

Isolation and characterization of two new morphogenetically active peptides from *Hydra vulgaris*

Sabine A. H. Hoffmeister

Zentrum für Molekulare Neurobiologie II, Haus 42, Martinistrasse 52, D-20246 Hamburg, Germany

SUMMARY

Foot-specific differentiation processes in hydra are controlled by activating and inhibiting potentials. In an attempt to understand the molecular mechanisms underlying these processes, two substances were isolated from *Hydra vulgaris* that stimulate foot-specific differentiation measured as acceleration of foot regeneration. These substances were shown to be peptides of 13 and 21 amino acids, respectively, with sequences that bear no significant

homology to known peptides or proteins. Polyclonal antibodies were raised against both peptides. The data obtained by biological and radioimmunoassays show that the shorter peptide, pedin, is an excellent candidate for a major component of the 'foot-activating potential'.

Key words: hydra, foot regeneration, pattern formation, morphogenetically active peptide, pedin

INTRODUCTION

The freshwater coelenterate hydra is in many respects comparable to an early stage embryo, because its body column comprises three major stem cell populations, which are retained throughout the life-time of this primitive organism. The three different stem cell populations in its gastric column are the epithelial stem cells of the ectoderm and endoderm, and interstitial cells (Marcum and Campell, 1978; Sugiyama and Fujisawa 1978; Smid and Tardent, 1986). The head, with a hypostome and tentacles, and the foot, with a peduncle and a basal disk, are considered to be composed mainly of terminally differentiated cells. Due to the presence of the stem cells in the body column there exists a pool of proliferating cells, which occasionally may be induced to undergo specific differentiation processes. Epithelial stem cells of the ectoderm can undergo differentiation into either hypostomal and tentacle cells of the head or peduncle and basal disk cells of the foot. Interstitial stem cells may differentiate into distinct types of nematocytes or into nerve cells. The decision to take up a specific developmental fate is carefully regulated and very well balanced. This is due at least partly to the existence of morphogenetically active substances that regulate head-specific or foot-specific differentiation. Processes leading to head formation are regulated by head activator and head inhibitor, whereas foot formation is controlled by foot activator and foot inhibitor (Schaller et al., 1979).

The inhibitors have been previously biochemically characterized as nonpeptides with an overall positive charge and a molecular mass below 500 (Berking, 1977; Schmidt and Schaller, 1980). Head activator was shown to be a peptide with a molecular mass of 1124 and the amino acid composition is known (Schaller and Bodenmüller, 1981). The action of the four factors is specific and they exert their effects at concen-

trations below 10^{-8} M (Schaller et al., 1979). All four factors occur as gradients of sources within the animal, the head factors with a maximum in the head and the foot factors with a maximum in the foot region.

For foot activator, it has been shown that it specifically accelerates foot regeneration, does not influence head regeneration and occurs as a concentration gradient decreasing in the foot-to-head direction (Grimmelikhuijzen and Schaller, 1977; Grimmelikhuijzen, 1979). These properties are in agreement with what has been described as foot-forming potential as deduced from transplantation experiments (Burt, 1925; Yao, 1945; Hicklin and Wolpert, 1973). A first biochemical characterization of the foot activator by Grimmelikhuijzen (1979) showed that the foot activator is a peptide with a molecular mass between 500 and 1500 and with an estimated effective concentration of less than 10^{-8} M.

The data presented in this paper show the isolation of two peptides from *Hydra vulgaris*. These peptides were named pedin and pedibin because they stimulate foot-specific differentiation measured as acceleration of foot regeneration. Biological and biochemical properties of pedin fit very well with the properties of a foot activator which is thought to be the major component of the foot-activating potential. The data obtained for pedibin do not allow final conclusions regarding its function for foot regeneration.

MATERIALS AND METHODS

Animals

Animals of *Hydra vulgaris* and *Hydra oligactis* were originally obtained from the laboratory of P. Tardent, Zürich. They were cultured in a medium consisting of 1 mM CaCl_2 , 0.1 mM KCl, 0.1 mM MgCl_2 and 0.5 mM NaH_2PO_4 , pH 7.6. The temperature of the

medium was kept at $19 \pm 2^\circ\text{C}$. The animals were fed daily between 9 and 10 a.m. with nauplii of *Artemia salina* and washed 6 hours later. Animals without buds that had starved for 24 hours were used for all experiments.

Peroxidase assay for foot activator

Foot activator was assayed in the peroxidase assay as described before (Hoffmeister and Schaller, 1985). Budless animals of *Hydra vulgaris* were collected from the culture and feet were removed at time 0. The footless animals were then incubated in pure medium for the controls or in medium containing increasing amounts of test solutions. Three times 20 animals were assayed for each concentration and the control. After 23 hours, the animals were transferred to 5 ml tubes and the medium was aspirated. Subsequently 1 ml of 0.1 M citrate buffer (adjusted to pH 4.5 with concentrated HCl) containing 0.1% 2,2'-azino-bis-(3-ethylbenzthiazoline-sulfonic acid 6) ammonium salt (ABTS, Sigma) and 0.0003% hydrogen peroxide was added to each tube. One tube without animals served as reference. The reaction was stopped after 30 minutes by addition of 10 μl of 40 mM sodium azide and the optical density of the medium was measured at 415 nm. Activation was determined as percentage activation (A) of the treated (T) over the untreated (C) sample:

$$A = (T - C) / C \times 100 (\%) .$$

Chromatography

As a first purification step, after homogenization, extracts were processed over Sep-Pak C₁₈ cartridges (Waters). The probes were dissolved in water or 10 mM ammonium bicarbonate buffer (ABC), pH 7.8 and applied to the cartridges equilibrated with 10 mM ABC. After washing with 10 ml of ABC buffer, peptides were desorbed by elution with 10 ml of 80% methanol in 10 mM ABC. After evaporation of the solvent, the residues were dissolved in 100 mM NaCl, 10 mM Tris-HCl, pH 7.5 for assaying the biological activity or in an appropriate solvent for further purification steps. Anion-exchange chromatography was performed batchwise with DE-52 cellulose (Whatman). Peptide separation was carried out on a high-pressure liquid chromatograph (HPLC, Gradient Master by Laboratory Data Control, P 100 and Visi Duo by Latak). The HPLC columns used were Partisil ODS-3, 5 μm (Latak) and Superspher 100 RP-18 endcapped, 4 μm (Merck). Further details are given in the results.

Tyrosylation of the peptides

For the introduction of an iodinated tyrosyl residue, the peptides were reacted with *N*-succinimidyl-3-(4-hydroxyphenyl) propionate, the Bolton Hunter reagent, according to the protocol of the supplier (Pierce).

Iodination of the peptides

Radioactive iodine, ¹²⁵I, was incorporated into the peptides by use of *N*-chloro-benzenesulfonamide (sodium salt) immobilized on nonporous, polystyrene beads, IODO-BEADS, according to the protocol of the supplier (Pierce). Following the iodination reaction, the peptides were separated from the unbound iodine over Sep-Pak C₁₈ cartridges as described above with the exception that the peptides were eluted in 3×1 ml of 80% methanol for minimization of the volume.

Coupling of the peptides to keyhole limpet hemocyanin

The peptides were coupled to keyhole limpet hemocyanin (KLH, Sigma) via glutaraldehyde. For that purpose, 100 mg of the carrier, KLH, were dissolved in 5 ml of 100 mM Na₂HPO₄. This solution was carefully added dropwise to 5 ml of a fresh sample of 25% glutaraldehyde (Sigma), adjusted to pH 7.4 with Na₂HPO₄ and reacted overnight at room temperature on a magnetic stirrer. Subsequently the activated KLH was dialyzed against two liters of phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8.3 mM Na₂HPO₄ and 1.5 mM

KH₂PO₄, PBS) overnight at 4°C, the buffer was changed twice. The KLH solution was split into two halves and to each half 5 ml of peptide solution containing 1 mg/ml of each peptide, respectively, dissolved in 100 mM NaHCO₃ were added. These mixtures were again reacted overnight on a magnetic stirrer. Remaining free reactive aldehyde groups were reduced with NaBH₄ (10 mg/ml final concentration) and the samples were adjusted to a final dilution of 1 mg KLH/ml.

Synthetic peptides

Peptides according to the sequences obtained from the purification were synthesized in the laboratory of Dr R. Frank, ZMBH, Heidelberg.

Immunization

Rabbits were immunized with 500 μl of antigenic conjugate containing an equivalent of 120 μg peptide, respectively, and an equal volume of Freund's complete adjuvant (Sigma). Following the first immunization, the rabbits were boosted at monthly intervals with 500 μl of conjugate mixed with an equal amount of Freund's incomplete adjuvant and serum was obtained 7-8 days after each immunization. For a first analysis of antibody titers, the sera were assayed in an enzyme-linked immunosorbent assay (unpublished).

Radioimmunoassay (RIA)

The incubation buffer for the RIA (RIA buffer) was 10 mM NaH₂PO₄, pH 7.4, 25 mM NaCl for pedin and PBS for pedibin containing 0.1% bovine serum albumin and 0.1% sodium azide. Peptide standard and unknown sample were incubated at 4°C overnight with the antiserum (1:10,000, final dilution) and the tracer (10,000 cts/minute/sample) in a total volume of 500 μl . For the precipitation of the serum-bound tracer, 200 μl of 1% immunoglobulin G in RIA buffer and 1.5 ml of 20% polyethylenglycol in RIA buffer containing 0.03% Triton X-100 were added to each sample. After mixing thoroughly, the samples were centrifuged for 30 minutes at 2,000 *g*, the supernatants were discarded and the pellets were measured in a γ counter (Canberra Packard).

Preparation of extracts for RIA

For the determination of the respective content of pedin and pedibin in different hydra tissues, a 10-fold excess of 90% methanol in 35 mM ammonium sulfate was added to lyophilized tissue. After sonification, the homogenous suspension was centrifuged at 3,000 *g* for 10 minutes and reextracted twice. The pooled supernatants were evaporated to dryness and then applied to Sep-Pak C₁₈ cartridges and processed as described above. After a final evaporation, the samples were dissolved in an appropriate volume of RIA buffer.

Assays for effects at the cellular level

To study possible effects of pedin on interstitial cell proliferation and on nerve cell differentiation, animals of *Hydra oligactis* were used, because these animals contain a higher percentage of interstitial cells including nerve cell precursors than animals of *Hydra vulgaris* (Hoffmeister and Schaller, 1987, Hoffmeister, 1991). Large budless animals were selected from the culture and transferred to dishes with 10 ml of medium. Three animals were used for each concentration and the control. After 105 minutes for the determination of the mitotic index and after 8 hours for the determination of the nerve cell density, heads and feet of the animals were excised, and the gastric columns were collected and dissociated into single cells by maceration in a solution containing 7% acetic acid and 7% glycerol (David, 1973).

RESULTS

Purification of pedin and pedibin from hydra tissue

The purification of biologically active substances requires the

availability of a reliable assay that can be performed in a reasonable time course. Foot-specific differentiation processes are massively induced during foot regeneration after the removal of a foot. Therefore, monitoring the effect of a substance on foot regeneration provides a suitable tool for testing its ability to either stimulate or inhibit foot formation. The property of foot-specific cells to secrete mucous, which enables the animals to adhere to a substratum has been used as a criterion for a regenerated foot. For this assay, one has to monitor the animals over a time period from 18-24 hours after cutting in hourly intervals. Less time consuming is an assay that makes use of the fact that foot-specific ectodermal epithelial cells contain a peroxidase (Hoffmeister and Schaller, 1985). During foot regeneration, the reappearance of the peroxidase coincides with the time at which the animal reacquies its ability to stick to a substratum. Measuring the peroxidase at 23 hours after foot removal allows quantitation of the amount of regenerated foot-specific cells in treated compared to untreated animals. This peroxidase assay was used for the measurement of the biological activity of given fractions derived from the different steps of the purification procedure.

For the isolation of the foot-regeneration-stimulating activity, 10×2 g of lyophilized *Hydra vulgaris* (about 400,000 animals) were homogenized batchwise with a Teflon homogenizer in iso-osmolar buffer. After centrifugation at 45,000 g for 30 minutes, the supernatant was collected and the pellet

was dissolved in dilute buffer without sucrose to ensure rupture of membranes by osmotic shock and release of granule-stored material into the supernatant. After another round of centrifugation, the pellet was discarded and the supernatants were processed over Sep-Pak C₁₈ cartridges. The biologically active components eluted with 80% methanol in 10 mM ABC. This material was further purified by batchwise adsorption to the anion-exchanger DE-52. To 30 ml of extract, respectively, 10 g of DE-52 cellulose was added, which was equilibrated with 5 mM ammonium acetate and adjusted to pH 6.0. This mixture was shaken for 30 minutes and then centrifuged at 2,000 g for 10 minutes. The supernatant was once again mixed with fresh DE-52 cellulose and treated as before. After centrifugation, the pellets were washed twice with 30 ml of 5 mM ammonium acetate, pH 6.0. For the elution of the biologically active components, the pellets were washed three times with ammonium acetate, pH 3.5. The pooled supernatants were concentrated by rotary evaporation and desalted by Sep-Pak C₁₈ cartridges as described before.

The active fractions were further purified by reversed-phase HPLC (Fig. 1A-D). In the first step, a comparatively broad gradient was used which gave a crude separation and a first hint that the active components eluted with about 25-30% acetonitrile under acidic conditions (Fig. 1A). According to these results, in the next step a gradient was used that was narrower in its range. This resulted in an additional enrichment with no

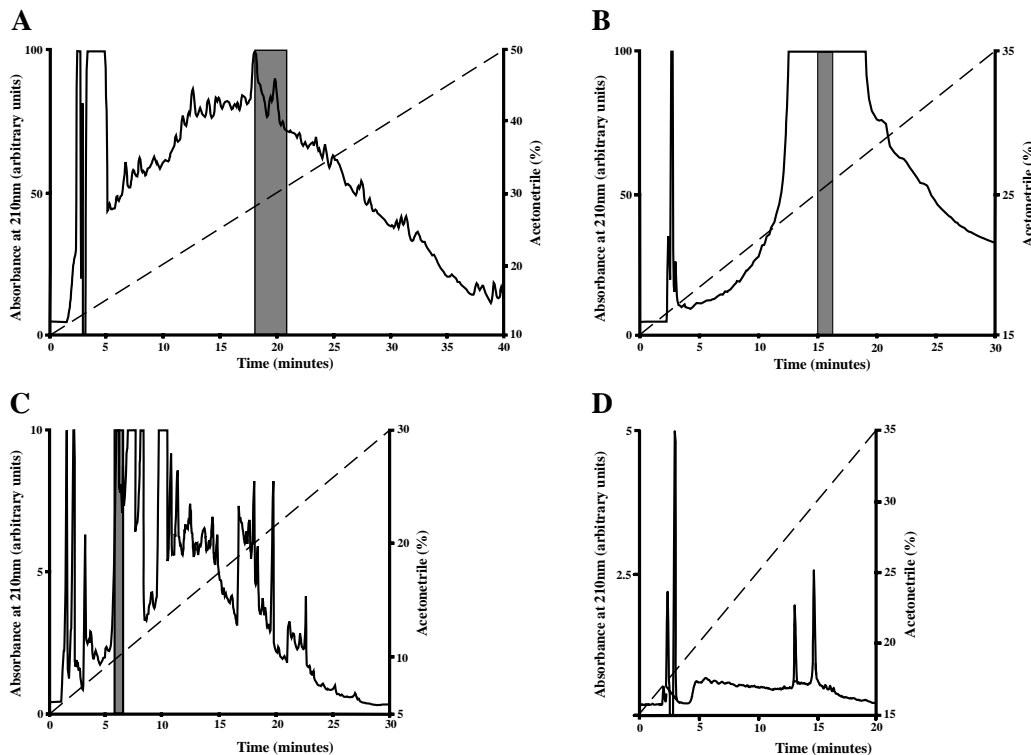


Fig. 1. Purification of the foot-regeneration-stimulating activity by HPLC. Vertical stippled area represents the fraction in which biological activity was found in the peroxidase assay.

(A) Chromatogram of one from ten consecutive runs performed with the pooled active fractions from the batchwise anion-exchange chromatography. The column (Partisil ODS-3 C₁₈; particle size, 5 μ m; pore diameter, 6 nm; dimensions, 4 \times 250 mm) was equilibrated with 10% acetonitrile in 0.1% trifluoroacetic acid (TFA). After injection of the sample, a linear gradient of 10-50% acetonitrile in 0.1% TFA was immediately started, the flow rate was 1 ml / minute. The duration of the gradient was 40 minutes. The active fractions of each individual run were pooled, respectively. (B) Chromatogram of one

from four consecutive runs performed with the pooled fractions 18. The column used was the same as in A, with a linear gradient of 15-35% acetonitrile in 0.1% TFA, a duration of 30 minutes and a flow rate of 1 ml/minute. (C) The active fractions 16 of B were pooled and further processed over a Superspher 100 RP-18 endcapped column (particle size 4 μ m; dimensions 4 \times 250 mm). The column was equilibrated with 10% acetonitrile in 5 mM ammonium bicarbonate (ABC), pH 7.8. Immediately after injection of the sample, a linear gradient of 10-30% acetonitrile in 5 mM ABC was started with a duration of 30 minutes and a flow rate of 1 ml/minute. (D) Chromatogram of the last run performed with the active fraction 7 of C. The column was the same as in C equilibrated with 15% acetonitrile in 0.1% TFA and eluted with a gradient of 15-35% acetonitrile in 0.1% TFA, in 20 minutes at 1 ml/minute. For all runs, the absorbance was measured at 210 nm and is expressed in arbitrary units.

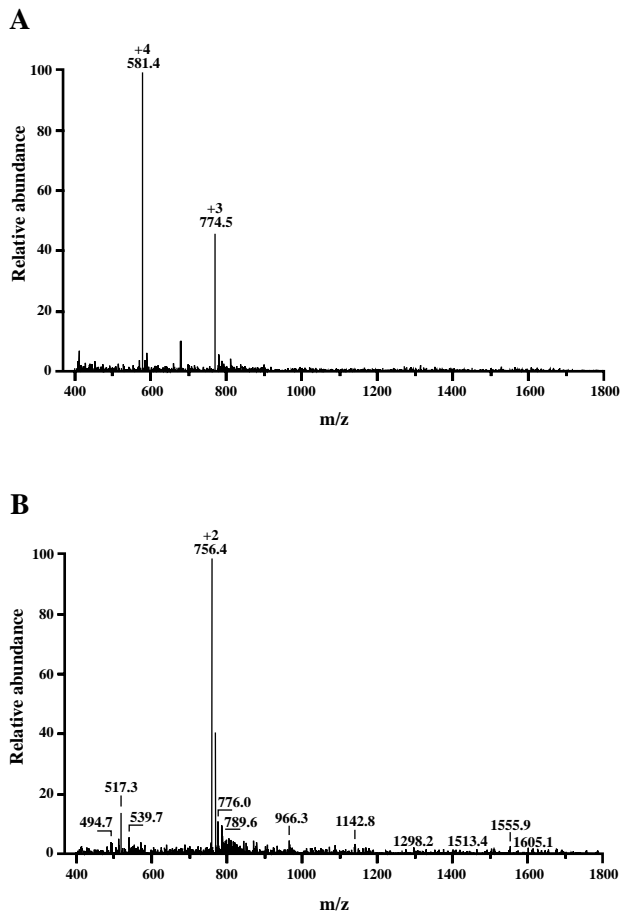


Fig. 2. ES/MS spectra of the two pure fractions 14 (A) and 16 (B). The calculated mass was 2321.4 for fraction 14 and 1510.7 for fraction 16. This determination of the mass of the peptides was performed by Dr C. Schulze, ZMNH, Hamburg.

resolution into single peaks (Fig. 1B). The first two steps had been performed using the same type of column material and acidic conditions. As a third step, therefore, a neutral gradient was chosen for elution allowing an improved separation. Fig. 1C shows that this was true for the biologically active components from hydra. As a final step, the active fraction 7 of this run was further purified using acidic conditions and column

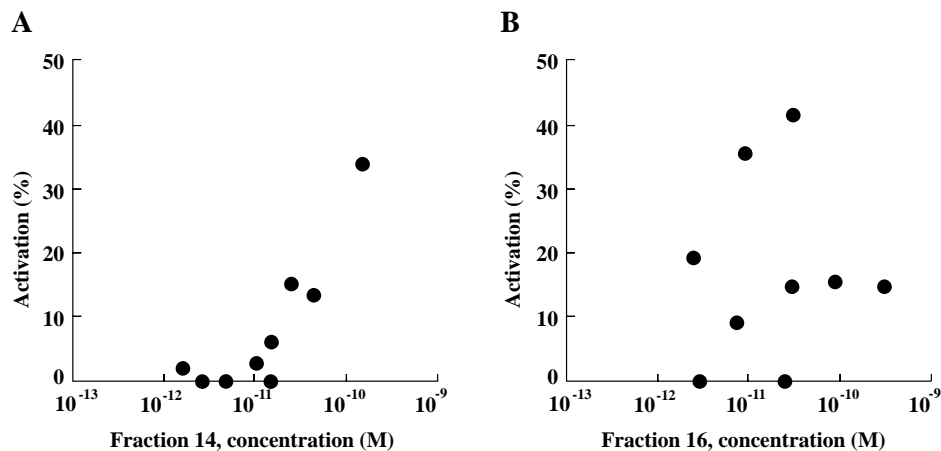


Fig. 3. Biological activity of fraction 14, pedibin (A) and 16, pedin (B), respectively assayed as stimulation of foot regeneration in the peroxidase assay. The concentrations of the peptides were estimated from the relative absorbance in the chromatogram shown in Fig. 1D. For each individual point 3×20 animals were assayed.

material that was different from that of the first two steps. This last chromatogram showed two distinct single peaks, each containing one component only as shown by mass spectrometry (Fig. 2A,B) and sequence analysis. Fraction 14 contained a peptide of 21 amino acids: AGEDVSHELEEKEKALANHSE, which we have called pedibin; fraction 16 contained a peptide of 13 amino acids: EELRPEVLPDVSE, which we have called pedin. These peptides show no significant sequence homology to known sequences. Both peptides stimulated foot regeneration in *Hydra vulgaris* which was measured in the peroxidase assay (Fig. 3A,B).

Generation of antibodies and iodinated tracers

On the basis of the obtained sequences from fraction 14 and 16 of the purification procedure, corresponding peptides were synthesized. The synthetic peptides have the same mass as the native ones. The respective elution times of the synthetic peptides on HPLC were identical to those of the native ones with pedibin eluting in the 14th minute as for fraction 14 and pedin eluting in the 15th minute as for fraction 16 (Fig. 4).

For a further examination of the peptides, polyclonal antibodies were raised in rabbits. As the peptides as such were not immunogenic enough to get a reasonable immune response, they were coupled to KLH as a carrier via glutaraldehyde (see Materials and methods). After 13 boosts, the α -pedibin serum could be used for immunoassays for pedibin and, after 7 boosts, the α -pedin serum was suited for use in radioimmunoassays for pedin. For the radioimmunoassays, both peptides had to be radioactively labeled. As pedin does not contain any iodinated amino acid residue, this peptide was labeled by reaction with the Bolton-Hunter reagent, which introduces a tyrosyl-group at the amino-terminal end of the peptide (Bolton and Hunter, 1973). Pedibin has two histidine residues in its sequence, and therefore this peptide could be iodinated directly at the two histidine residues without tyrosylation (see Materials and methods).

Distribution of pedin and pedibin in different hydra tissues

In a first attempt to further characterize the peptides, radioimmunoassays were established for both peptides. The radioimmunoassay for pedin was more sensitive than that for pedibin. This was due to the better titer of the α -pedin serum, which could be diluted 1:10,000, compared to the α -pedibin serum

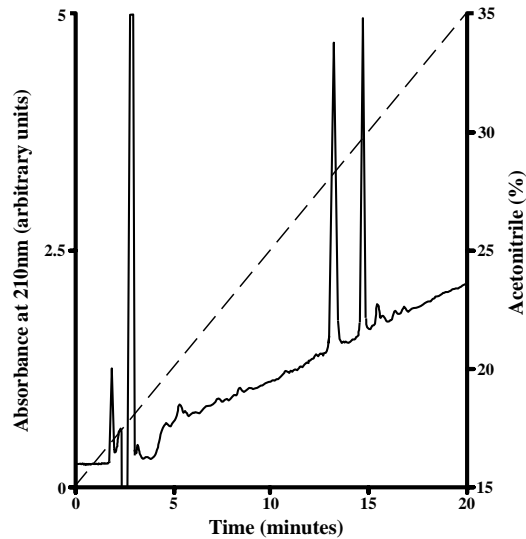


Fig. 4. HPLC behavior of the synthetic peptides. The column and the conditions used were as described for the native peptides in Fig. 1D. The synthetic peptides were eluted at the same retention times as the native peptides.

which was used at a dilution of 1:500. As can be seen in Fig. 5A and C, for a 50% competition, 2×10^{-9} M pedibin was needed and 1.5×10^{-10} M pedin. To determine the respective

content of pedin and pedibin, hydra extract was prepared from 2 g of lyophilized *Hydra vulgaris* as described in Materials and methods. The obtained results showed that *Hydra vulgaris* contains 54 fm pedin and 286 fm pedibin per mg dry weight which equals 2.7 fm pedin and 14.3 fm pedibin per animal. Thus *Hydra vulgaris* contains a factor of five more pedibin than pedin (Fig. 5B,D).

To correlate the presence of the peptides with the formerly described foot-differentiation-stimulating activity, the content of pedin and pedibin in a foot-regeneration-deficient strain of *Hydra oligactis* was determined. These animals have been examined for their content of morphogenetically active substances and it was found that the foot-differentiation-stimulating activity was reduced to 42% in comparison to *Hydra vulgaris* (Hoffmeister, 1991). Therefore, it was expected that either pedin or pedibin or both would be reduced in these animals. The results obtained by radioimmunoassays showed that *Hydra oligactis* contains 4 fm pedin and 55 fm pedibin per mg dry weight (Fig. 5B,D), i.e., *Hydra oligactis* contains a factor of 13.5 times less pedin and 5 times less pedibin as compared to *Hydra vulgaris*.

Since foot activator was shown to occur in the animal as a gradient declining in the foot-to-head direction, the distribution of the peptides along the axis of *Hydra vulgaris* (Grimmelikhuijzen, 1977) was studied. 10,000 animals of *Hydra vulgaris* were cut batchwise, about 50 animals per 5 minutes, into two halves and immediately frozen to prevent loss of peptides by diffusion. After lyophilization, the collected

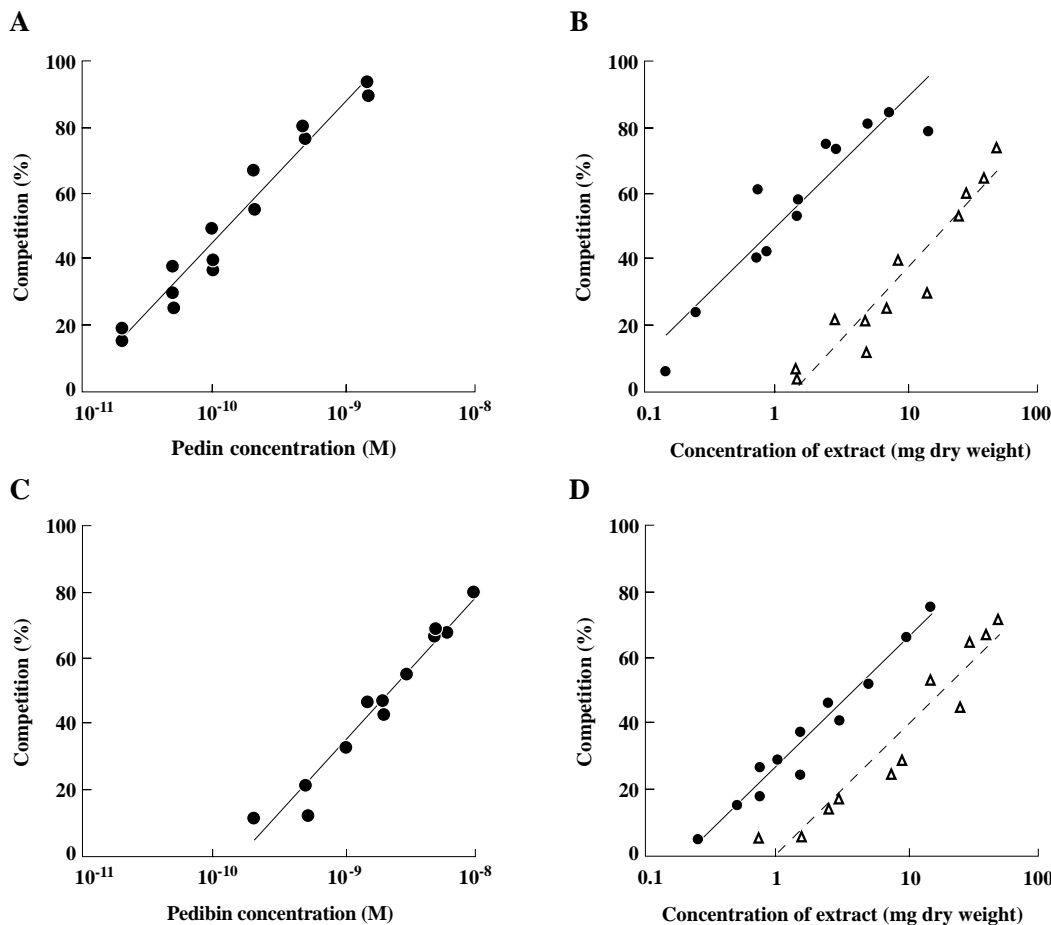


Fig. 5. Determination of the content of pedin and pedibin in *Hydra vulgaris* in comparison to *Hydra oligactis* by radioimmunoassays. (A) The standard curve for pedin, (B) competition curves for pedin with extracts from *Hydra vulgaris* (●) and *Hydra oligactis* (Δ), (C) the standard curve for pedibin, (D) competition curves for pedibin with extracts from *Hydra vulgaris* (●) and *Hydra oligactis* (Δ).

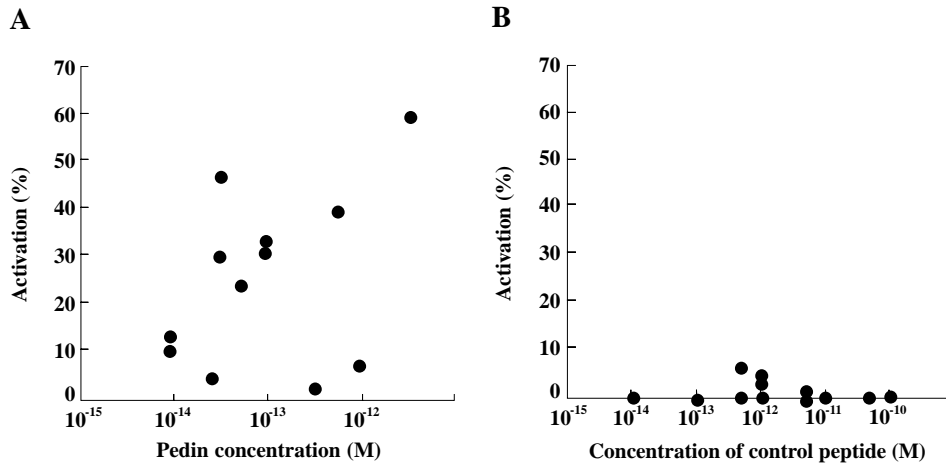


Fig. 6. The biological activity of the synthetic peptide pedin was measured in the peroxidase assay (A). As a negative control, the biological activity of the peptide AGEDVSHELEEKEKALANHKP was also measured in the peroxidase assay (B). This peptide was inactive, whereas pedin started to be active between 10^{-14} and 10^{-13} M. For individual points animals were assayed in independent experiments on different days.

material was extracted as described above. The results obtained showed that pedibin was more or less evenly distributed over the animal with the upper halves containing more of the peptide (214 fm/mg dry weight), than the lower ones (140 fm/mg dry weight). For pedin the distribution was more 'foot activator-like', with 48 fm/mg dry weight in the lower halves compared to 24 fm/mg dry weight in the upper halves. However, there was some loss of the peptides during the cutting and collecting procedure, because, for both peptides, the total amount per animal obtained in this experiment is less than before.

Biological activity of pedin

As the preceding experiments had shown pedin to be a good candidate for representing at least part of the foot activator activity, the synthetic peptide was tested for its biological activity in the foot regeneration assay. Fig. 6A shows the dose-response curve that was obtained for pedin in the peroxidase assay. The peptide starts to be active at a concentration of 10^{-14} to 10^{-13} M, which again is in accordance to what had been suggested for foot activator (Grimmelikhuijzen, 1979). To ascertain that the effect of pedin on foot regeneration was specifically brought about by this peptide, a modified form of

pedibin which had been synthesized for other reasons was used as a negative control. As can be seen clearly in Fig. 6B, this peptide did not exert a stimulating effect at any concentration tested. Besides the stimulating effect on foot regeneration, foot activator was shown to stimulate the proliferative activity of interstitial cells and epithelial cells as well as the differentiation of nerve cells (Hoffmeister, 1989). If pedin represented the foot activator or part of this described activity, then it should also be active in a comparable way at the cellular level. For testing the effects of pedin at the cellular level, *Hydra oligactis* was chosen. The animals of this strain have in their gastric tissue relatively higher fractions of undifferentiated interstitial cells but lower fractions of their differentiation products, namely nematocytes and nerve cells (Hoffmeister, 1991). The reduced fraction of nerve cells correlates with an increased fraction of big interstitial cells. This is due to the fact that *Hydra oligactis* contains a high percentage of nerve-cell precursors that are arrested in the G₂-phase (Hoffmeister and Schaller, 1987). Therefore, these animals constitute a large pool of putative target cells for the action of pedin. The assays were performed as described in Materials and methods. Fig. 7 shows that pedin exerts a stimulatory effect on the proliferative activity of big interstitial cells in the gastric column of *Hydra oligactis* and

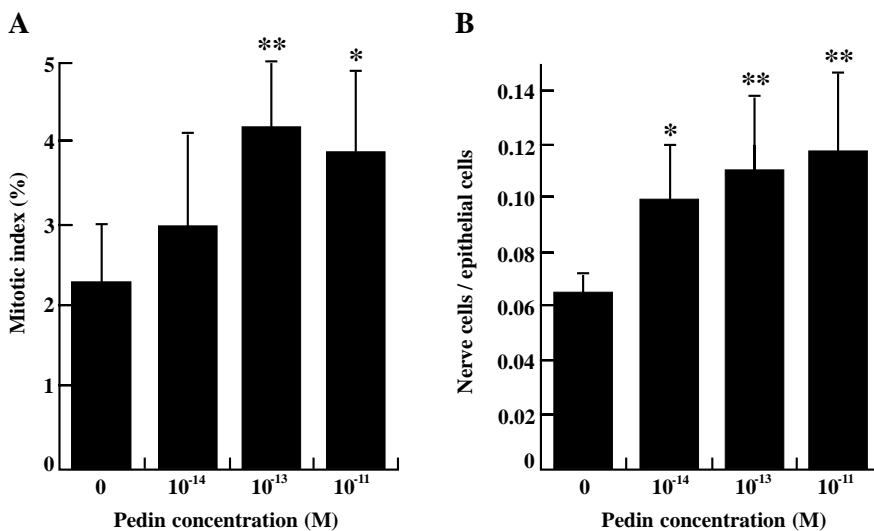


Fig. 7. Effect of pedin on the mitotic index of big interstitial cells (A) and the nerve cell density (B), measured as ratio of nerve cells to epithelial cells. For each value, 6×400 cells, respectively, were counted for the mitotic index and 6×500 cells, respectively, for the nerve cell density. The error bars give the standard error of the respective mean values. The differences between the control values and the values labeled with stars are statistically significant (t -test, * $P \leq 0.05$, ** $P \leq 0.01$).

that it also stimulates the differentiation of nerve cells in a concentration range from 10^{-14} to 10^{-11} M. Taken together, these results imply that pedin is playing a major role in the control of foot regeneration as well as in the control of cell proliferation and differentiation processes.

DISCUSSION

Hydra is not only evolutionary very old but has also been the subject of research for developmental biologists for many years. It was already known from the work of Burt (1925), Yao (1945) Shostak (1972), Hicklin and Wolpert (1973), MacWilliams and Kafatos (1974) and Grimmelikhuijzen and Schaller (1977) that, in *hydra*, the foot represents an autonomous center of organization, and that there exists a foot formation potential as a gradient declining from the foot to the head, which was thought to be built up by a substance termed foot activator. This name was given in analogy to another substance, the head activator, which was isolated and sequenced in 1981 by Schaller. Head activator is a neuropeptide of 11 amino acids and was shown to stimulate head-specific differentiation processes in *hydra*, to act as a mitogen (Schaller, 1976a) and to stimulate the determination as well as the differentiation of nerve cells in *hydra* (Schaller, 1976b; for a review see Schaller et al., 1989).

In this paper, the isolation is reported of two new peptides, which have been purified because of their stimulatory effect on foot regeneration. Both peptides coeluted under all chromatographic conditions used and it was only in the last step of the purification procedure that they could be separated. Surprisingly, it was found that both peptides stimulated foot-specific differentiation in the foot regeneration assay, with pedin being more potent than pedibin. Up to now, it is not clear in what way these peptides exert their effect: whether both of them compete for the same receptor, activate the same cascade of second messengers while binding to different receptors or are both members of the same stimulatory cascade, or whether the stimulatory effect of the larger peptide, pedibin, is a pure side product of an essentially different signal transduction pathway.

According to the data shown in this paper, the properties of pedin, the shorter of the two peptides, fit very well to what has been described for the foot-differentiation-stimulating activity, implying that pedin is responsible for the regulation of foot-specific pattern formation. The stimulatory effect on foot regeneration found for the native peptide (fraction 16) was confirmed with the synthetic peptide. There are two major reasons for the large variance in the foot regeneration assay. The first reason is that *hydra*'s ability to take up substances from the medium is highly variable. Since *hydra* is a freshwater animal, it has to protect itself from osmotic collapse by tightening its surface. In this respect, *hydra* is different from its marine relatives. These can be shown to take up substances like BrdU from the medium much more easily than *hydra* (Plickert and Kroiher, 1988). Nevertheless, the described assay system can be used for the analysis of foot-regeneration-stimulating effects, since the negative control confirms that the obtained effects are specific. As a second reason, in analogy to what has been found out for head activator, the foot-regeneration-stimulating activity appears to occur bound to a large

carrier molecule in its native form (Schaller et al., 1986 and unpublished observations). In this native form, head activator is protected from degradation and self inactivation by dimerization (Bodenmüller et al., 1986). According to the amino acid sequence one would not expect pedin to undergo the same type of self-inactivating conformational interaction that was described for head activator, but there may exist different mechanisms for self inactivation for this molecule. Conformational peculiarities, putative modifications and interactions of pedin with a larger carrier molecule will be the subjects for further investigations.

Another proof of the relevance of this peptide for foot-specific differentiation processes is the fact that it is highly under-represented in a foot-regeneration-deficient strain of *Hydra oligactis*. A previous analysis of the foot regeneration deficiency of *Hydra oligactis* had shown that it contains a factor of two less foot activator per animal; pedibin was also a factor of five less concentrated in *Hydra oligactis* compared to *Hydra vulgaris*. The data of the radioimmunoassays with extracts of upper and lower halves of *Hydra vulgaris* show that the lower halves contain a factor of two more pedin than the upper halves. These differences might be even more pronounced, since it appears that a certain percentage of the peptides (10-25%) was lost during the cutting/collecting/freezing process that precedes the extraction. The correlation for the higher concentration of the peptide in the foot region did not hold for pedibin. For this reason pedin was examined in more detail. Finally, the effects of pedin on the mitotic activity of interstitial cells and on the differentiation of nerve cells were tested. It was shown that also in this respect the action of pedin is comparable to that of foot activator.

Taken together, these data show that pedin and pedibin are two new peptides isolated from *hydra* tissue and enriched about 10^6 -fold. Both peptides stimulate foot regeneration in a concentration range starting from 10^{-13} M for pedin and 10^{-12} M for pedibin. Whereas the role of pedibin is yet unclear, pedin was shown to be directly involved in the control of foot-specific differentiation processes in *hydra*.

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Note added in proof

The accession numbers are: P80577 for pedibin and P80578 for pedin (SWISS-PROT protein sequence database and PIR International protein sequence databases).

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