In vitro degradation of the Neb-Trypsin Modulating Oostatic Factor (Neb-TMOF) in gut luminal content and hemolymph of the grey fleshfly, Neobellieria bullata

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Abstract

The unblocked hexapeptidic Trypsin Modulating Oostatic Factor of the fleshfly, an inhibitor of both trypsin and ecdysone biosynthesis, resists very well proteolytic breakdown by enzymes present in the lumen of the gut of previtellogenic fleshflies. However, when incubated in hemolymph of adult flies, females and males, its half-life time is a mere 0.5 min. In hemolymph of last instar larvae, this value increases to about 1.5 min. Whereas PMSF, a potent inhibitor of serine proteases has no effect, captopril and lisinopril, both known to be specific inhibitors of mammalian angiotensin I converting enzyme (ACE), effectively inhibit TMOF breakdown in fly hemolymph. Digestion of Neb-TMOF by recombinant Drosophila AnCE on itself results in identical degradation products as with total hemolymph. In both cases ESI-Qq-oa-Tof mass spectrometry demonstrated the appearance of peptide fragments with the sequences NPTN, LH and NP. These observations not only confirm the reported presence of circulating ACE-like activity in flies but also strongly suggest that in flies this hemolymph ACE-like activity might be involved in the regulation of the oostatic activity as exerted by Neb-TMOF. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Neb-TMOF; Neobellieria bullata; Diptera; Hemolymph; Midgut; Proteolytic breakdown; Angiotensin converting enzyme (ACE)

1. Introduction

The hazards of the use of conventional insecticides stimulated the search for alternative agents interfering with the endocrine system of insects. Analogues or agonists of juvenile hormone (JH) (e.g. methoprene, precocene) and ecdysone (e.g. RH-5992) are already used for insect control as insect growth regulators (IGRs). Peptidic regulatory molecules (e.g. neuropeptides) that control key physiological processes such as molting, metamorphosis, diuresis, JH- and ecdysone production, are also considered as potential candidates. Although these signalling peptides are unlikely to be used as insecticides per se, knowledge of their chemistry and biological action can be applied in novel approaches to insect control. Furthermore, the protein nature of neuropeptides makes them amenable to this control by the use of recombinant DNA technology and genetic engineering to have such peptides produced by transgenic crop plants or other suitable organisms.

The primary requirements for such a potential use are: (a) In order to be active, the peptides should not be blocked at either its N- or C-terminus, because plants cannot make these modifications. (b) It should not be quickly degraded by proteolytic enzymes in the gut lumen. (c) The peptide itself should be able to permeate the epithelium of the gut unaltered. (d) If it is partially degraded in the lumen, some of the degradation products should still retain physiological activity upon permeation through the epithelium. (e) Upon arrival in the hemolymph compartment, it should remain active long enough to exert its action (De Loof, 1996).

Dipteran Trypsin Modulating Oostatic Factors (TMOFs) have been forwarded as potential insecticides,
since they meet at least some of the criteria mentioned above (De Loof, 1996). The first identified TMOF (YDPAPPPPPP) originates from the mosquito Aedes aegypti and was named Aea-TMOF (Borovsky et al., 1990). It is produced by vitellogenic ovaries. A second TMOF-like hormone (NPTNLH) was subsequently purified from extracts of vitellogenic ovaries of the grey fleshfly Neobellieria bullata (Bylemans et al., 1994). Both TMOFs inhibit the de novo biosynthesis of trypsin by the midgut cells. This results in a lack of free amino acids for vitellogenin production in the fat body and consequently a termination of oocyte growth, hence the name TMOF. Neb-TMOF has no structural similarity with Aea-TMOF. However, computer modelling demonstrated that in both molecules, an aromatic amino acid (tyrosine for Aea-TMOF and histidine for Neb-TMOF) is exposed from the molecular axis (Bylemans et al., 1994). Strated that in both molecules, an aromatic amino acid with Aea-TMOF. However, computer modelling demonstrated that in both molecules, an aromatic amino acid (tyrosine for Aea-TMOF and histidine for Neb-TMOF) sticks out of the molecular axis (Bylemans et al., 1994). In Calliphora vomitoria, Neb-TMOF is also a very potent inhibitor of edcsyne biosynthesis (Hua et al., 1994). It is likely that TMOFs are widespread among insects and that, upon sequence determination, a pronounced structural variability and species specificity will show up (De Loof, 1996).

In order to answer the question about the stability of Neb-TMOF, in this study we investigated turnover rates of Neb-TMOF in both gut extracts and hemolymph of the fleshly Neobellieria bullata (Bylemans et al., 1994). In Calliphora vomitoria, Neb-TMOF is also a very potent inhibitor of edcysone biosynthesis (Hua et al., 1994). It is likely that TMOFs are widespread among insects and that, upon sequence determination, a pronounced structural variability and species specificity will show up (De Loof, 1996).

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2. Materials and methods

2.1. Insects

Neobellieria bullata was reared in our laboratory at 25°C with a 13:11 light–dark cycle. During the first 3 days of adult life, flies were fed a sugar–water diet. On day 4, when the first batch of ovarian follicles completes previtellogenic development, flies were offered pieces of beef liver as a protein source required for synthesizing vitellogenins. On day 7, the follicle cells synthesize the chorion and yolk synthesis is terminated. The eggs then descend into the uterus and embryonic development takes place during the next 3–4 days.

2.2. Peptide synthesis

Peptide acids were synthesized in the solid phase and on the 2-chlorotrotyl chloride resin (Barlos et al., 1991) using the Fmoc/t-Bu strategy while for the protection of the side chain of His the trityl (Trt) group was used.

Peptide chain elongation was performed on the H-His(Trt)-O-2-chlorotrotyl resin using exactly the procedure previously described (Poulos et al., 1994) except that the benzotriazolylester of the Fmoc-amino acids used in the coupling steps were prepared using diisopropylcarbodiimide (DIC) instead of dicyclohexylcarbodiimide (DCC).

Completion of the acylation reaction was indicated by a negative Kaiser test. After incorporation of each amino acid, resin samples were treated with a mixture of acetic acid (AcOH)/trifluoroethanol (TFE)/dichloromethane (DCM) (1:2:7 v/v) for 2 min. The intermediate peptide with the linker intact released from the resin was checked for purity by thin layer chromatography (TLC) in the solvent system toluene/AcOH/methanol (MeOH) (70:15:15) and/or reverse-phase high-pressure liquid chromatography (RP-HPLC). Retention times (tR) were measured by RP-HPLC Nucleosil C18 column 250×4.6 mm with the following solvent systems: A 0.1% trifluoroacetic acid (TFA) in water, B 0.1% TFA in CH3CN. Conditions: (S1) linear gradient from 95–5% (A:B) to 65–35% (A:B) for 30 min, (S2) linear gradient from 98–2% (A:B) to 85–15% (A:B) for 30 min, (S3) linear gradient from 80–20% (A:B) to 20–80% (A:B) for 30 min, UV detection at 214 nm, flow rate 1 ml/min.

The final products were obtained in two stages. In the first stage the peptide resin, after removing the Fmoc group, was stirred in 30 ml of TFE/DCM (3:7 v/v) for 2 h. Then the resin was filtered and washed three times with 10 ml of the above mixture. The combined filtrates were concentrated in vacuo and the residue was solidified upon the addition of dry ether to yield the corresponding side chain protected peptides. The Trt- and t-Bu side chain protected groups were removed by treating the above peptide with 20 ml of a mixture TFA/DCM/anisole/water (9:1:10:0.3 v/v) for 1 h. The solvent was then evaporated in vacuo and the residue was taken up in distilled water and lyophilized to yield the desired peptide. Purification of peptides was performed on a RP-HPLC Nucleosil C18 column 250×10 mm with the corresponding solvent systems used in analytical HPLC except that running time was 70 min, UV detection at 225 nm and flow rate 3 ml/min.

Pepites synthesized with the above procedure are:

- H–Asn–Pro–Thr–Asn–Leu–His–OH (Neb-TMOF): m/z 696 (M+H)\(^+\), tR=11.6 min (S1).
- H–Asn–dehydroPro–Thr–Asn–Leu–His–OH: m/z 694 (M+H)\(^+\), tR=9.9 min (S1).
- H–Asn–Pro–Thr–Asn–OH: m/z 445 (M+H)\(^+\), tR=5.8 min (S2).
- H–Thr–Asn–OH: m/z 234 (M+H)\(^+\), tR=5.1 min (S2).
- H–Leu–His–OH: m/z 267 (M+H)\(^+\), tR=10.6 min (S2).

The dipeptide H–Asn–Pro–OH was synthesized in the liquid phase and finally purified by semi-preparative RP-HPLC to yield the desired peptide: m/z 223 (M+H)\(^+\), tR=7.2 min (S2).

For tritation of Neb-TMOF the precursor compound H–Asn–dehydroPro–Thr–Asn–Leu–His–OH was used.
The reduction of the dehydroPro (catalytic tritiation) and removal of labile tritium was done by Cambridge Research Chemicals, UK. The crude material was further purified by HPLC as described before.

2.3. Protease inhibitors and enzymes

The protease inhibitors such as captopril, lisinopril, ethylenediamine tetraacetate (EDTA) and phenylmethanesulfonyl fluoride (PMSF) were purchased from Sigma. Recombinant Drosophila ACE-like enzyme (AnCE) was a kind gift of Dr E. Isaac, School of Biology, University of Leeds, UK. One microliter of this original enzyme preparation produced 24.3 nmol of hippurate/min when incubated at 37°C in 100 mM phosphate buffer (pH 8.3) containing 10 μM ZnSO4, 300 mM NaCl and 5 mM Hip–His–Leu.

2.4. Collection of hemolymph and gut contents

The hemolymph of 1-day-old, CO2 dazed adult flies was taken in a 4 μl glass capillary after cutting a leg and slightly squeezing the thorax. From older flies (2–8 day old females, 1-day-old males), that contain much less hemolymph, collection was done by centrifugation. The mouth was covered with a paraplast drop to avoid exudation of gut content. Small holes were made by a sharp needle in the cuticle between the eyes and on the dorsal side of thorax. The fly was then placed into a 200 μl pipette tip with its head forwards. The tip was inserted in an conical tube, and span down in a bench centrifuge at 2000 rpm for 5 min. Usually, hemolymph from 20 to 40 animals was pooled. Gut contents were collected in a similar way. In this case, the mouth was left open and no hole was made in the cuticle. Hemolymph from larvae was directly taken from a small wound made by fine scissors cutting in the head region. It was pooled and stored on ice until use.

2.5. Incubation of Neb-TMOF

In order to study its proteolytic breakdown, 1 μl of 3H-labeled peptide (20,000±2000 dpm/μl) was incubated at 30°C in 40 μl of gut content or hemolymph. Aliquots (5 μl) were taken at different time intervals (0.5, 5, 15, 30 and 60 min) and immediately mixed with 95 μl of 0.1% TFA in MilliQ water to stop enzyme activity. All samples were stored on ice until further analysis by HPLC. Experiments were repeated at least 3 times.

For the purification of Neb-TMOF degradation products, 30 μg of synthetic “cold” Neb-TMOF was incubated in 20 μl hemolymph from 1-day-old female flies at 30°C. After 0.5 and 5 min, 5 μl of hemolymph was removed and added to 95 μl of 0.1% TFA in MilliQ water.

2.6. HPLC analysis

Samples obtained during incubation of labeled and unlabeled Neb-TMOF were centrifuged at 13,000 rpm for 5 min at 4°C and analyzed by RP-HPLC on a Microsorb-MV C18 column (4.6×520 mm, Varian, USA). The gradient used was 0.1% TFA in MilliQ water for 5 min followed by a linear increase to 50% CH3CN containing 0.1% TFA in 30 min at a flow rate of 1 ml/min. The absorbance at 214 nm was monitored with an UV wavelength detector. Synthetic samples served as references. Fractions were collected at intervals of 0.5 min. For the analysis of [3H]Neb-TMOF cleavage products, radioactivity of each fraction was assayed with a liquid scintillation counter (Beckman LS 9000). Fractions gained from the incubation experiment with cold Neb-TMOF were used for the determination of the amino acid sequence of the degradation products.

2.7. Determination of amino acid sequence of the degradation products

Nanoflow electrospray (ESI) Quadrupole collision (Qq) orthogonal acceleration (oa) Time of Flight (ToF) mass spectrometry was performed on a Q-Tof hybrid ESI-Tof system (Micromass, UK) (Mozzis et al., 1996). One microliter of acetonitrile/water/formic acid (50:49:1) of the fractions containing the peptides, obtained from the HPLC run, was loaded in a gold coated capillary (Micromass type A nanoflow needle). This sample was sprayed at a flow rate of about 30 nl/min giving extended analysis time in which an MS spectrum as well as several MS/MS spectra were obtained. During MS/MS or tandem mass spectrometry, fragment ions are generated from a selected precursor ion by collision induced dissociation (CID). Argon was used as the collision gas. Since not all peptide ions fragment with the same efficiency, the collision energy is typically varied between 20 and 35 eV so that the parent ion is fragmented in a satisfying number of different daughter ions.

2.8. Inhibition of proteolytic breakdown in the hemolymph

The protease inhibitors captopril, lisinopril, EDTA and PMSF were dissolved in Hepes buffer in concentrations of 0.1–1000 μM, exceptionally up to 40 mM. Five microliters of these solutions were preincubated in 5 μl of hemolymph of 1-day-old flies for 20 min at 30°C. Reactions were initiated by adding 1 μl of [3H]-Neb-TMOF and the breakdown was studied as described before. Incubation of the radioactive peptide in hemolymph, diluted with an equal amount of Hepes buffer without such inhibitors, served as control.

Hemolymph of 4-day-old female flies, heat inactivated
in a water bath for 30 min at 65°C and further incubated with \[^{3}H\]Neb-TMOF, was used to verify the presence of proteases.

2.9. Hydrolysis of Neb-TMOF by recombinant Drosophila AnCE

Twenty micrograms of Neb-TMOF was incubated with recombinant Drosophila ACE-like enzyme (1 μl of pure enzyme preparation in 10 μl of Hepes buffer) for 10 min. The enzymatic reaction was stopped by adding 90 μl of 0.1% TFA in MilliQ. The samples were further purified on HPLC and fractions were analyzed by Q-Tof mass spectrometry as described above.

3. Results

3.1. Breakdown of Neb-TMOF in gut content and hemolymph

The degradation of labeled Neb-TMOF in gut content and hemolymph was followed over a period of 1 h. Analysis by HPLC showed that Neb-TMOF was found to be rather stable in the gut content of previtellogenic flies, which were only fed sugar (Fig. 1). Even 1 h after incubation, more than 90% of intact \[^{3}H\]Neb-TMOF could still be found in the incubation mixture. This peptide, however, was less stable in gut content of older flies fed liver. Only 20% intact peptide was detected after 15 min of incubation. Neb-TMOF was rapidly degraded in the hemolymph of female flies of the same age. Within 30 s, only about 20% of Neb-TMOF were left. Besides the intact peptide, two fractions with increased radioactivity could be registered [Fig. 2(A)]. One peak, representing 45% of total dpm, appeared at a retention time of about 14 min under the HPLC conditions used and was product P1. The other product (P2) had fewer radioactivities (18% of that of total incubated peptide) and eluted at about 12 min.

The amount of P1 decreased during the next minutes, whereas the radioactivity of P2 increased to about 24% during 5 min of incubation. At this time, <10% of intact \[^{3}H\]Neb-TMOF was found in the hemolymph. After 15 min of incubation, the combined amounts of \[^{3}H\]Neb-TMOF as well as that of the degradation products had fallen to 5–7% of the total radioactivity. More than 80% of the radioactivity had shifted towards the first fractions that eluted soon after starting the HPLC-run (with MilliQ water containing 0.1% TFA, further called "wash"). Thirty minutes after incubation, both degradation products could no longer be registered. The wash contained more than 90% and the fraction, corresponding to intact Neb-TMOF, <5% of the total dpm applied initially.

Incubation of \[^{3}H\]Neb-TMOF in hemolymph of 1-, 2- and 3-day-old female flies (data not shown) gave very similar degradation curves. The half-life time in all of these samples was found to be less than 0.5 min.

\[^{3}H\]-Neb-TMOF was also incubated in hemolymph samples of 5-, 6-, 7-, and 8-day-old female flies [Fig. 2(B)]. After collection of hemolymph, the flies were dissected, and the stage of ovarian development was determined under a microscope. Hemolymph fractions from females with aberrant ovarian development for a
given day were discarded. Although TMOF is supposed to be most active towards the end of vitellogenesis, the measured half-life time of [3H]Neb-TMOF in these hemolymph samples was still very short. In all the hemolymph samples we tested, more than 50% of radiolabeled peptide was degraded within 0.5 min. The appearance of degradation products P1 and P2 was comparable to incubation experiments with hemolymph of previtellogenic flies.

To examine if this peptide, which is synthesized by the ovaries of female flies, can also be degraded in the hemolymph of the male flies, [3H]Neb-TMOF was incubated in hemolymph taken from 1-day-old adult males [Fig. 2(C)]. Within 0.5 min, 65% of the peptide was degraded. Two degradation products appeared in the hemolymph and disappeared completely after 30 min of incubation, comparable to the situation in female flies.

TMOF was also incubated in hemolymph taken from unsexed last instar larvae. The degradation of [3H]Neb-

TMOF was found to be slower in larval hemolymph [Fig. 2(D)]. The half-life of this peptide was calculated to be 1–1.5 min. The degradation products were similar to those found in adult hemolymph (same retention time) but the products P1 and P2 persisted for a longer time in the larval hemolymph.

3.2. Purification and identification of degradation products

When non-labeled Neb-TMOF was incubated in hemolymph of 1-day-old adult females for 0.5 min, two peaks of degradation products appeared in the HPLC chromatogram with retention times of about 14 and 15 min corresponding to 15 and 17% CH3CN. After incubation for 5 min, a third, more polar peak with a retention time of about 12 min (12% CH3CN) was registered (Fig. 3). The retention times of the first and third peak were similar to those of the degradation products found...
Fig. 3. HPLC chromatogram of the Neb-TMOF degradation products P1, P2 and P3.

during the study with [3H]Neb-TMOF, which were called P1 and P2.

The masses of the purified degradation products (P1, P2 and P3), measured using Q-Tof-MS, were found to be 445.17, 229.97 and 269.21 Da, respectively. These masses correspond to the calculated values of NPTH, NP and LH (Table 1). Fragmentation of these ions by CID on an ESI-Qq-oa-Tof mass spectrometer confirmed these sequences (Figs. 4 and 5). The retention times found during HPLC analysis were similar to those obtained with the synthetic peptides.

3.3. Inhibition of proteolytic breakdown in the hemolymph

The fast degradation of [3H]-Neb-TMOF by fly hemolymph indicates the existence of a protease(s) in the fly hemolymph. To check if the hydrolysis of TMOF is due to enzymatic activity, hemolymph of female adult flies was heated at 65°C for 30 min. Neb-TMOF remained very stable in such heat-inactivated hemolymph. One hour after incubation, about 90% of the intact radioactive peptide was still present (data not shown). In contrast, [3H]Neb-TMOF was degraded very fast in the control samples.

Further studies were done incubating Neb-TMOF with protease inhibitors. In order to see if a serine protease inhibitor could stop the breakdown, hemolymph of 1-day-old adult females was mixed with PMSF. This compound could not inhibit the degradation of [3H]Neb-TMOF, even at 20 mM (Fig. 6).

Incubation with EDTA (10 mM) terminated the degradation of Neb-TMOF in the hemolymph of 1-day-old female flies and pointed towards metalloprotease activity being responsible for the fast degradation. It is interesting that incubation with captopril (0.5 mM) also stopped the enzymatic degradation in the hemolymph of 1-day-old male and female flies. The results indicated that an angiotensin converting enzyme-like (ACE) protease, of which captopril is a specific inhibitor, is present in the hemolymph of the fleshfly N. bullata.

Moreover, lisinopril, another specific ACE inhibitor, also blocked degradation of Neb-TMOF. Detailed inhibition studies with various concentrations showed that the IC50 is about 1 μM, which is lower than that of captopril which is about 10 μM (Fig. 7).

3.4. Hydrolysis of Neb-TMOF by recombinant Drosophila AnCE

Two degradation products that have identical retention times as those of P1 and P3 were identified by ESI-Qq-Tof-MS after degradation of TMOF with Drosophila AnCE. The measured masses of these peptides are 445.17 and 269.21, which is identical to the calculated masses of NPTN, and LH (see Table 1). Fragmentation analysis by CID further confirmed these sequences. We were not able to identify degradation product P2 due to its low concentration in the HPLC fraction. However, in studies with labeled Neb-TMOF, P2 (radioactivity) appeared with the same retention time as a peak seen in the chromatogram of samples incubated in either fleshfly hemolymph or fruitfly AnCE. A longer incubation time for the Neb-TMOF with Drosophila AnCE was needed to see the peak of P2 in the HPLC chromatogram.

4. Discussion

The major result of this paper is that Neb-TMOF resists much better to degradation by proteases present in the gut than to the ones circulating in the hemolymph. About 90% of [3H]Neb-TMOF remained intact after 1 h of incubation in gut contents taken from previtellogenic flies that were fed sugar and water only. In liver fed 4-day-old animals, the degradation was faster, although after about 17 min, still 25% of the administered TMOF remained intact. This relatively high stability of Neb-TMOF in this protease-rich environment is not totally unexpected because the sequence NPTNLH does not contain any of the typical cleavage sites for the
known trypsins and chymotrypsins. The difference could be explained by the fact that intake of liver juice either stimulates the secretion by the midgut cells of additional enzyme types, or the non-sterile food source introduces such proteases. In the perspective of the potential use of TMOF for insect control purposes, the observed stability in the gut lumen is acceptable. Indeed, the transepithelial transport of Neb-TMOF from the lumen into the hemolymph compartment is a fast process that only takes minutes (Zhu et al., in preparation). Effective transepithelial transport of TMOF is also supported by data from feeding experiments which show that oral uptake of Neb-TMOF results in inhibition of trypsin biosynthesis and ovarian development in this fleshfly (Janssen et al., in preparation).

It is surprising that Neb-TMOF is so quickly degraded in the hemolymph of all the life stages we tested: larvae, previtellogenic and vitellogenic females as well as males. The half-life of the peptide in adult flies is less than 0.5 min. The metabolism of the degradation products is slower. Most of the products disappeared within 20 min after incubation in adult hemolymph. The fast
proteolytic cleavage of Neb-TMOF in the hemolymph indicates that, compared to the situation in the gut, the hemolymph contains other or additional proteases. This was supported by the fact that the serine protease inhibitor PMSF could not inhibit the degradation of Neb-TMOF in hemolymph.

In addition to the selective inhibition of the proteolytic degradation of Neb-TMOF by both captopril and lisinopril and the demonstrated ability of recombinant *Drosophila* AnCE to process Neb-TMOF in an identical manner as does crude hemolymph, there are several arguments that an ACE-like protease is responsible for the breakdown of TMOF in the hemolymph of flies (Fig. 8). Indeed, there is exhaustive evidence that ACE-like enzymes are present in different fly species (Lamango et al., 1996; Wijffels et al. 1996, 1997; Schoofs et al., 1998; Ekbote et al., 1999). Isaac and Lamango (1994) priorly reported the occurrence of such enzymes in free circulation and this observation was later confirmed from gene sequencing data which indicated the presence of an amino-terminal signaling peptide for secretion (Williams et al., 1996) in the pre-enzyme. Similar to mammalian ACE, the fly ACE-equivalent preferentially cleaves C-terminal dipeptides as observed in our experiments. Finally the observation of insect ACE being a candidate modulator of ecdysone synthesis (Loeb et al., 1998) is in good agreement with the knowledge of Neb-TMOF being a potent inhibitor of ecdysone biosynthesis (Hua et al., 1994).

Martensen et al. (1998) investigated the degradation of Neb-TMOF by homogenated and soluble membrane fractions of the blowfly *Calliphora vicina*. The authors found that in this particular species the peptide was hydrolyzed by a proline-specific dipeptidyl peptidase releasing the fragment NP from the N-termmini. Obviously, the enzyme differs from that found in the hemolymph of *N. bullata* which cleaves dipeptides from the C-terminal side of Neb-TMOF and was selectively inhibited by captopril, not by PMSF.
Nevertheless, we had expected to find a slower degradation of Neb-TMOF towards the end of vitellogenesis when this ovarian peptide starts to exert its oostastic activity by modulating trypsin biosynthesis in the gut at the translational level (Borovsky et al., 1996). Even in this stage, the breakdown is very fast and seemingly puzzling. It should however be noticed that very low concentrations of Neb-TMOF still efficiently inhibit trypsin biosynthesis in Neobellieria (Bylemans et al., 1994; Janssen et al., 1998). At this moment it can not be excluded either that Neb-TMOF itself acts as a precursor for a cleavage product (NPTN or and LH) that by itself is the most active inhibitor. In mammals, ACE also activates the precursor angiotensin I to the active vasopressor angiotensin II. Experiments to investigate this suggested trypsin modulating activity by Neb-TMOF cleavage products LH, NPTN and/or other fragments will be started soon.

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References


