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Aminopeptidases in *Caenorhabditis elegans* and *Panagrellus redivivus*: detection using peptide and non-peptide substrates

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Abstract

Aminopeptidase activities were detected in extracts of the free-living nematodes *Caenorhabditis elegans* and *Panagrellus redivivus* using the aminoacyl substrate L-alanine-4-nitroanilide. The activities exhibited similarities in Km (*C. elegans* = 2.22 mM; *P. redivivus* = 2.09 mM) and specific activity (*C. elegans* = $1.38 \pm 0.43\,\text{mAU}\,\text{min}^{-1}\,\mu\text{g}^{-1}$; *P. redivivus*, $1.23 \pm 0.18\,\text{mAU}\,\text{min}^{-1}\,\mu\text{g}^{-1}$). Each is inhibited competitively by amastatin (*C. elegans* $IC_{50} = 0.46\,\mu\text{M}$; *P. redivivus* $IC_{50} = 15.90\,\mu\text{M}$) and non-competitively by leuhistin (*C. elegans* $IC_{50} = 3.00\,\mu\text{M}$; *P. redivivus* $IC_{50} = 37.35\,\mu\text{M}$). The bioactive peptides adipokinetic hormone and substance P decrease the apparent aminopeptidase activities of each extract suggesting that the peptides compete with the Ala-pNA as substrates. With each extract, adipokinetic hormone appeared to be the more effective substrate. Digestion of adipokinetic hormone by *C. elegans* and *P. redivivus* extracts in the presence and absence of 1 mM amastatin produced distinct chromatographic profiles that suggest different digestion patterns for the two species. However, amastatin had clear effects on chromatographic profiles from each species indicating that an aminopeptidase is involved in the digestion of the peptide substrates. The data presented indicate that extracts of free-living nematodes are capable of metabolizing peptide hormones, and that this metabolism involves substrate-selective aminopeptidases.

Introduction

Regulated proteolysis is an essential molecular component in all animal cells. Proteolytic enzymes are critical to anabolic and catabolic pathways in development and metabolism, including elimination of proteins during normal turnover, peptide signal attenuation, and gene regulation. In nematodes, proteases also are involved in sex determination (Sokol & Kuwabara, 2000) and neuropeptide degradation (Sajid & Isaac, 1995; Sajid *et al.*, 1996, 1997). Neuropeptides play key roles in all aspects of invertebrate physiology, and have been studied in a number of invertebrate groups including crustaceans (Keller, 1992), molluscs (Muneoka & Kobayashi, 1992), and most extensively in insects (Masler *et al.*, 1993; Nassel, 1993). Evidence for the presence of neuropeptides in nematodes continues to increase (Davis & Stretton, 1995; Shaw, 1996), with genetic and physiological studies uncovering roles in neurotransmission, muscular activity, and egg laying (Nelson *et al.*, 1998; Brownlee & Fairweather, 1999; Day & Maule, 1999; Waggoner *et al.*, 2000). There is immunological and physiological evidence for the presence of molecules similar to the insect neuropeptides allatostatin (Smart

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et al., 1995) and adipokinetic hormone (Davenport *et al.*, 1991) in nematodes. The biochemistry of adipokinetic hormone metabolism is particularly well characterized in insects (Rayne & O'Shea, 1992; Masler *et al.*, 1996), and an aminopeptidase is essential to the regulated catabolism of the hormone. A gene for an aminopeptidase has been cloned in *Caenorhabditis elegans*, and one suggested role for the enzyme is in neuropeptide degradation (Baset *et al.*, 1998). In the present report, evidence is presented for the presence of aminopeptidase activity in extracts of two free-living nematodes, *C. elegans* and *Panagrellus redivivus*, detected using an aminoacyl substrate and specific aminopeptidase inhibitors. In addition, evidence is presented for the interaction of peptides as substrates for the enzyme, and the involvement of nematode aminopeptidase in the degradation of the insect neuropeptide, adipokinetic hormone.

Materials and methods

Caenorhabditis elegans and *P. redivivus* were reared in liquid culture at 22°C (Chitwood *et al.*, 1995). Worms were exhaustively washed with water to remove culture medium prior to extraction, then processed in 10–20 volumes of water by disruption with a Polytron homogenizer (Brinkman Instruments, Westbury, New York). After centrifugation (48,000 × *g*, 30 min, 10°C), the supernatant was collected, and the pellet re-extracted with the Polytron in 4–5 volumes of water and centrifuged as above. The two supernatants were pooled, aliquots dried by vacuum centrifugation, and stored at −15°C. Aliquots were dissolved in 100 mM TRIS-HCl, pH 8.1 (assay buffer) as needed. Total protein was estimated using the microBCA assay (Pierce Chemical, Rockford, Illinois) following the manufacturer's instructions.

Kinetic assays using L-alanine-4-nitroanilide hydrochloride (Ala-pNA; Fluka Chemical Co., Ronkonkoma, New York) as substrate were done at 37°C in 96-well polystyrene microtitre plates (Corning Easy Wash Assay Plate; Corning, Inc., Corning, New York). Samples in 150 µl assay buffer were combined with 30 µl of substrate, with final Ala-pNA concentrations of 0.7 to 8 mM. Absorbance was monitored at 405 nm using a microplate reader (Biotek Instruments, Winooski, Vermont). Reaction rate (V) is expressed as milli-absorbance units per minute. For inhibition assays, aminopeptidase inhibitors (amastatin, arphaminine B, bestatin, epiamastatin, epibestatin, leuhistin; Sigma Chemical Co., St Louis, Missouri) or peptides (locust adipokinetic hormone, pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr and substance P, Arg-Pro-Lys-Pro-Asn-Asn-Phe-Phe-Gly-Leu-Met; Peninsula Laboratories, Belmont, California) were dissolved in assay buffer, then added to extract in microtitre wells to a combined volume of 150 µl. Substrate (30 µl) was then added to start the reaction. Km was estimated using the Lineweaver-Burk equation, and IC_{50} was estimated by linear regression.

Samples used in the chromatographic assay were prepared by incubating worm extract (5–6 µg) alone, extract plus 2 µg adipokinetic hormone (AKH), or extract, AKH and 1 mM amastatin (Sigma) in 100 mM TRIS-HCl, pH 8.1, 5 µl total volume, 37°C, for 4 h. Reactions were stopped by the addition of 50 µl of 0.1% trifluoroacetic acid (TFA) in acetonitrile (CH_3CN). The mixtures were dried under vacuum, dissolved in 0.1% TFA, centrifuged (9800 × *g*, 1 min, RT) and injected onto the column. The chromatographic system used a reversed-phase C_8 column (Hewlett-Packard 5 mm id × 150 mm, 5 µm particle size, Hewlett-Packard, Avondale, Pennsylvania), flow rate = 0.5 ml min^{-1}. A linear gradient of 5% to 45% CH_3CN in 0.1% TFA was run over 20 min (2% CH_3CN min^{-1}). Absorbance was monitored at 210 nm, and retention times determined by on-board integration software (ChemStation, Hewlett-Packard).

Results

Aminopeptidase-like activity was detected in both *C. elegans* and *P. redivivus* supernatants. Km values for the two extracts were similar (*C. elegans*, 2.22 mM; *P. redivivus*, 2.09 mM). Specific activities (n = 6–8; mean±SD) were; *C. elegans*, 1.38 ± 0.43 mAU min^{-1} μg^{-1} and *P. redivivus*, 1.23 ± 0.18 mAU min^{-1} μg^{-1}. Assays were done with 8 mM Ala-pNA.

Six aminopeptidase inhibitors were individually screened against *C. elegans* extract at 90–100 µM each, using the colorimetric assay. In descending order of efficacy, indicated by percent inhibition, they were: amastatin (95%) = leuhistin (95%) > epiamastatin (84%) > arphaminine B (77%) ⩾ bestatin (17%) > epibestatin (15%). Amastatin and leuhistin were examined further. Each was tested against *C. elegans* and *P. redivivus* preparations (fig. 1). Amastatin and leuhistin inhibited *C. elegans* aminopeptidase in a dose-responsive manner (fig. 1A). Amastatin was 6-fold more effective (IC_{50} = 0.46 µM) than leuhistin (IC_{50} = 3.00 µM). *Panagrellus redivivus* activity also responded to each inhibitor in a dose-responsive manner (fig. 1B), but was less sensitive than *C. elegans* aminopeptidase. As with *C. elegans*, *P. redivivus* aminopeptidase was more sensitive to amastatin (IC_{50} = 15.90 µM) than to leuhistin (IC_{50} = 37.35 µM).

The interaction of amastatin with *C. elegans* aminopeptidase was competitive. Km increased from 2.2 mM (fig. 2A, control, circles) to 9.3 mM with 7.5 µM amastatin (fig. 2A, triangles). Amastatin was also a competitive inhibitor of *P. redivivus* aminopeptidase (fig. 2B), with an increase in Km from 2.1 mM (control, circles) to 9.9 mM with 12.5 µM amastatin (triangles). Leuhistin was a non-competitive inhibitor of *C. elegans* aminopeptidase (fig. 2A), with a slight increase in Km from the control value (2.2 mM) to 3.4 mM at 4.4 µM leuhistin (squares). Increased inhibitor concentration (10.9 µM leuhistin, not shown) did not further increase Km (3.3 mM). Leuhistin appeared to be a competitive inhibitor of *P. redivivus* aminopeptidase (fig. 2B), increasing Km to 6.6 mM at 10 µM leuhistin (squares) compared with 2.1 mM for the control (circles).

The insect neuropeptide adipokinetic hormone and the vertebrate peptide substance P each caused a dose-dependent decrease in detected aminopeptidase activity when tested against *C. elegans* extract (fig. 3A). Adipokinetic hormone (fig. 3A, circles) was 5.4-fold more effective (IC_{50} = 20.04 µM) than substance P (IC_{50} = 108.95 µM; fig. 3A, squares) in decreasing aminopeptidase activity. These peptides also decreased

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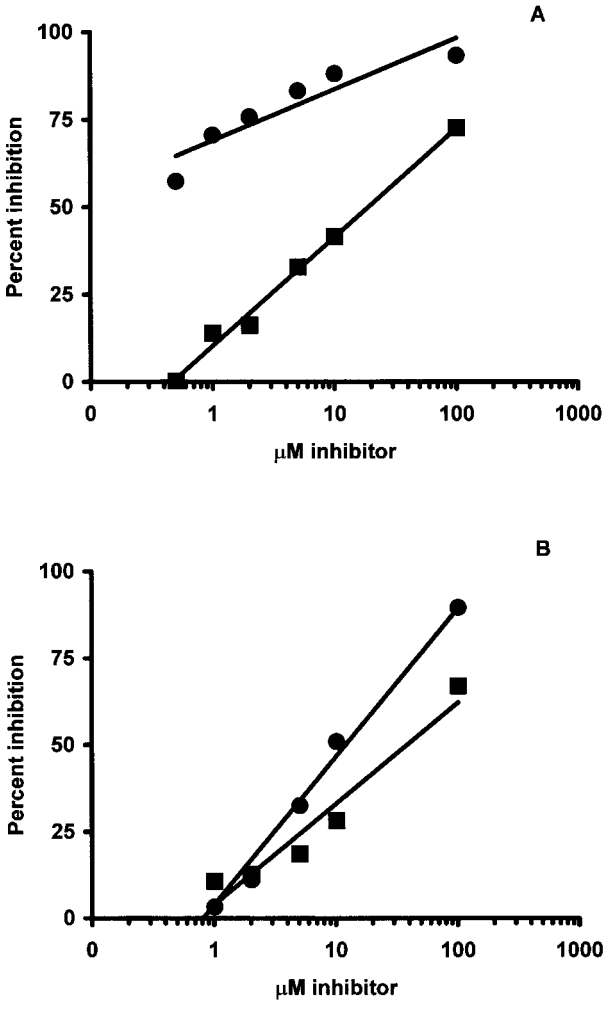

Fig. 1. Inhibition of *Caenorhabditis elegans* and *Panagrellus redivivus* aminopeptidase-like activities by amastatin and leuhistin. (A) *C. elegans* extract. (B) *P. redivivus* extract. Reactions contained 8 mM Ala-pNA, 5 μg extract protein, and 0.5–100 μM inhibitor. Incubations were at 37°C. Data are expressed as the average of two to three separate determinations. ●, amastatin; ■, leuhistin.

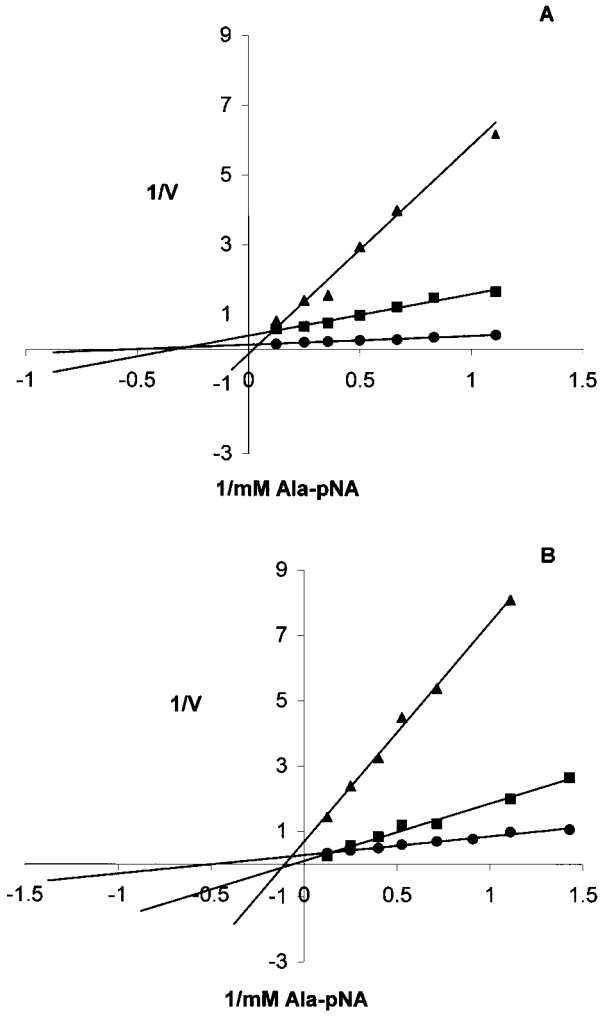

Fig. 2. Inhibition characteristics of amastatin and leuhistin. Lineweaver-Burk plots were generated for (A) *Caenorhabditis elegans*, 4.4 μM leuhistin (■) and 7.5 μM amastatin (▲), (B) *Panagrellus redivivus*, 10 μM leuhistin (■) and 12.5 μM amastatin (▲). Reactions contained varying concentrations of Ala-pNA (0.7–8.0 mM) and 5 μg of extract protein. Control curves (●) contained no inhibitor. Incubations were at 37°C.

aminopeptidase activity in *P. redivivus* extracts (fig. 3B). Adipokinetic hormone (fig. 3B, circles) was 2.4-fold more effective ($IC_{50} = 46.12\ \mu M$) than substance P (fig. 3B, squares; $IC_{50} = 108.46\ \mu M$). The interaction of AKH with nematode extracts was then examined further by chromatographic assay.

Three prominent peaks were evident in *C. elegans* extract following incubation at 37°C (fig. 4A; elution times of, 9.2 12.9 and 15.4 min). At least four additional peaks resulted from incubation of extract with AKH (fig. 4B, asterisks). The peaks at 12.6, 13.6, 16.7 and 17.2 min were absent from incubations of the extract alone. When amastatin was included in the incubation (fig. 4C), the peak at 17.2 min increased in absorbance 3.5-fold over the corresponding peak without amastatin present (fig. 4B), suggesting the accumulation of an AKH metabolite. An additional peak at 14.1 min (fig. 4C) was not observed without amastatin. The chromatographic profile of incubated *P. redivivus* extract (fig. 5A) consisted of smaller but more numerous peaks than those observed in *C. elegans* extract (fig. 4A), with no quantitatively dominant UV-peaks present. Incubation with AKH yielded a prominent peak at 15.4 min (fig. 5B), which may have obscured the 15.6 min peak that was seen in the extract alone (fig. 5A). The size of the 15.4 min peak (fig. 5B) decreased considerably in the presence of amastatin (fig. 5C), while a new prominent peak appeared at 14.1 min (fig. 5C).

Discussion

The aminopeptidase activities prepared from *C. elegans*

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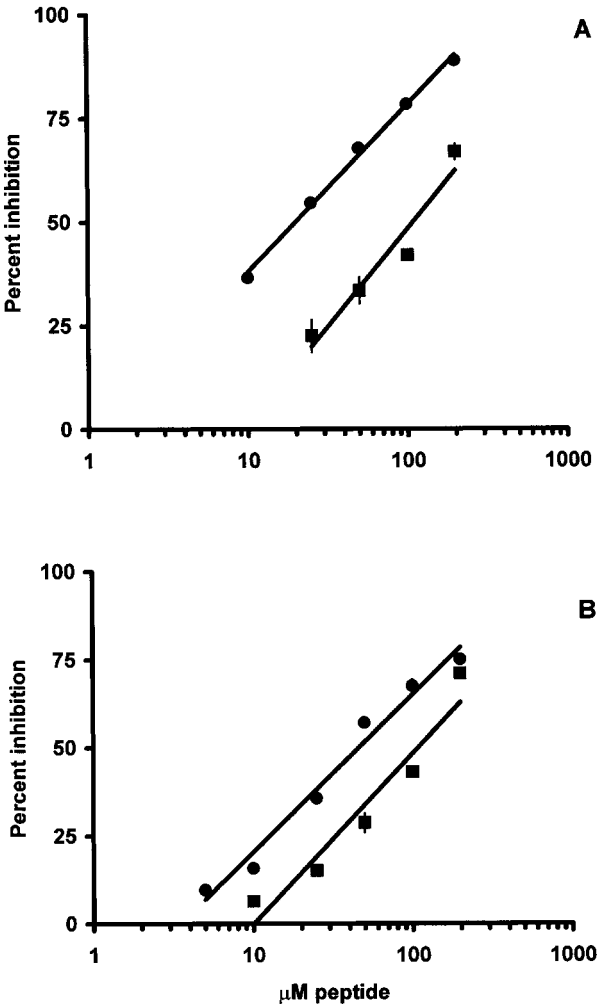

Fig. 3. Effect of neuropeptides on the apparent activity of aminopeptidase in extracts of *Caenorhabditis elegans* (A) and *Panagrellus redivivus* (B). Each reaction contained 8 mM Ala-pNA, 5 μg of extract protein and 0.5–200 μM of either locust adipokinetic hormone (●) or vertebrate substance P (■). Each data point is the mean±SD of at least three separate determinations. Points with no error bars had deviation too small to illustrate. Incubations were at 37°C.

and *P. redivivus* are similar in a number of basic characteristics including Km and specific activity, and are the first aminopeptidases described from extracts of any free-living nematode. Amastatin sensitive activity was reported in *C. elegans* extract (Gimenez-Pardo *et al.*, 1999), suggesting that aminopeptidase was present. A recombinant enzyme with aminopeptidase activity (AP-1) was cloned from *C. elegans* (Baset *et al.*, 1998), with a preference for Arg-pNA as an aminoacyl substrate (Km = 0.43 mM), although Ala-pNA was also an effective substrate with a Km (5.53 mM) somewhat similar to those in the present study. However, whereas bestatin was a poor inhibitor of extracted aminopeptidases from *C. elegans*, it was a very potent inhibitor of the expressed recombinant enzyme (IC_{50} = 2.6 μM; Baset *et al.*, 1998). Amastatin or leuhistin were not tested on the recombinant aminopeptidase. An aminopeptidase purified from secretions of the animal parasite *Ascaris suum* (Rhoads *et al.*, 1998) was inhibited significantly by either 100 μM amastatin (90%) or 100 μM bestatin (74%). Comparisons between enzyme activity in extracts and that of a pure enzyme must be interpreted cautiously. However, the aminopeptidase activities detected in *C. elegans* and *P. redivivus* extracts may well represent enzymes different from, or in addition to, *C. elegans* AP-1, and appear to differ from that purified from *A. suum*.

The aminopeptidases prepared from *C. elegans* and *P. redivivus* were each inhibited by amastatin or leuhistin, each was more sensitive to amastatin than to leuhistin, and amastatin was a competitive inhibitor of both enzymes. However, the *C. elegans* enzyme was much more sensitive to either inhibitor, as judged by IC_{50} values, by a factor of more than 10, than was the enzyme prepared from *P. redivivus*. Also, the type of inhibition of the *P. redivivus* activity by leuhistin (competitive vs. non-competitive) was not so clear as in *C. elegans*. One factor that could influence apparent activities is the concentration of total protein in either the extract or in the assay itself. However, extract protein concentrations and specific activities were similar for the two species, so the differences in relative responses to the inhibitors may be due to other factors. For example, protease inhibitors have been reported in extracts of *A. suum* and *Trichuris suis* (Zenka & Prokopic, 1985; Rhoads *et al.*, 2000). This suggests that endogenous molecules are present in nematode extracts that can affect the response of enzymes to substrates, and perhaps to inhibitors as well. Assay conditions such as pH, buffer ion concentrations and temperature may also influence enzyme-substrate and enzyme-inhibitor association. Gimenez-Pardo *et al.* (1999) reported that response to a number of inhibitors of a variety of enzyme activities in *C. elegans* extracts varied dramatically depending upon assay temperature. Whatever the basis is for the observed differences in inhibitor response between the *C. elegans* and *P. redivivus* enzymes, the *C. elegans* aminopeptidase was consistently more sensitive to inhibition. Aminopeptidases have been implicated in larval moulting in animal parasitic nematodes (Hong *et al.*, 1993; Rhoads *et al.*, 1998), and amastatin can affect moulting. It would be of interest to compare the effect of aminopeptidase inhibitors on larval development in *C. elegans* and *P. redivivus* in the light of their different biochemical responses to aminopeptidase inhibitors.

Comparisons of the two extracts were expanded to include peptide substrates. Adipokinetic hormone was chosen since extracts of *P. redivivus* evoke adipokinetic responses in insects (Davenport *et al.*, 1991), and substance P was chosen for comparison as an unrelated peptide of similar size, with a free N-terminus. Each peptide caused a reduction in the apparent aminopeptidase activity in either *C. elegans* or *P. redivivus* preparations. Substance P decreased activity equally in the two species. Adipokinetic hormone was about two-fold more effective in *C. elegans* than in *P. redivivus*. Interestingly, the N-terminally blocked AKH was the more effective 'inhibitor' in each extract. If these peptides serve as substrates, the reduction in activity probably

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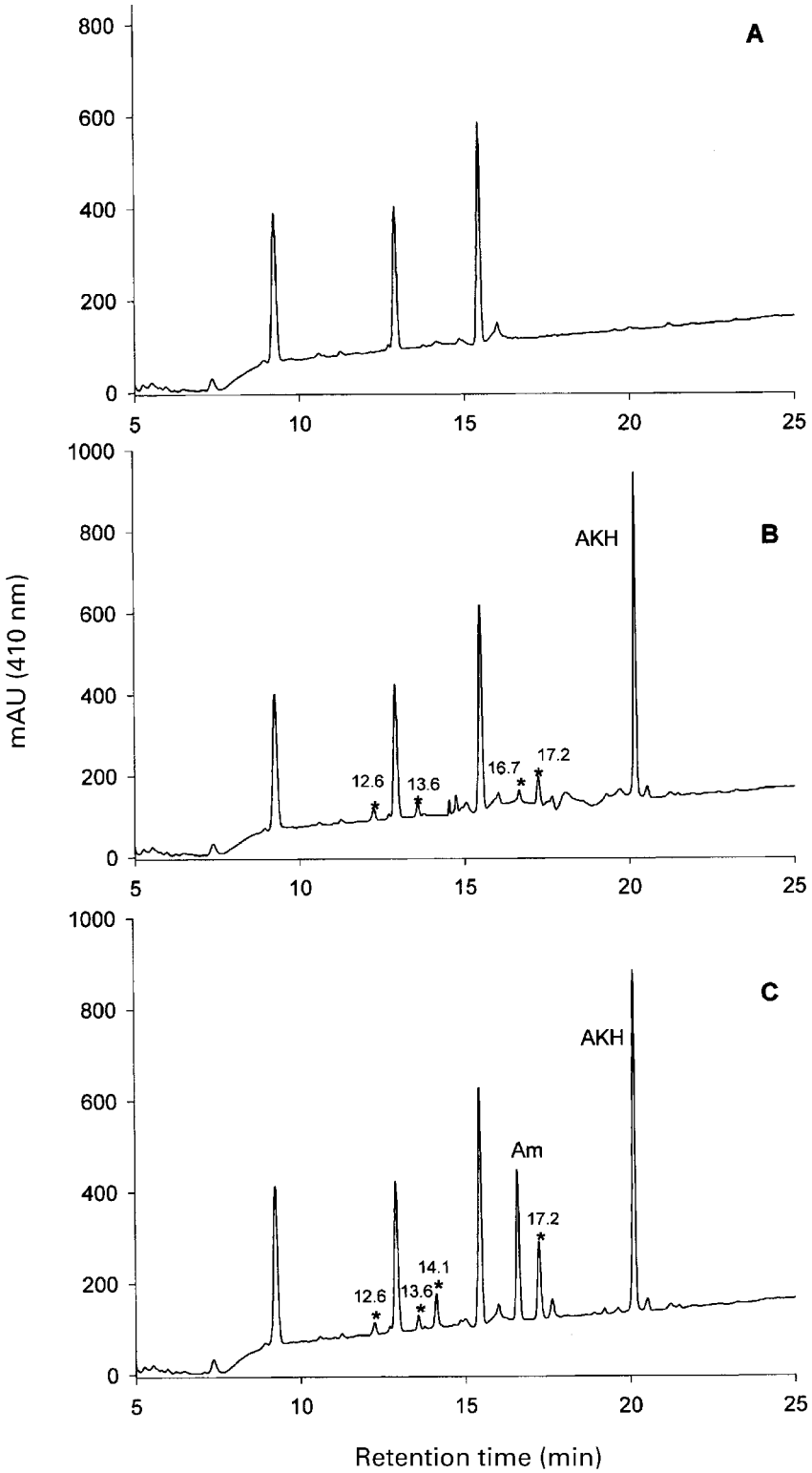

Fig. 4. RP-HPLC profiles of *Caenorhabditis elegans* extract incubated at 37°C. UV detection was at 210 nm. A-extract (5–6 μg) only; B-extract with 2 μg AKH; C-extract, AKH and 1 mM amastatin. Peaks not observed in extract alone are indicated with an asterisk (*). Numbers indicate retention time.

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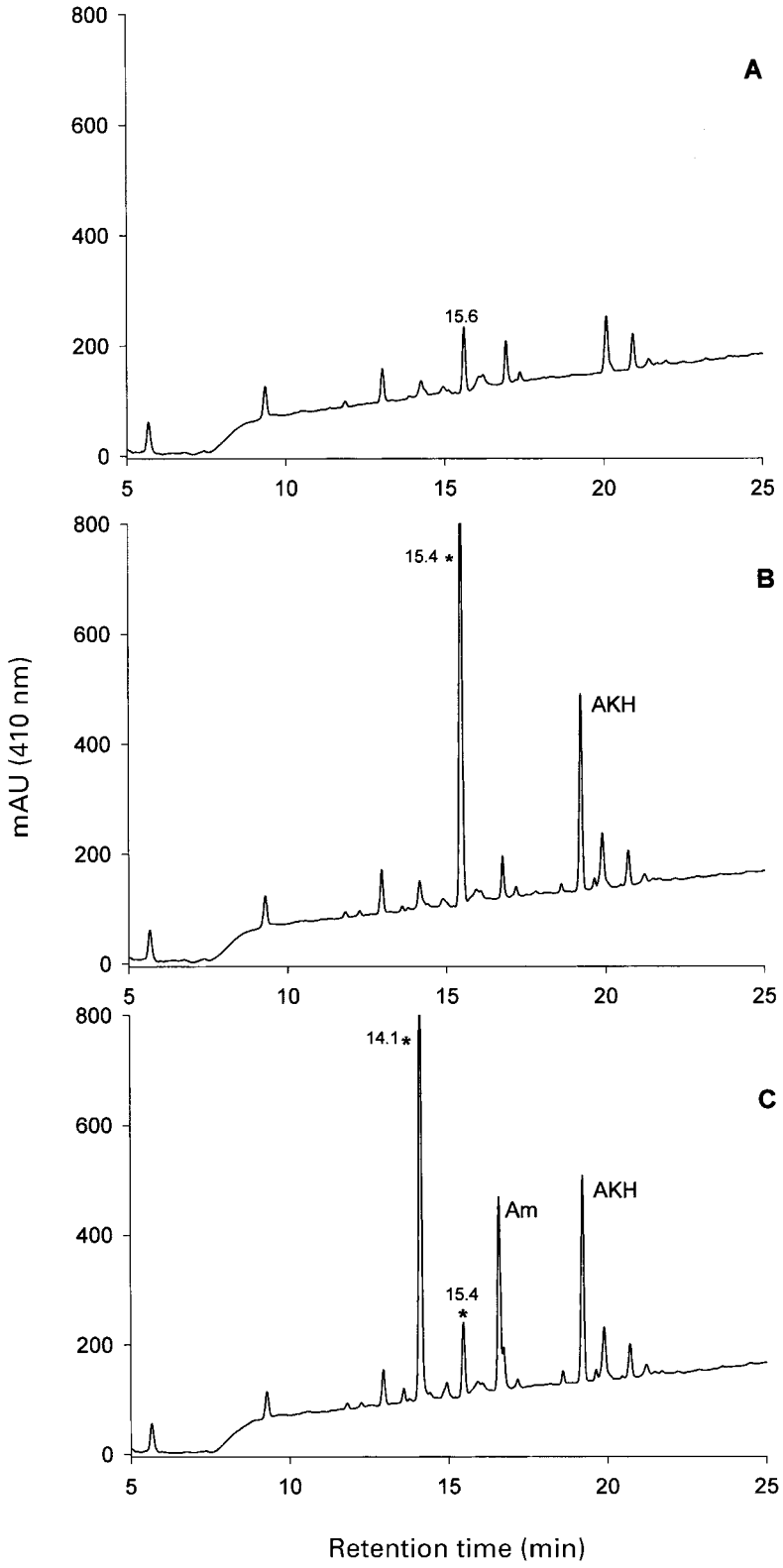

Fig. 5. RP-HPLC profiles of *Panagrellus redivivus* extract incubated at 37°C. UV detection was at 210 nm. A-extract (5–6 μg) only; B-extract with 2 μg AKH; C-extract, AKH and 1 mM amastatin (Am). Peaks not observed in extract alone are indicated with an asterisk (*). Numbers indicate retention time.

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represents competition between the peptides and Ala-pNA for the enzyme. Since the assay involved extract and not pure aminopeptidase, the neuropeptides certainly were subject to proteolytic digestion in addition to the action of any aminopeptidase. Indeed, AKH cannot serve as an aminopeptidase substrate until an initial internal cleavage exposes a free amino terminus. Thus, under the assay conditions used, a mixture of peptide substrates was most likely present. This mixture would consist of intact adipokinetic hormone and various fragments produced by proteolytic digestion. The same situation should occur with substance P. It is not clear, however, why adipokinetic hormone was the more effective substrate.

A screen of substance P fragments (data not shown) revealed that C-terminal fragments (e.g. Pro-Asn-Asn-Phe-Phe-Gly-Leu-Met or shorter) reduced activity by only 1–10%. Also, the short N-terminal fragment Arg-Pro-Lys-Pro had little effect on aminopeptidase in either extract, reducing activity by only 4–12%. However, N-terminal fragments that included the first 6–9 residues (i.e. Arg-Pro-Lys-Pro-Asn-Asn to Arg-Pro-Lys-Pro-Asn-Asn-Phe-Phe-Gly) were very effective inhibitors, reducing aminopeptidase activity by 50–87%. One explanation is that the 6–9 residue N-terminal fragments are more structurally similar to substance P than are the C-terminal fragments. Another possibility is that the N-terminal fragments are more stable in the presence of endogenous proteases than are the C-terminal fragments. In this context, it is interesting to note that in a study on the nematode myotropic neuropeptide Lys-Pro-Asn-Phe-Ile-Arg-Phe, Kubiak *et al.* (1996) determined that Pro^2 contributes to the metabolic stability of the peptide in the presence of endogenous aminopeptidases, and suggest that it is required to maintain the physiologically preferred conformation of the peptide. Perhaps similar roles are served by the substance P Pro^2.

Adipokinetic hormone appears to be an effective substrate for *C. elegans* and *P. redivivus* aminopeptidases. Chromatographic fractionation of digests in which AKH was present revealed UV peaks that were absent from extracts alone. In addition, the presence of amastatin altered both the number of such peaks as well as the relative amount (UV-absorbance) of some of them. This was apparent in both *C. elegans* and *P. redivivus* extracts, but the chromatographic patterns were markedly different for the two species. Incubation of *C. elegans* extract yielded three quantitatively major peaks, while in *P. redivivus* extract a number of small peaks were present. Digestion of AKH resulted in what appeared to be a single large peak in the *P. redivivus* system, but at least four small metabolite peaks in the *C. elegans* incubations. Despite such differences, amastatin caused a distinct change in the chromatographic profile of each extract. The accumulation of a UV-peak or appearance of new UV-peaks in the presence of amastatin clearly demonstrates the presence of an aminopeptidase. It is interesting to note that in both extracts, the presence of amastatin and AKH results in the detection of a UV-peak at 14.1 min. Thus, while *C. elegans* and *P. redivivus* extracts present distinctly different chromatographic profiles, each digests AKH as a peptide substrate and each responds to amastatin as an aminopeptidase inhibitor.

The attenuation of peptide signals is regulated in large part through the action of endo- and exopeptidases. This process has been extensively studied in vertebrates, and among invertebrates is well characterized in the insects where metabolism of AKH has been described. Evidence presented here demonstrates that *C. elegans* and *P. redivivus* have aminopeptidases that can use biologically active peptides as substrates, and that this activity is selective as evidenced by preferences for AKH and specific substance P fragments. Although both nematodes possess enzymes, including amastatin-sensitive aminopeptidases, that can digest AKH, the digestion profiles are distinctly different. The respective aminopeptidases also demonstrate some quantitative differences in response to aminopeptidase inhibitors. The aminopeptidases in the two species may indeed be different, which would not conflict with the classification of *C. elegans* and *P. redivivus* in separate phylogenetic clades (Dorris *et al.*, 1999). It is clear that various neuropeptides have physiological effects in nematodes (Davenport *et al.*, 1991; Davis & Stretton, 1995; Brownlee & Fairweather, 1999), and that in animal parasitic nematodes, proteases are present that can regulate neuropeptide signals (Sajid & Isaac, 1995; Sajid *et al.*, 1996, 1997). The demonstration of neuropeptide degrading activity in free-living nematode extracts expands these observations, and adds to the evidence that nematodes use and metabolize peptide hormones.

References

Baset, H.A., Ford-Hutchinson, A.W. & O'Neill, G.P. (1998) Molecular cloning and functional expression of a *Caenorhabditis elegans* aminopeptidase structurally related to mammalian leukotriene A_4 hydrolases. *Journal of Biological Chemistry* **273**, 27978–27987.

Brownlee, D.J.A. & Fairweather, I. (1999) Exploring the neurotransmitter labyrinth in nematodes. *Trends in Neurosciences* **22**, 16–24.

Chitwood, D.J., Lusby, W.R., Thompson, M.J., Kochansky, J.P. & Howarth, O.W. (1995) The glycosylceramides of the nematode *Caenorhabditis elegans* contain an unusual, branched-chain sphingoid base. *Lipids* **30**, 567–573.

Davenport, T.R.B., Isaac, R.E. & Lee, D.L. (1991) The presence of peptides related to the adipokinetic hormone/red pigment-concentrating hormone family in the nematode, *Panagrellus redivivus*. *General and Comparative Endocrinology* **81**, 419–425.

Davis, R.E. & Stretton, A.O.W. (1995) Neurotransmitters of helminths. pp. 257–287 *in* Marr, J.J. & Muller, M. (*Eds*) *Biochemistry and molecular biology of parasites*. New York, Academic Press.

Day, T.A. & Maule, A.G. (1999) Parasitic peptides. The structure and function of neuropeptides in parasitic worms. *Peptides* **20**, 999–1019.

Dorris, M., De Ley, P. & Blaxter, M.L. (1999) Molecular analysis of nematode diversity and the evolution of parasitism. *Parasitology Today* **15**, 188–193.

Gimenez-Pardo, C., Vazquez-Lopez, C., de Armas-Serra, C. & Rodriguez-Caabeiro, F. (1999) Proteolytic activity in *Caenorhabditis elegans*: soluble and insoluble fractions. *Journal of Helminthology* **73**, 123–127.

Hong, X., Bouvier, J., Wong, M.M., Yamagata, G.Y.L. & McKerrow, J.H. (1993) *Brugia pahangi*: identification and purification of an aminopeptidase associated with larval molting. *Experimental Parasitology* **76**, 127–133.

Keller, R. (1992) Crustacean neuropeptides: structures, functions and comparative aspects. *Experientia* **48**, 439–448.

Kubiak, T.M., Maule, A.G., Marks, N.J., Martin, R.A. & Wiest, J.R. (1996) Importance of the proline residue to the functional activity and metabolic stability of the nematode FMRFamide-related peptide, KPNFIRFamide (PF4). *Peptides* **17**, 1267–1277.

Masler, E.P., Kelly, T.J. & Menn, J.J. (1993) Insect neuropeptides: discovery and application in insect management. *Archives of Insect Biochemistry and Physiology* **22**, 87–111.

Masler, E.P., Wagner, R.M. & Kovaleva, E.S. (1996) *In vitro* metabolism of an insect neuropeptide by neural membrane preparations from *Lymantria dispar*. *Peptides* **17**, 321–326.

Muneoka, Y. & Kobayashi, M. (1992) Comparative aspects of structure and action of molluscan neuropeptides. *Experientia* **48**, 448–456.

Nassel, D.R. (1993) Neuropeptides in the insect brain: a review. *Cell and Tissue Research* **273**, 1–29.

Nelson, L.S., Kim, K., Memmott, J.E. & Li, C. (1998) FMRFamide-related gene family in the nematode, *Caenorhabditis elegans*. *Molecular Brain Research* **58**, 103–111.

Rayne, R.C. & O'Shea, M. (1992) Inactivation of neuropeptide hormones (AKH I and AKH II) studied *in vivo* and *in vitro*. *Insect Biochemistry and Molecular Biology* **22**, 25–34.

Rhoads, M.L., Fetterer, R.H. & Urban, Jr., J.F. (1998) Effect of protease class-specific inhibitors on *in vitro* development of the third- to fourth-stage larvae of *Ascaris suum*. *Journal of Parasitology* **84**, 686–690.

Rhoads, M.L., Fetterer, R.H. & Hill, D.E. (2000) *Trichuris suis*: a secretory serine protease inhibitor. *Experimental Parasitology* **94**, 1–7.

Sajid, M. & Isaac, R.E. (1995) Identification and properties of a neuropeptide-degrading endopeptidase (neprilysin) of *Ascaris suum* muscle. *Parasitology* **111**, 599–608.

Sajid, M., Keating, C., Holden-Dye, L., Harrow, I.D. & Isaac, R.E. (1996) Metabolism of AF1 (KNEFIRF-NH_2) in the nematode, *Ascaris suum*, by aminopeptidase, endopeptidase and deamidase enzymes. *Molecular and Biochemical Parasitology* **75**, 159–168.

Sajid, M., Isaac, R.E. & Harrow, I.D. (1997) Purification and properties of a membrane aminopeptidase from *Ascaris suum* muscle that degrades neuropeptides AF1 and AF2. *Molecular and Biochemical Parasitology* **89**, 225–234.

Shaw, C. (1996) Neuropeptides and their evolution. *Parasitology* **113**, S35–S45.

Smart, D., Johnston, C.F., Maule, A.G., Halton, D.W., Hrckova, G., Shaw, C. & Buchanan, K.D. (1995) Localization of *Diploptera punctata* allatostatin-like immunoreactivity in helminths: an immunocytochemical study. *Parasitology* **110**, 87–96.

Sokol, S.B. & Kuwabara, P.E. (2000) Proteolysis in *Caenorhabditis elegans* sex determination: cleavage of TRA-2A by TRA-3. *Genes and Development* **14**, 901–906.

Waggoner, L.E., Hardaker, L.A., Golik, S. & Schafer, W.R. (2000) Effect of a neuropeptide gene on behavioral states in *Caenorhabditis elegans* egg-laying. *Genetics* **154**, 1181–1192.

Zenka, J. & Prokopic, J. (1985) Aminopeptidase inhibitor from *Ascaris suum*. *Folia Parasitologica* **32**, 247–253.

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