BIOORGANIC CHEMISTRY **26,** 131–140 (1998)
ARTICLE NO. BH981092

The C-Terminus Shortened Analogs of the Insect Peptide Oostatic Hormone with Accelerated Activity

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Received February 11, 1998

Shortened analogs **1a–1f** of the oostatic hormone **1g** with a decreased number or an omission of the C-terminal Pro residues were synthesized for investigation of their effect on oogenesis in the flesh fly *Sarcophaga bullata Parker* (Diptera). Peptides **1b–1g** were prepared by solid-phase synthesis on polystyrene/divinylbenzene polymer with a 2-chlorotrityl linker using Fmoc/tBu strategy in dimethylformamide (DMF). Tetrapeptide **1a,** devoid of all the carboxy-terminal Pro residues, was prepared by stepwise procedure in DMF solution from HCl-H-Ala-OMe using Boc-Pro-OH, Z-Asp(OtBu)-OSu, and Boc-Tyr-OSu. In general, the peptides assayed affected processes of egg development in 20–80% of ovarioles causing changes in the follicular epithelium, proliferation of its nuclei, and cell division toward the inner part of the egg chamber. This process leads to the occurrence of a multilayered follicular epithelium which later fills up almost the whole egg chamber and then degenerates. Such ovarioles are never able to produce eggs. The shortening of the peptide sequence accelerated the observed effects on egg development. The application of the ^{125}I-labeled peptides made possible to express quantitatively the time dependence of the radioactivity distribution in the insect body, including ovaries through which it is transferred to the next F1 generation. The radioactivity was measured in larvaes and, consequently, in newborn flies. For different peptide sequences applied, different radioactivity distributions in the insect body were found using the same radiolabeling.

INTRODUCTION

Oostatic or antigonadotropic insect hormones have been found in a number of insect species (*1*). Some time ago, Borovsky *et al.* isolated another member of this class of reproductive and developmental hormones from mosquito and described it (*2*) to be an unusual decapeptide **1g** containing six C-terminal Pro (*3*) residues. This molecule was postulated to affect oogenesis through modulation of ovarian ecdysteroid synthesis, gut trypsin synthesis, or egg development neurosecretory hormone release in the Diptera and to influence a juvenile hormone action on ovarian yolk uptake in some Hemiptera. The peptide was designed to function as a signal that terminates vitellogenesis.

In our preliminary communication (*4*) we described the solution synthesis and oostatic properties of some peptides with the sequence of the oostatic hormone **1g** and its shorter analogs **1d–1f,** differing in a number of the C-terminal Pro residues.

0045-2068/98 $25.00

A comparative study of the hormone **1g** and one of the shorter analogs (**1e**) revealed certain effects on processes of egg development. They were manifested by disturbances of yolk deposition, changes of follicular epithelium, and formation of nonviable eggs. The presence of Tyr in the peptides prepared allowed us to carry out the radiotracer distribution studies using ^{125}I-labeled molecules of **1d** and **1g** and we quantitatively described a presence of the labeled peptide material in various parts of the insect body (ovaries, head, intestine).

H-Tyr-Asp-Pro-Ala-Pro$_n$-OH
1a $n = 0$, **1b** $n = 1$, **1c** $n = 2$, **1d** $n = 3$, **1e** $n = 4$, **1f** $n = 5$, **1g** $n = 6$
2a Boc-Pro-Ala-OMe
2b Z-Asp(OtBu)-Pro-Ala-OMe
2c Boc-Tyr-Asp(OtBu)-Pro-Ala-OMe
2d Boc-Tyr-Asp(OtBu)-Pro-Ala-OH

The results obtained in our first study have inspired us with an idea to perform similar studies on analogs with a peptide sequence even more shortened from the C-terminus. Therefore, we synthesized peptides **1a–1c** to compare their oostatic properties with peptides described earlier, including the native decapeptide **1g.**

Tetrapeptide **1a,** devoid of all C-terminal Pro residues, was prepared by stepwise procedure in solution. The synthesis was started from HCl · H-Ala-OMe which was coupled to Boc[1]-Pro-OH in DMF using DCC and HOBt in the presence of DIEA. The protected dipeptide **2a** was treated with TFA and the resulting TFA.H-Pro-Ala-OMe acylated by Z-Asp(OtBu)-OSu in DMF to give the protected tripeptide **2b.** After removal of the Z-protecting group by hydrogenolysis on Pd black in MeOH, the Boc-Tyr-OSu was used to complete the sequence of the protected tetrapeptide **2c** which was saponified to acid **2d** which was finally deprotected by the mixture TFA–anisole (9 : 1).

Peptides **1b** and **1c** and the larger peptides described previously (**1d–1g,** Ref. 4) were prepared by solid-phase approach using a polymer with a 2-chlorotritylchloride linker. We have utilized Fmoc/tBu protection of amino acids which were coupled in DMF by means of DCC/HOBt. The Fmoc protecting group has been removed by piperidine in DMF.

In the last step of the syntheses the peptides were deprotected and split off the resin by trifluoroacetic acid in the presence of anisole.

The peptides synthesized were purified by preparative HPLC and characterized by means of amino acid analysis, mass spectrometry, and analytical HPLC.

To illustrate the fate of the shortened analogs after their application to insect, basic morphological, histological, and radiotracer studies were carried out using tetrapeptide **1a** and pentapeptide **1b,** respectively, including their ^{125}I labeling. The results were compared with those obtained for the larger peptides.

[1] Abbreviations used: Boc, *tert*-butyloxycarbonyl; DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DMF, dimethylformamide; HOBt, hydroxybenzotriazole; OSu, succinimide ester; tBu, *tert*-butyl; TDM, 4,4′-tetramethyldiamino-diphenylmethane; TFA, trifluoroacetic acid.

MATERIAL AND METHODS

Materials

Z-Asp(OtBu)-OSu was purchased from Bachem (Switzerland), whereas all other amino acid derivatives were prepared in our laboratory following general protocols (*5*) and were checked for their purity by TLC, HPLC, elemental analysis, and mass spectrometry. 2-Chlorotritylchloride resin (1.3 mmol/g) was purchased from Calbiochem–Novabiochem AG (Switzerland).

General Methods

TLC of the protected amino acids and peptides prepared was performed on precoated Silufol$_{254}$ plates (Kavalier, Czech Republic) in the following systems: 2-butanol–98% formic acid–water (75 : 13.5 : 11.5) (S1); 2-butanol–25% aqueous ammonia–water (85 : 7.5 : 7.5) (S2); 1-butanol–acetic acid–water (4 : 1 : 1) (S3); and 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S4). The compounds were visualized directly under an ultraviolet light or by ninhydrin and chlorine–TDM detection (*6*). Melting points were measured on a Kofler block and were not corrected. Solvents were evaporated *in vacuo* on a rotary evaporator (bath temperature 30°C); DMF was evaporated at 30°C and 150 Pa. Analytical electrophoresis was carried out in a moist chamber on Whatman 3MM paper (20 V/cm) in 6% acetic acid (pH 2.4) and in a pyridine–acetate buffer (pH 5.7) for 60 min. Optical rotations were obtained on a Perkin–Elmer 141 MCA polarimeter at 20°C. The samples for amino acid analysis were hydrolyzed by 6 M HCl at 110°C for 20 h with 3% phenol. The amino acid analyses were performed on a Durum D-500 amino acid analyzer (Durum Instruments, USA). The molecular weights of the peptides were determined using mass spectrometry with FAB technique (VG Analytical, England). For HPLC, a Spectra Physics instrument with an SP 8800 pump, an SP 8450 UV detector, and an SP 4290 integrator was used. Peptides **1a–1g** were purified by preparative HPLC on a 35 × 2.5-cm RP-18 column, 10-μm (Vydac, The Separations Group, Hesperia, USA). Analytical HPLC was carried out on a 250 × 4-mm RP-18 column, 5-μm (LiChrospher WP-300, Merck Darmstadt, Germany). The samples for the elemental analysis performed at the analytical department of the above institute were dried over P_2O_5 at room temperature at 150 Pa.

Syntheses

tert-Butyloxycarbonyl-prolyl-alanine methyl ester (**2a**). A solution of Boc-Pro-OH (13.8 g; 70 mmol), HOBt (10.3 g; 77 mmol), and DCC (14.4 g; 70 mmol) in DMF (40 ml) was stirred at −10°C for 30 min and then filtered to a cooled solution of HCl.H-Ala-OMe (10 g, 72 mmol) in DMF (10 ml) and pH 7.5 adjusted by DIEA. The reaction mixture was stirred for 2 h at 0°C followed by stirring overnight at room temperature. The mixture was then cooled in refrigerator and the precipitated dicyclohexylurea separated by filtration and DMF evaporated. The residue was dissolved in ethyl acetate (EtOAc) and the solution washed three times with 1 M $NaHCO_3$, H_2O, 20% citric acid, and a saturated solution of Na_2SO_4. After drying

by anhydrous Na_2SO_4 the EtOAc solution was evaporated and the resulting product crystallized from EtOAc–light petroleum to give 13.4 g of the protected dipeptide **2a** with m.p. 77–80°C. $[\alpha]_D$ −57.7° (c 0.5, DMF), −93.3° (c 0.4, MeOH). For $C_{14}H_{24}N_2O_5$ (300.4) calculated: 55.99% C, 8.05% H, 9.33% N; found: 55.78% C, 8.01% H, 9.21% N. Mass spectrum (FAB), *m/z* 301.2 (M^+ +1). Amino acid composition: Ala (1), Pro (1.02).

N-Benzyloxycarbonyl-O-tert-butylaspartyl-prolyl-alanine methyl ester (**2b**). The Boc-Pro-Ala-OMe (**2a,** 6.5 mmol, 1.95 g) was treated with TFA (10 ml) for 30 min and the resulting TFA · H-Pro-Ala-OMe ($E^{2,4}_{Gly}$ 1.44; $E^{2,4}_{His}$ 0.95; $E^{5,7}_{His}$ 0.99) was acylated by Z-Asp(OtBu)-OSu (7 mmol, 2.8 g) in DMF (15 ml) in the presence of HOBt (6.5 mmol, 0.87 g) at room temperature and pH 7 was maintained by DIEA. The reaction mixture was worked up after 2 days similarly to preparation of **2a** to give 3.9 g of the protected tripeptide **2b.** The pure product with m.p. 103–105°C was obtained on crystallization from EtOAc–light petroleum. $[\alpha]_D$ −67.2° (c 0.3, DMF). For $C_{25}H_{35}N_3O_8$ (505.6) calculated: 59.39% C, 6.98% H, 8.31% N; found: 59.32% C, 7.01% H, 8.45% N. Mass spectrum (FAB), *m/z*: 506.2 (M^+ +1). Amino acid composition: Asp (1.02), Ala (1.0), Pro (0.95).

N-tert-Butyloxycarbonyltyrosyl-O-tert-butylaspartyl-prolyl-alanine methyl ester (**2c**). The Z-Asp(OtBu)-Pro-Ala-OMe (**2b,** 2.02 g, 4 mmol) in MeOH (100 ml) was hydrogenated in the presence of Pd black for 3 h, and the resulting H-Asp(OtBu)-Pro-Ala-OMe ($E^{2,4}_{Gly}$ 0.99; $E^{2,4}_{His}$ 0.67; $E^{5,7}_{His}$ 0.76) was acylated by Boc-Tyr-OSu (4 mmol, 1.5 g) in DMF (15 ml) in the presence of HOBt (4 mmol, 0.54 g) for 2 days. The reaction mixture was worked up similarly to the synthesis of **2a** with a yield of the amorphous **2c** (2.4 g). TLC: 0.73(S1), 0.77(S2). For $C_{31}H_{46}N_4O_{10}$ (634.7) calculated: 58.66% C, 7.30% H, 8.83% N; found: 58.44% C, 7.41% H, 8.97% N. Mass spectrum (FAB), *m/z* 635.3 (M^+ +1). Amino acid composition: Tyr (0.97), Asp (1.02), Ala (1.0), Pro (1.01).

N-tert-Butyloxycarbonyltyrosyl-O-tert-butylaspartyl-prolyl-alanine (**2d**). The solution of the Boc-Tyr-Asp(OtBu)-Pro-Ala-Pro-OMe (**2c,** 1.27 g; 2 mmol) in acetone (30 ml)–water (3 ml) was stirred with 2 M NaOH (1 ml; 2 mmol) at room temperature for 3 h. Water was added (10 ml), acetone evaporated, and the alkaline solution washed with EtOAc and acidified by 20% citric acid to pH 3.5. The oily product was taken to EtOAc and the EtOAc solution washed by water, dried by anhydrous Na_2SO_4, and after filtration evaporated to dryness. The residue was solidified from ether–light petroleum to give the protected tetrapeptide acid **2d** (0.98 g) with TLC: 0.57 (S2), 0.62 (S4). $[\alpha]_D$ −112° (c 0.4; DMF). For $C_{30}H_{44}N_4O_{10}$ (620.7) calculated: 58.05% C, 7.15% H, 9.03% N; found: 57.81% C, 7.04% H, 9.29% N. Mass spectrum (FAB), *m/z* 621.3 (M^+ +1). Amino acid composition corresponded to that of the methyl ester **2c.**

Tyrosyl-aspartyl-prolyl-alanine (**1a**). The protected tetrapeptide **2d** (0.19 g; 0.3 mmol) was dissolved in a mixture of TFA (5 ml)–anisole (0.5 ml) at room temperature and after 1 h the solution was evaporated and the residue dissolved in 10% AcOH (200 ml), washed three times with ethyl acetate, and freeze dried. The peptide was purified by preparative HPLC on a 30 × 2.5-cm column filled with Vydac stationary phase with a gradient of 5–20% ACN in 0.05% TFA in 30 min

TABLE 1
Analytical Data on Peptides **1a–1c**

Compound[a] HPLC[e]	Formula[b] MW/(M^+ + 1)	AAA[c] Tyr	Asp	Ala	Pro	$E^{2.4,d}$ E_{gly}	E_{his}	$E^{5.7}$ E_{Pic}
HTyrAspProAlaOH 8.59 (**1a**)	$C_{21}H_{28}N_4O_8$ 464.8/465.3	1.03	1.01	1.0	0.98	0.75	0.51	0.29
HTyrAspProAlaProOH 11.80 (**1b**)	$C_{26}H_{35}N_5O_9$ 561.2/562.2	1.02	1.02	1.0	2.1	0.72	0.48	0.27
HTyrAspProAlaPro$_2$OH 14.17 (**1c**)	$C_{31}H_{42}N_6O_{10}$ 658.7/659.2	1.04	1.03	1.0	2.9	0.68	0.44	0.24

[a] Analytical data of peptides **1d–1g,** see (4).
[b] Determined with FAB technique (VG Analytical, England).
[c] Amino acid analyses were performed on a Durum D-500 AA analyzer (Durum Instruments, Corp., Palo Alto, CA).
[d] Analytical electrophoresis was carried out in a moist chamber on Whatman 3MM paper (20 V/cm) in 6% acetic acid (pH 2.4) and in a pyridine–acetate buffer (pH 5.7) for 60 min.
[e] Retention time in minutes, 25 × 0.4-cm RP-18 column, 5-μm (LiChrospher WP-300, Merck Darmstadt, Germany), flow rate 60 ml/h, detection at 222 nm, gradient 5–50% of acetonitrile in 0.05% aqueous TFA, 25 min; Spectra Physics 8800 HPLC pump with SP 8450 UV/VIS detector and SP 4290 integrator.

and characterized by mass spectrometry, amino acid analysis, electrophoresis, and analytical HPLC (Table 1).

Solid-Phase Synthesis of Peptide **1b**

The solution of Fmoc-Pro-OH (0.92 g; 2.73 mmol) in dry DCM (30 ml) containing 2 ml of dry DMF (just enough to make the amino acid dissolve in DCM) was added to the 2-chlorotritylchloride resin (3 g) together with about one-third of the total amount (1.2 ml, 7 mmol) of DIEA. After 5 min of stirring the remainder of the DIEA in DCM (1 : 1) was added. Vigorous stirring was prolonged for an additional 60 min. At the end of this time an excess of HPLC-grade MeOH (3 ml) was added and stirring continued for 10 min to end cap the remaining trityl groups on the resin. The resin was then washed three times with 30-ml portions of DCM, DMF, methanol, and diethylether and dried over KOH in a dessicator (3.6 g).

The Fmoc-Pro-2-chlorotrityl resin (0.6 g) was swollen in DMF/DCM (1 : 1) and deprotected by 5% piperidine in DMF for 10 min (30 ml) followed by 20% piperidine in DMF (30 ml) for 15 min. The free amino acid resin was then gradually acylated with three equivalents of Fmoc-Ala-OH (0.29 g), Fmoc-Pro-OH (0.31 g), Fmoc-Asp(tBu)-OH (0.38 g), and Fmoc-Tyr(tBu)-OH (0.43 g) activated by HOBt/DCC mixture (0.19 g, 1.4 mmol/0.21 g/1.02 mmol) in DMF (30 ml). At each step cleavage of the Fmoc group has been done by 20% piperidine in DMF (2× 30 ml, 30 and

45 min). Finally, the TFA/anisole mixture (10 ml; 9:1) was used to carry out the simultaneous cleavage of the side-chain tBu protecting groups and peptide **1b** from the resin.

The Fmoc-Pro-2-chlorotrityl resin was further treated similarly to the preparation of **1b** using corresponding amino acids with regard to a number of Pro residues in the C-terminus of the resulting peptides **1c–1g**.

Peptides **1b–1g** were purified and characterized under the same conditions used for peptide **1a.** The analytical data of the new peptides **1a–1c** are shown in Table 1. Those of the newly synthesized peptides **1d–1g** were identical to data of the peptides synthesized early by a solution method as described in our previous paper (4).

Iodination with ^{125}I

A solution of corresponding peptide (5 mg) in 0.05 M sodium phosphate buffer, pH 7.5 (25 ml), was placed in an IODO-GEN tube (*7*) washed earlier with this phosphate buffer. To this solution the 10 ml of $Na^{125}I$ (corresponding to 37 MBq) was added and after 60 min the reaction was stopped by transporting the reaction mixture out of the IODO-GEN tube to a test tube and adding the 35 ml of 0.05 M KI. Separation of the iodinated peptide from unreacted $Na^{125}I$ was performed on a column of Sephadex G-25 (55 × 1.4 cm) which was equilibrated with 0.02 M sodium phosphate buffer.

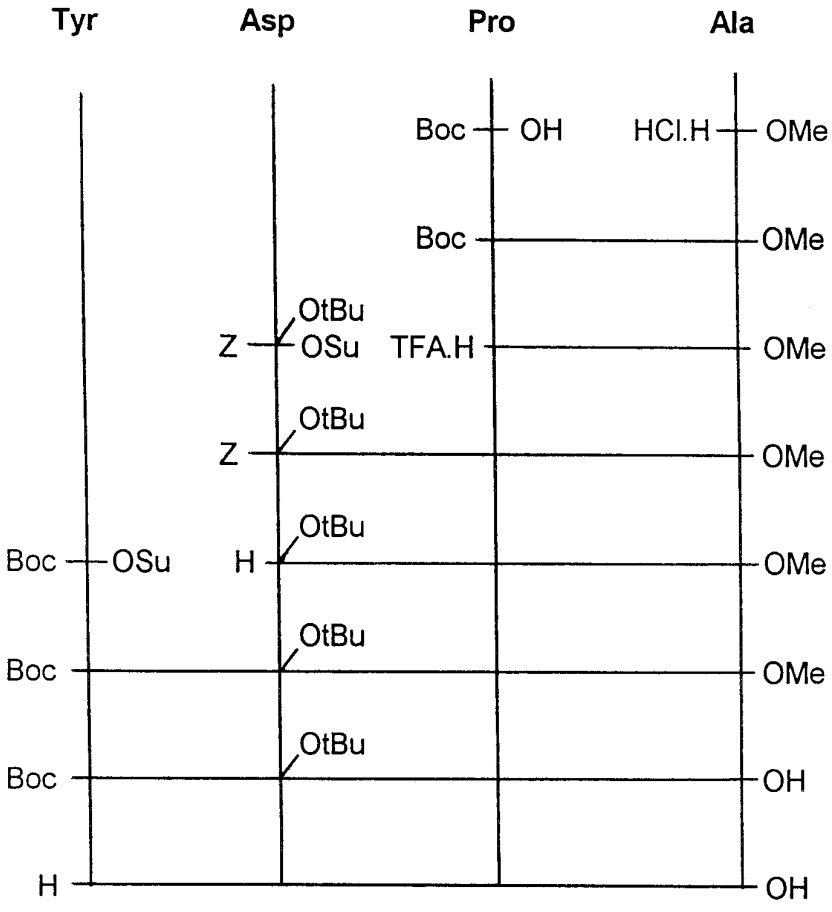
Bioassays and Radiotracer Distribution Studies

The peptides under study were separately injected in Ringer solution (1 μg/ml, 3 μl per female) into the upper part of the thorax of freshly emerged adult females of the flesh fly *Sarcophaga bullata Parker* (Diptera). The flies were dissected at regular time intervals (on days 1, 2, 7, 8, and 15 after application) and their ovaries were examined for changes in egg development during the first gonotrophic cycle. Egg hatchability was determined on deposited eggs in the uterus. The ovaries undergoing the second gonotrophic cycle were examined as well. Histological preparations made of suspect ovaries of both gonotrophic cycles were prepared by standard procedure. Differences in the degree of inhibition of correct egg development were evaluated with regard to the regular time intervals after peptide application and the peptide length. A general relationship between a decreasing number of the C-terminal Pro residues in peptides **1a–1g** and the beginning of the oostatic effect was evaluated as an acceleration phenomenon.

For radioactivity microdistribution within an insect body, a dissected organ or an appropriate body part was dissolved in 1 ml of a scintillation cocktail (Soluene 350, Packard) using shaking at 40°C for 48 h. Liquid scintillation spectrometry (Beckman, LS 6500) was then used for radioactivity measurement.

RESULTS AND DISCUSSION

For the synthesis of peptides **1b–1g** the solid phase approach was chosen. After the first Fmoc-Pro residue was bound to the resin and the Fmoc protecting group was split off by 20% piperidine in DMF, the polymer material was used for synthesis



SCHEME 1. Synthesis of tetrapeptide **1a.**

of the corresponding peptides with a gradually growing chain. The solid-phase synthesis was carried out with the 2-chlorotrityl linker which is particularly suited to the synthesis of the Pro peptides. The extreme steric hindrance of the trityl group means that formation of dioxopiperazine at the dipeptide step, with consequent loss of peptide from the resin, is totally suppressed.

Tetrapeptide **1a** without the C-terminal Pro residue was prepared in solution similarly to the first series of our oostatic peptides described in our previous paper (*4*) (Scheme 1).

Morphology and Histology

Using tetrapeptide **1a** and pentapeptide **1b** for application, morphological and histological observations revealed changes in ovarian development. Morphologically affected egg chambers have irregular appearance; their shape is often distorted.

Histological evaluation revealed the following events: (1) a division of the nucleolus of the follicular cell which is followed by nuclear division and cell division; and (2) the rapid cell division and migration of such cells into yolk as well as into a region of nutritive cells, followed by a complete desintegration of the yolk and nutritive cells. In this way, the whole inner space of the egg chamber is filled up by those proliferating cells. Finally, resorption of such an affected egg chamber occurred.

The occurrence of those changes varies according to the length (a number of the Pro residues) of the C-terminus for the whole series of the peptides investigated (**1a–1g**). A shortening of the peptide molecules from the C-terminus was followed by reaching the given biological effect in the shorter time period. The changes appear mostly in the development of the second batch of eggs; the changes in the first gonotrophic cycle are manifested by decreased hatchability of eggs in the uterus. For example, comparing decapeptide **1g** which allows up to 80% of egg hatchability, the pentapeptide **1b** application decreases egg hatchability to 40% and that of tetrapeptide **1a** up to 20%. As a result the tetra- and pentapeptides **1a** and **1b** were found to effect more ovarioles in comparison to the larger peptides **1c–1f** and even more to the native decapeptide **1g** due to reaching the oostatic effect at the beginning of the bioassay. On the other hand, larger peptides **1c–1g** including the native one needed more time to evoke a similar biological effect.

Comparing the effects of tetrapeptide **1a** and pentapeptide **1b** with those of the larger peptides **1d–1g,** the final effect after day 15 is the same—resorption, but the initial changes—proliferation and cell migration detected within the first period of the bioassay (the first gonotrophic cycle)—are most pronounced using tetrapeptide **1a** and pentapeptide **1b.** It follows from a comparison to the previously assayed peptide series including decapeptide **1g** (*4*) that the shortest peptides reveal significantly accelerated activity (*8*).

Radioactivity Microdistribution

The radiolabeled forms of tetrapeptide **1a** and pentapeptide **1b** with the most pronounced oostatic effect were used to describe the transportation of the applied radioactivity through the insect body and its transfer from the treated female body to the next (F1) generation. Such investigations could considerably contribute to analyses of the mode of action of the peptides under investigation within the treated body and to the description of the transfer to the next generation including that affecting of this generation (*8*).

It follows from the results given in Table 2 that the excretion of tetrapeptide **1a** shortly after its application is more intensive than that of pentapeptide **1b** under the same experimental conditions. Therefore, a lower radioactivity remains available for distribution within the insect body during days 1 and 2 after application of tetrapeptide. Nevertheless, as it was described, proliferation and cell migration representing the initial effects are more pronounced using tetrapeptide. All obtained findings so indicate an accelerated activity of tetrapeptide in comparison to pentapeptide. Twenty-four hours after separated application of both radiolabeled peptides, radioactivity was already detected in all other analyzed body parts, i.e., head, fat body, and ovaries.

TABLE 2
Relative Mean Radioactivities (in Percentage of the Applied Radioactivity) after Application of the Radiolabeled Tetrapeptide (T) or Pentapeptide (P), Respectively

Days after application	1		2		7		15	
Peptide	T	P	T	P	T	P	T	P
Gut	21.3	17.3	10.1	4.7	8.9	4.6	0.7	1.6
Uterus with eggs							4.5	5.5
Uterus with larvae							7.4	6.4

Note. $N = 10$. SD of each radioactivity measurement $< \pm 1\%$.

The main result of the paper on freshly emerged *Sarcophaga bullata* female flies is to demonstrate that the development of the egg chamber and yolk deposition were affected by the injection of both the oostatic hormone and peptide sequences with a lower number of proline residues, in that way, shortening of the carboxy terminus enhances the observed effects by affecting the first gonotrophic cycle as well and thus shortens the time interval after injection at which time the morphological changes appear.

From this it could be suggested that the oostatic hormone with the six C-terminal proline residues can play, under physiological conditions, a role of hormonogene or prohormone. A space arrangement of the proline residues could exhibit an important role in active sequence protection against premature enzyme degradation during peptide transport to a corresponding receptor and even more in binding of the peptide to this receptor. Once a conformation of the decapeptide is changed within a complex hormone receptor, an active amino-terminal tetrapeptide part can be released for the next biological function including a transduction of the biological effect. Our shortened peptides, especially tetrapeptide **1a** without C-terminal proline residues, could participate in biological activity transduction directly, thus accelerating oostasis and also causing total damage and resorption of the egg.

As to the radiolabeling study performed and described in our paper, the main goals of application of the radiolabeled peptides were:

(1) To prove different changes in radioactivity distribution within an insect body in different time intervals after injection. In this way it has been relatively simply demonstrated that the kinetics of the different labeled peptide molecules under study in the insect body are different. The determination of the radioactivity distribution within the fly body as an introductory act was important for seeing what part of the body is worth detailed structure analysis of the isolated peptide material to be performed during further research.

(2) To prove that the radioactivity is transported to the next F1 generation of the treated flies. The radioactivity transfer to male or female body parts of the F1 generation, respectively, was proven on one side by measurement of the radioactivity of the uterus with eggs and larvae of the treated fly (Table 2) and, on the other,

by the radioactivity measurement of the males and females of the F1 generation, respectively. The difference between male and female body parts was not significant for both peptides applied. The mean of 8-day-old flies (n = 10) was found to be 0.03% of the radioactivity applied to the ''mother'' body. This value roughly corresponds to the value of the uterus given in Table 2.

(3) To find out whether peptides with an iodinated tyrosine residue (either radio- or non-radiolabeled) trigger similar oostatic effects as the peptides containing the tyrosine residue only. Our preliminary results suggest that iodination of the tyrosine probably does not interfere with recognition and binding of corresponding peptides to a receptor.

We consider the analysis of the radiolabeled degradation products as a next step which represents a different experimental approach, especially due to very low amounts of the analyzed fractions after radiochromatography and to the necessity of their isolation with sufficient high purity for identification. Our previous experiments and analytical measurements indicate some modification of the peptide molecule structures during peptide application and their action in biological material.

ACKNOWLEDGMENT

This study was supported by grant of the Grant Agency of the Czech Republic (505/95/1030).

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