent review (Laurent & Frazer, 1992). It could soon be shown that the spreading factor present in extracts from testes and other sources was an enzyme degrading HA (Chain & Duthie, 1939). This work, sub-

Reprint requests to: Gunther Kreil, Institute of Molecular Biology, Austrian Academy of Sciences, Billrothstrasse 11, A-5020 Salzburg, Austria; e-mail gkreil@oeaw.ac.at.

sequently confirmed and extended by other groups, supplied a link between the biological phenomenon of diffusion in connective tissue and a defined enzymatic activity. Moreover, this work also demonstrated that mature mammalian testes contain high amounts of hyase.

In subsequent years, it was demonstrated that at least three types of hyases exist (see Table 1) and that they hydrolyze HA via different mechanisms (Meyer, 1971). The first group includes the endo- β -N-acetyl-D-hexosaminidases that degrade the high-molecular weight substrate to the tetrasaccharide as the main end product. In the case of the testicular enzyme, which is the prototype of this group, it has been shown that it also catalyzes transglycosylation reactions (Laurent, 1989; Cramer et al., 1994; Takagaki et al., 1994) so that hexa-, di-, and octasaccharides are also formed during hydrolysis of HA. A second type is represented by the hyase from leeches, which is a β -endoglucuronidase. Enzymes of this type may also be present in hookworms (Hotez et al., 1994). Lastly, the bacterial hyases are lyases that act via β -elimination, yielding disaccharides as the main end products. The sequence of the genes for two streptococcal enzymes of this type has recently been determined (Berry et al., 1994; B. Lin et al., 1994) and these are about 50% identical. Two additional prokaryotic hyases that are not related to the streptococcal enzymes have been characterized. One is from *Clostridium perfringens* (Canard et al., 1994) and the other from a bacteriophage isolated from a strain of *Streptococcus pyogenes* (Hynes & Ferretti, 1989). A variety of assays have been described to measure the activity of these different enzymes (see review by Hynes & Ferretti, 1994).

Hyases from animal venoms

Hyase activity has been detected in the venom of snakes, fish, bees, wasps, scorpions, spiders, etc. These enzymes supposedly serve as "spreading factors" by degrading HA, thereby facilitating the diffusion of other venom constituents. A few of these enzymes have been characterized to some extent (Tu & Hendon, 1983; Ramanaiah et al., 1990; Poh et al., 1992). In particular, the enzyme from bee venom has been investigated in detail. This is a

glycoprotein that was purified to homogeneity and its amino-terminal sequence was determined (Kemeny et al., 1984; Jacobsen et al., 1992). Based on these results, the amino acid sequence of bee venom hyase could be elucidated via cDNA cloning (Gmachl & Kreil, 1993). This enzyme is derived from a precursor containing a signal peptide and a short pro-segment. The mature hyase is composed of 349 amino acids including four cysteine residues. In the sequence, three potential sites for N-glycosylation are present. Expression of the cloned cDNA in *Escherichia coli* yielded a polypeptide with hyase activity. Recently, the amino acid sequence of the enzyme from hornet venom has also been determined via cDNA cloning (Lu et al., 1995). This hyase comprises 331 amino acids, of which 56% are identical to the bee venom hyase. Like other components of the venom of Hymenoptera, the hyases are potential allergens in susceptible individuals.

Testicular hyase

As already mentioned, it has been known for a long time that extracts from mammalian testes contain hyase activity. Preparations of partially purified hyases from bovine and ovine testis have for many years been available from several companies. It was thus surprising that no efforts had been made to characterize any of these enzymes in more detail. This was, in fact, the reason why it seemed of some interest to us to elucidate the sequence of the bee venom hyase (Gmachl & Kreil, 1993). It was then found that significant homology existed between this insect enzyme and a protein termed PH-20, which had earlier been detected with monoclonal antibodies (Primakoff & Myles, 1983). This protein is present on the posterior head and the acrosomal membrane of mammalian sperm. Via cDNA cloning, the amino acid sequence of the guinea pig protein could be elucidated (Lathrop et al., 1990). PH-20 is originally synthesized as a polypeptide with an apparent molecular weight of 64 kDa, and it is bound to the membrane via a glycosyl-phosphatidyl-inositol (GPI) anchor. In the course of sperm maturation, part of PH-20 is processed into two fragments, linked by disulfide bridges: an amino-terminal domain of 41–48 kDa, and a carboxy-terminal domain of 27 kDa. It has been shown that PH-20 plays an essential role in fertilization; guinea pigs immunized with their own PH-20 protein are sterile (Primakoff et al., 1988).

A comparison of the sequences of bee venom hyase and the guinea pig PH-20 protein revealed that these are 36% identical in a region encompassing about 300 amino acids. This raised the possibility that the PH-20 protein possesses hyase activity. This notion was corroborated for the human (Gmachl et al., 1993) and the monkey and mouse PH-20 proteins (Y. Lin et al., 1994). Expression of the cloned cDNAs encoding these proteins in rabbit kidney RK13 or insect cells yielded polypeptides with hyase activity. Moreover, treatment of rabbit kidney RK13 cells expressing the membrane-bound PH-20 protein with phosphoinositol-specific phospholipase C yielded a soluble enzyme with 5–10-fold higher activity (Gmachl et al., 1993). This may be due to the fact that the substrate, a polysaccharide with a molecular mass of up to several millions, may have only limited access to the membrane-bound enzyme. In addition, GPI-anchored proteins are known to form clusters on cell membranes (see e.g., Rothberg et al., 1990) and this could also be responsible for the lower specific activity of the enzyme.

Table 1. Enzymes that hydrolyze HA

A. Hyaluronate glycanohydrolase (EC 3.2.1.35)	
Testes-type hyase	
Endo- β -N-acetyl-D-hexosaminidases that degrade HA to the tetrasaccharide (GlcUA-GlcNAc-GlcUA-GlcNAc) ^a as the main product; also act on chondroitin sulfate.	
Present in mammalian spermatozoa, lysosomes, venoms of hymenoptera, snakes, etc.	
B. Hyaluronate glycanohydrolase (EC 3.2.1.36)	
Hyase from leech	
Endo- β -D-glucuronidase that degrades HA mainly to the tetrasaccharide (GlcNAc-GlcUA-GlcNAc-GlcUA).	
Present in the salivary gland of leeches.	
C. Hyaluronate lyase (EC 4.2.99.1)	
Bacterial hyases that act as N-acetyl-D-hexosaminidases by β -elimination yielding disaccharides that contain glucuronosyl residues with a double bond between carbons 4 and 5.	

^a GlcUA = glucuronic acid, GlcNAc = N-acetylglucosamine.

As mentioned earlier, extracts of mammalian testis contain hyase activity. Whether these soluble hyases are derived from or related to the PH-20 protein remains to be investigated. Using Northern blot analysis, the PH-20 mRNA could only be detected in testis (Y. Lin et al., 1993). Also, no evidence for the presence of PH-20 mRNA or related mRNAs in different mouse tissues and mouse embryos could be obtained (M.F. Meyer, unpubl. experiments). In this context, it is noteworthy that the mRNA encoding the bee venom enzyme could also be detected in testes from drones but not in other bee tissues (Gmachl & Kreil, 1993). Upon fertilization, mammalian sperm must first traverse a layer of cumulus cells that surround the egg. These cells are embedded in a matrix rich in HA (Salustri et al., 1992) that may be digested by the PH-20 hyase. Indeed, it has been shown (Y. Lin et al., 1994) that recombinant PH-20 protein can digest this outer layer. Inhibitors of this enzyme could thus block fertilization and thereby conceivably act as contraceptives.

Comparative aspects

Based on the homology between bee venom hyase and the PH-20 proteins, it seems likely that the enzymatic activity resides in the amino-terminal domain of the latter. A comparison of the sequence of bee and hornet venom hyases (Gmachl & Kreil, 1993; Lu et al., 1995) with those of human, monkey, mouse, and guinea pig PH-20 protein (Lathrop et al., 1990; Y. Lin et al., 1993, 1994; Gmachl et al., 1993) demonstrates that in a common region encompassing the 330–340 amino acids of the insect hyases, 57 are conserved in all of them. These include four cysteine residues forming two disulfide bridges. Moreover, it seems likely that the residues essential for substrate binding and catalysis are also conserved between the hymenopteran and mammalian enzymes. At present, nothing is known about the active site of hyases. However, by analogy with other enzymes that hydrolyze polysaccharides, certain assumptions can be made. Studies on the 3D structure of lysozyme (Philips, 1967), amylase (Mikami et al., 1994), xylanase (Törrönen et al., 1994), cellobiohydrolase (Divne et al., 1994), and other endoglycosidases (Van Roey et al., 1994) have all shown that two acidic amino acids, mostly two glutamic acid residues, are part of the active site. Of these, one acts as a nucleophile and the other as the acid-base catalyst. This has in some instances also been demonstrated by site-directed mutagenesis (Watanabe et al., 1993; Wakarchuk et al., 1994) and biochemical methods (Bray & Clark, 1994). In addition, it has been shown that the region where the polysaccharide binds to some of these enzymes is lined with the aromatic side chain of several tryptophan and tyrosine residues. Lastly, one can assume that, in the particular case of hyases, which act on a polysaccharide that is a polyanion, positively charged amino acids should be essential for binding the substrate to the enzyme. In keeping with these analogies, it is noteworthy that in the insect and the PH-20 hyases studied so far, six acidic, five basic, and nine tryptophan plus tyrosine residues have been conserved. Using the recombinant bee venom enzyme, we are currently testing by in vitro mutagenesis which of these are essential for catalytic activity.

Hyase from liver and serum

Hyase activity can be detected in many mammalian tissues. A rich source for this enzyme is the liver. HA and its fragments present in serum are taken up by the liver and degraded in ly-

sosomes. The liver hyase is a glycoprotein with a molecular mass of 55–70 kDa (Joy et al., 1985). A similar enzyme has been purified from serum (Afify et al., 1993). This enzyme has a pH optimum of 3.7 and it may thus represent a lysosomal enzyme that is secreted to some extent. It has been shown that the liver is the main site of catabolism of circulating HA (Laurent & Fraser, 1992). HA is taken up by the liver via specific HA receptors (Forsberg & Gustafson, 1991). Using the amino-terminal sequence of a preparation of pig liver hyase, cDNAs encoding this enzyme have recently been isolated from a corresponding library (Zhu et al., 1994). From the sequence of these cDNAs it was deduced that the pig liver hyase was more than 60% identical to that of human and rat hemopexin (Takahashi et al., 1985; Nikkila et al., 1991). However, more recent results indicate that the suggestion that hemopexin has hyase activity may have been based on an artifact (R. Stern, pers. comm.). The structure of liver, serum, and other hyases thus remains to be elucidated.

Concluding remarks

For many years, investigations on the ECM have largely dealt with collagens and its numerous other protein constituents. Only recently has the role HA, a ubiquitous component of this matrix, also received some interest. This was largely due to studies showing that growth of normal and tumor cells is influenced by the interaction of cellular HA receptors with HA of the ECM (Sherman et al., 1994). In this context, the enzymes catalyzing the biosynthesis and the degradation of this polysaccharide and the factors that control their activity are of increasing importance. The structure of a prokaryotic HA synthase has been elucidated recently (DeAngelis et al., 1993, 1994) and one can expect that these findings will soon be extended to higher organisms. As summarized in this review, one type of animal hyase has now been characterized. These are the PH-20 hyases from mammalian sperm, which are related to the corresponding enzyme from bee and hornet venom. It will be of great interest to test whether the PH-20 hyase or related enzymes also participate in the many physiological situations where HA catabolism takes place.

Acknowledgments

The work from the author's laboratory mentioned in this review has been supported by grants from the Austrian Fonds zur Förderung der wissenschaftlichen Forschung (grant P09888) and the Medizinische Forschungsgesellschaft Salzburg.

References

- Afify AM, Stern M, Guntenhöner M, Stern R. 1993. Purification and characterization of human serum hyaluronidase. *Arch Biochem Biophys* 305: 434–441.
- Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. 1990. CD44 is the principal cell surface receptor for hyaluronate. *Cell* 61:1303–1313.
- Berry AM, Lock RA, Thomas SM, Rajan DP, Hansman D, Paton JC. 1994. Cloning and nucleotide sequence of the *Streptococcus pneumoniae* hyaluronidase gene and purification of the enzyme from recombinant *E. coli*. *Infect Immunol* 62:1101–1108.
- Bray MR, Clarke AJ. 1994. Identification of a glutamate residue at the active site of xylanase A from *Schizophyllum commune*. *Eur J Biochem* 219: 821–827.
- Canard B, Garnier T, Saint-Joanis B, Cole ST. 1994. Molecular genetic analysis of the nagH gene encoding a hyaluronidase from *Clostridium perfringens*. *Mol Gen Genet* 243:215–224.
- Chain E, Duthie ES. 1939. A mucolytic enzyme in testis extracts. *Nature* 144:977–978.
- Cramer JA, Bailey LC, Bailey CA, Miller RT. 1994. Kinetic and mechanis-

- tic studies with bovine testicular hyaluronidase. *Biochim Biophys Acta* 1200:315-321.
- DeAngelis PL, Papaconstantinou J, Weigel PH. 1993. Molecular cloning, identification, and sequence of the hyaluronan synthase gene from group A *Streptococcus pyogenes*. *J Biol Chem* 268:19181-19184.
- DeAngelis PL, Yang N, Weigel PH. 1994. The *Streptococcus pyogenes* hyaluronan synthase: Sequence comparison and conservation among various group A strains. *Biochem Biophys Res Commun* 199:1-10.
- Divne C, Stahlberg J, Reinikainen T, Ruohonen L, Petterson G, Knowles JKC, Teeri TT, Jones A. 1994. The three-dimensional crystal structure of the catalytic core of cellobiohydrolase I from *Trichoderma reesei*. *Science* 265:524-528.
- Duran-Reynals F. 1928. Exaltation de l'activité du virus vaccinal par les extraits de certains organes. *CR Séances Soc Biol Fil* 99:6-7.
- Forsberg N, Gustafson S. 1991. Characterization and purification of the hyaluronan receptor on liver endothelial cells. *Biochim Biophys Acta* 1078:12-18.
- Gmachl M, Kreil G. 1993. Bee venom hyaluronidase is homologous to a membrane protein of mammalian sperm. *Proc Natl Acad Sci USA* 90:3569-3573.
- Gmachl M, Sagan S, Ketter S, Kreil G. 1993. The human sperm protein PH-20 has hyaluronidase activity. *FEBS Lett* 336:545-548.
- Hardwick C, Hoare K, Owens R, Hohn HP, Hook M, Moore D, Crippa V, Austen L, Nance DM, Turley EA. 1992. Molecular cloning of a novel hyaluronan receptor that mediates tumor cell motility. *J Cell Biol* 117:1343-1350.
- Hotez P, Cappello M, Hawdon J, Beckers C, Sakanari J. 1994. Hyaluronidases of the gastrointestinal invasive nematodes *Ancylostoma caninum* and *Anisakis simplex*: Possible functions in the pathogenesis of human zoonoses. *J Infect Dis* 170:918-926.
- Hynes WL, Ferretti JJ. 1989. Sequence analysis and expression in *E. coli* of the hyaluronidase gene of *Streptococcus pyogenes* bacteriophage H4489A. *Infect Immunol* 57:533-539.
- Hynes WL, Ferretti JJ. 1994. Assays for hyaluronidase activity. *Methods Enzymol* 235:606-616.
- Jacobson RS, Hoffman DR, Kemeny DM. 1992. The cross-reactivity between bee and vespid hyaluronidases has a structural basis. *J Allergy Clin Immunol* 89:292 [Abstr].
- Joy MB, Dodgson KS, Olavesen AH, Gacesa P. 1985. Purification and some properties of pig liver hyaluronidase. *Biochim Biophys Acta* 838:257-263.
- Kemeny DM, Dalton N, Lawrence AJ, Pearce FL, Vernon CA. 1984. The purification and characterization of hyaluronidase from the venom of the honey bee, *Apis mellifera*. *Eur J Biochem* 139:217-233.
- Klewes L, Turley EA, Prehm P. 1993. The hyaluronate synthase from a eukaryotic cell line. *Biochem J* 290:791-795.
- Lathrop WF, Carmichael EP, Myles DG, Primakoff P. 1990. cDNA cloning reveals the molecular structure of a sperm surface protein, PH-20, involved in sperm-egg adhesion and the wide distribution of the gene among mammals. *J Cell Biol* 111:2939-2949.
- Laurent TC. 1989. The biology of hyaluronan. *Ciba Foundation Symp* 143. New York: John Wiley & Sons. pp 1-298.
- Laurent TC, Fraser JRE. 1992. Hyaluronan. *FASEB J* 6:2397-2404.
- Lin B, Hollingshead SK, Coligan JE, Egan ML, Baker JR, Pritchard DG. 1994. Cloning and expression of the gene for group B streptococcal hyaluronate lyase. *J Biol Chem* 269:30113-30116.
- Lin Y, Kimmel LH, Myles DG, Primakoff P. 1993. Molecular cloning of the human and monkey sperm surface protein PH-20. *Proc Natl Acad Sci USA* 90:10071-10075.
- Lin Y, Mahan K, Lathrop WF, Myles DG, Primakoff P. 1994. A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the egg. *J Cell Biol* 125:1157-1163.
- Lu G, Kochoomian L, King TP. 1995. Sequence identity and antigenic cross reactivity of white face hornet venom allergen, also a hyaluronidase, with other proteins. *J Biol Chem* 270:4457-4465.
- Meyer K. 1971. Hyaluronidases. In: Boyer PD, ed. *The enzymes*, 3rd ed, vol V. New York: Academic Press. pp 307-320.
- Meyer K, Palmer JW. 1934. The polysaccharide of the vitreous humor. *J Biol Chem* 107:629-634.
- Mikami B, Degano M, Hehre EJ, Sacchettini JC. 1994. Crystal structures of soybean β -amylase reacted with β -maltose and maltal: Active site components and their apparent roles in catalysis. *Biochemistry* 33:7779-7787.
- Nikkila H, Gitlin JD, Muller-Eberhard U. 1991. Rat hemopexin. Molecular cloning, primary structural characterization, and analysis of gene expression. *Biochemistry* 30:823-829.
- Philips DC. 1967. The hen egg white lysozyme molecule. *Proc Natl Acad Sci USA* 249:380-386.
- Poh CH, Yuen R, Chung MC, Khoo HE. 1992. Purification and partial characterization of hyaluronidase from stonefish (*Synanceja horrida*) venom. *Comp Biochem Physiol (B)* 101:159-163.
- Primakoff P, Lathrop W, Woolman L, Cowan A, Myles DG. 1988. Fully effective contraception in male and female guinea pigs immunized with the sperm protein PH-20. *Nature* 335:543-546.
- Primakoff P, Myles DG. 1983. A map of the guinea pig sperm surface constructed with monoclonal antibodies. *Dev Biol* 98:417-428.
- Ramanaiah M, Parthasarathy PR, Venkaiah B. 1990. Isolation and characterization of hyaluronidase from scorpion (*Heterometrus fulvipes*) venom. *Biochem Int* 20:301-310.
- Rosa F, Sargent TD, Rebbert ML, Michaelis GS, Jamrich M, Grunz H, Jonas E, Winkles JA, Dawid IB. 1988. Accumulation and decay of DG42 gene products follow a gradient during *Xenopus* embryogenesis. *Dev Biol* 129:114-123.
- Rothberg KG, Ying YS, Kamen BA, Anderson RGW. 1990. Cholesterol controls the clustering of the glycopospholipid-anchored membrane receptor of 5-methyltetrahydrofolate. *J Cell Biol* 111:2931-2938.
- Salustri A, Yanagishita M, Underhill CB, Laurent TC, Hascall VC. 1992. Localization and synthesis of hyaluronic acid in the cumulus cells and mural granulosa cells of the preovulatory follicle. *Dev Biol* 151:541-551.
- Semino CE, Robbins PW. 1995. Synthesis of "Nod"-like chitin oligosaccharides by the *Xenopus* developmental protein DG42. *Proc Natl Acad Sci USA* 92:3498-3501.
- Sherman L, Sleeman J, Herrlich P, Ponta H. 1994. Hyaluronate receptors: Key players in growth, differentiation, migration and tumor progression. *Curr Opin Cell Biol* 6:726-733.
- Takagaki K, Nakamura T, Izumi J, Saitoh H, Endo M, Kojima K, Kato I, Majima M. 1994. Characterization of hydrolysis and transglycosylation by testicular hyaluronidase using ion-spray mass spectroscopy. *Biochemistry* 33:6503-6507.
- Takahashi N, Takahashi Y, Putnam FW. 1985. Complete amino acid sequence of human hemopexin, the heme-binding protein of serum. *Proc Natl Acad Sci USA* 82:73-77.
- Törrönen A, Harkki A, Rouvinen J. 1994. Three-dimensional structure of endo-1,4- β -xylanase II from *Trichoderma reesei*: Two conformational states in the active site. *EMBO J* 13:2493-2501.
- Tu AT, Hendon RR. 1983. Characterization of lizard venom hyaluronidase and evidence for its action as a spreading factor. *Comp Biochem Physiol (B)* 76:377-383.
- Van Roey P, Rao V, Plummer TH, Tarentino AL. 1994. Crystal structure of endo- β -N-acetylglucosaminidase F1, an alpha/beta barrel enzyme adapted for a complex substrate. *Biochemistry* 33:13989-13996.
- Wakarchuk WW, Campbell RL, Sung WL, Davoodi J, Yaguchi M. 1994. Mutational and crystallographic analysis of the active site residues of the *Bacillus circulans* xylanase. *Protein Sci* 3:367-475.
- Watanabe T, Kobori K, Miyashita K, Jujii T, Sakai H, Uchida M, Tanaka H. 1993. Identification of glutamic acid 204 and aspartic acid 200 as essential residues in chitinase A1 of *Bacillus circulans* WL-12 as essential residues for chitinase activity. *J Biol Chem* 268:18567-18572.
- Yang B, Yang BL, Savani RC, Turley EA. 1994. Identification of a common hyaluronan binding motif in the hyaluronan binding protein RHAMM, CD44 and link protein. *EMBO J* 13:286-296.
- Zhu L, Hope TJ, Hall J, Davies A, Stern M, Muller-Eberhard U, Stern R, Paslow TG. 1994. Molecular cloning of a mammalian hyaluronidase reveals identity with hemopexin, a serum heme-binding protein. *J Biol Chem* 269:32092-32097.