

# Isolation and amino acid sequence of a morphogenetic peptide from hydra

(hydra head activator/neuropeptide/high-pressure liquid chromatography/sequence microdetermination)

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**ABSTRACT** From *Anthopleura elegantissima* and from *Hydra attenuata*, a morphogenetic peptide—the head activator—was isolated in pure form. The sequence of the head activator was established by enzymatic and by chemical degradation of the whole peptide or fragments thereof and subsequent analysis of the amino acids by micromethods. The head activator from both sources was identical and has the sequence:

<pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe.

From the freshwater coelenterate hydra a substance can be isolated—the head activator—which acts as a head-inducing morphogen (1–3). Its presence is required to initiate head-specific, as opposed to foot-specific, growth and differentiation processes. The head activator affects growth by triggering cells to divide (4) and affects cellular differentiation by determining (for example) the multipotent interstitial cells to become nerve cells (5). In normal animals the head activator is produced by nerve cells and is stored there in neurosecretory granules (6, 7).

The head activator was found to be degraded by various proteolytic enzymes and, therefore, was assumed to be a peptide. Like other neuropeptides it is active at a very low concentration, 0.1 pM. As a consequence of this, we found that a hydra contains only 0.1 pg or 0.1 fmol of the head activator. For a chemical analysis, an amount of 10 nmol seemed desirable. Because hydra cannot be produced in such large quantities, we looked for other sources. We found that relatives of hydra, such as sea anemones, which are  $10^4$  times larger than hydra and are abundant in the ocean, all contain a substance that in its biological and chemical properties seemed to be identical to the head activator from hydra (8). In this paper we describe the isolation of the head activator from the sea anemone *Anthopleura elegantissima* and the analysis of its amino acid sequence. We also isolated the head activator from hydra and found that the peptides from the two sources are identical.

## MATERIALS AND METHODS

**Animals.** *Anthopleura elegantissima* (200 kg) were obtained deep frozen from Pacific Biomarine Laboratories, Venice, CA. They were stored at  $-70^\circ\text{C}$  before use. *Hydra attenuata* were cultured in our own laboratory. In the course of 10–12 yr we collected about 3 kg.

**Homogenization.** The frozen animals were transferred from  $-70^\circ\text{C}$  to  $-20^\circ\text{C}$  for one day to facilitate mechanical disruption. Large pieces (1–3  $\text{cm}^3$ ) were covered with a 2-fold excess of cold ( $-20^\circ\text{C}$ ) methanol and homogenized with an Ultraturrax (Janke and Kunkel) under ice, cooling three times for 10 min.

**Column Materials.** Sephadex G-10 and DEAE-Sephadex A-25 were from Pharmacia, Bio-Gel P-2 was from Bio-Rad, and LiChrosorb RP-8 powder (10- $\mu\text{m}$  particle size) and prepacked  $250 \times 4$  mm high-pressure liquid chromatography (HPLC) col-

umns containing LiChrosorb RP-8 or RP-18 (7- $\mu\text{m}$  particle size) were from Merck.

**Glassware for Microanalysis.** All glassware was subjected overnight to  $500^\circ\text{C}$  prior to use. As test tubes we used segments of tubing (3-cm length and 1.2-mm inner diameter) which were sealed by melting.

**Dansylation and Amino Acid Analysis.** All reagents used were sequanal grade and obtained from Pierce. For a total hydrolysis, the peptide (20–100 pmol) was hydrolyzed in 5  $\mu\text{l}$  of 6 M HCl at  $110^\circ\text{C}$  for 18 hr; for endgroup analysis, the dansylated peptide was hydrolyzed for 4 hr. Dansylation was carried out as described by Gray (9), and the dansylated amino acids were separated by thin-layer chromatography on  $3 \times 3$  cm polyamide sheets obtained from Schleicher & Schuell. For the dansyl Edman degradation, the peptide (20–50 pmol per vial) was treated with 6  $\mu\text{l}$  of the respective reagents. The degradation was carried out in the consecutive manner as described by Gray and Smith (10)—i. e., without butyl acetate extraction after each step. This was necessary because, after one or two cleavage steps, the residual head-activator peptide became so hydrophobic that it was extracted into the organic solvent and, therefore, removed from further degradation.

**Enzymic Digestions.** Carboxypeptidase A (EC 3.4.12.2), B (EC 3.4.12.3), and Y; aminopeptidase M (EC 3.4.11.2); and chymotrypsin (EC 3.4.21.1) were purchased from Boehringer Mannheim. Pyroglutamate aminopeptidase (EC 3.4.11.8) was from Serva (Heidelberg), trypsin TPCK (EC 3.4.21.4) was from Worthington, and the low molecular weight protease was a gift from R. Zwilling (University of Heidelberg) (11). The following enzyme solutions were used: carboxypeptidase A and B at 10  $\mu\text{g}/\text{ml}$  in 0.5 M NaCl/0.05 Tris-HCl, pH 7.6; carboxypeptidase Y at 100  $\mu\text{g}/\text{ml}$  in 0.1 M  $\text{NH}_4\text{OAc}$  (pH 6.0); and aminopeptidase M at 10  $\mu\text{g}/\text{ml}$ , trypsin at 10  $\mu\text{g}/\text{ml}$ , chymotrypsin at 100  $\mu\text{g}/\text{ml}$ , pyroglutamate amino peptidase at 1 mg/ml, and the low molecular weight protease at 1  $\mu\text{g}/\text{ml}$ , each in 0.05 M  $\text{NH}_4\text{OAc}$  (pH 7.6). All enzymes were analyzed for their action on model peptides, such as bradykinin, neurotensin, etc., before and while they were used on the head activator. The peptide (50–100 pmol) was incubated in 2  $\mu\text{l}$  of the respective enzyme solutions at  $37^\circ\text{C}$ . The reaction was stopped by freezing the samples in acetone/ $\text{CO}_2$ , followed by lyophilization. The free amino acids were analyzed after dansylation.

**HPLC.** Peptide separation was carried out on a high-pressure liquid chromatograph by Laboratory Data Control (Constametric III, Gradient Master, Spectromonitor III). The separation of dansylated amino acids was carried out on a Du Pont 830 apparatus with a Du Pont 836 fluorescence detector.

**Biological Assay.** Head activator was assayed as stimulation of bud outgrowth in hydra measured 3 hr after addition of the head activator to the medium (12).

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Abbreviation: HPLC, high-pressure liquid chromatography.

## RESULTS

**Isolation Procedure. *Anthopleura*.** Batches of *Anthopleura* (10–13 kg) were extracted by homogenization in methanol. After centrifugation at  $3000 \times g$  for 10 min, the sediments were suspended twice in methanol and reextracted. The collected supernatants were concentrated to 10–15 liters by rotary evaporation and extracted first with petroleum ether and then with chloroform. The organic phase was discarded, and the aqueous phase was lyophilized, reextracted with as little as possible pure methanol, and centrifuged. The methanol supernatant was evaporated to dryness, redissolved in 500 ml of distilled water, and adjusted to pH 7.6. Portions (250 ml) were applied to a 5.3-liter Sephadex G-10 column. The head activator adsorbed to Sephadex G-10 and was eluted more slowly than salt by at least a factor of two (Fig. 1; refs. 1 and 12). This very effective purification step led to a  $10^4$ -fold enrichment based on weight of the head activator over crude extracts, with a 90% yield.

The first 100 kg of *Anthopleura* was processed this way. Unfortunately, during the long course of our isolation efforts, the gel properties changed, with the result that any Sephadex G-10 purchased after 1979 no longer had this extreme adsorbing property for the head activator. A batchwise absorption to the anion exchanger DEAE Sephadex A-25 proved to be a good, although not as efficient, alternative first step in chromatography. To 500 ml of extract from 10–13 kg of *Anthopleura*, 100 g of DEAE Sephadex was added, which was adjusted to pH 7.6 and equilibrated with 5 mM ammonium acetate. This mixture was shaken for 30 min and then centrifuged at  $2000 \times g$  for 30 min. The supernatant was twice again mixed with fresh DEAE-Sephadex and treated as before. The supernatants were discarded. The sediment was brought to pH 3.5 with acetic acid, and the head activator was eluted by three additions of 500 ml of 5 mM ammonium acetate (pH 3.5), followed by centrifugation. The supernatants were concentrated to 50 ml by rotary

evaporation, brought to pH 7.6, and applied (25 ml) to a 250-ml column of Sephadex G-10 purchased before 1979 (Fig. 1b).

The fractions containing the head activator were combined, concentrated to 20 ml, and applied to a 660-ml Bio-Gel P-2 column. Like Sephadex G-10, this gel separates molecules primarily by molecular size. The head activator was eluted between void volume and salt (Fig. 1c). The active fractions were concentrated and rechromatographed first on a smaller Sephadex G-10 column (25 ml; Fig. 1d) and again on a Bio-Gel P-2 column (40 ml; Fig. 1e). In both cases, we added salt to the buffer to reduce loss of head activator due to unspecific binding to glass, gel, etc. Up to this step, the yield of the head activator was satisfactory ( $\approx 70\%$ ) with a 500,000-fold enrichment.

The active fractions were condensed to 0.5 ml and applied to a Pasteur capillary pipette containing 0.5 ml of the reverse-phase octyl silyl silica gel, LiChrosorb RP-8 (particle size, 10  $\mu\text{m}$ ), which was equilibrated with 20% (vol/vol) methanol in 5 mM ammonium bicarbonate. After washing the column with 3 ml of the same buffer, the head activator was eluted with 3 ml of 80% methanol in 5 mM ammonium bicarbonate. The methanol was evaporated, and the sample was applied to another 0.5-ml LiChrosorb RP-8 column, equilibrated with 20% (vol/vol) acetonitrile in 0.1% trifluoroacetic acid. After washing with 3 ml of the same buffer, the head activator was eluted with 3 ml of 40% acetonitrile in 0.1% trifluoroacetic acid. As a final criterion for purity, the head activator was subjected to reverse-phase HPLC on LiChrosorb RP-8 columns with acetonitrile (Fig. 2a) and methanol (Fig. 2b) as the mobile phase both isocratically (not shown) and with gradient elution. The retention times at a flow rate of 1 ml/min (i) for isocratic conditions in 30% acetonitrile/0.1% trifluoroacetic acid and in 50% methanol/5 mM ammonium bicarbonate were 8 min and 7.6 min, respectively, and (ii) for a 10-min gradient elution from 20% to 40% acetonitrile in 0.1% trifluoroacetic acid and from 40% to 60%

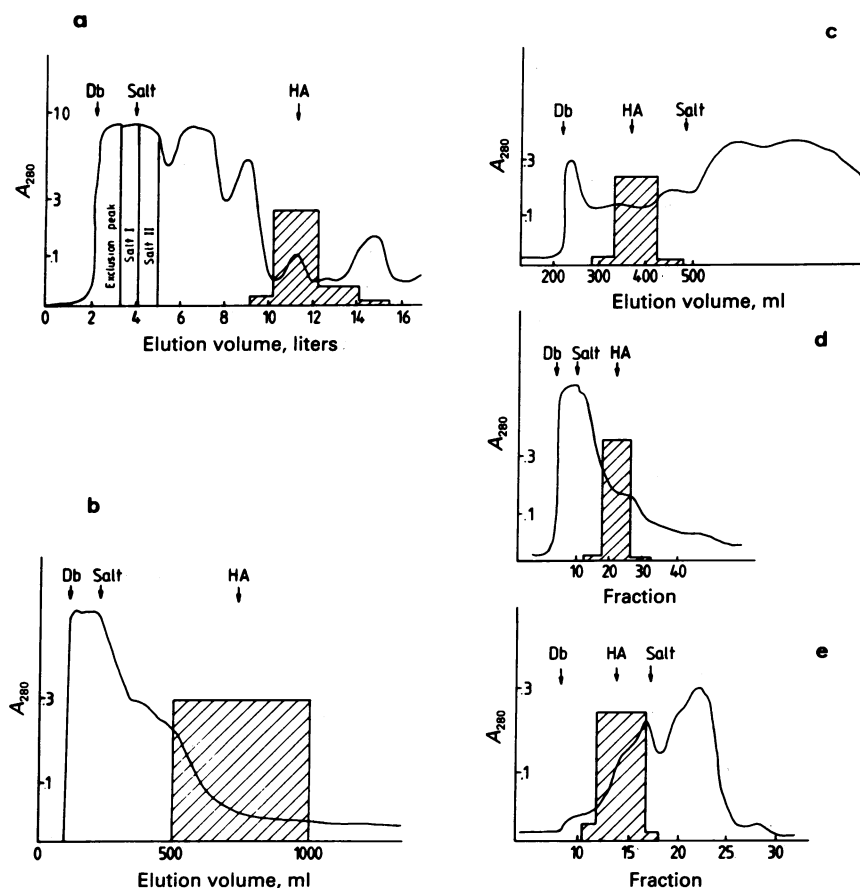


FIG. 1. Purification procedure for the head activator (HA) from *Anthopleura*. (a) Chromatography of an extract from *Anthopleura* (5–6 kg) on Sephadex G-10 (column size,  $10 \times 73.5$  cm; total volume, 5.3 liters; eluent, 20 mM ammonium bicarbonate). (b) Adsorption by batches of the same extract to DEAE-Sephadex at pH 7.6, elution at pH 3.5, and chromatography on Sephadex G-10 (column size,  $5.5 \times 13$  cm; total volume, 250 ml; eluent, distilled water). (c) Chromatography of the active fractions from a or b (9.5–14.0 liters or 500–1000 ml, respectively) on Bio-Gel P-2 (column size,  $4.5 \times 50$  cm; total volume, 660 ml; eluent, 5 mM ammonium bicarbonate). (d) Chromatography of the active fractions of c (300–450 ml) on Sephadex G-10 (column size,  $1.5 \times 16$  cm; total volume, 25 ml; eluent, 0.1 M NaCl/0.01 M Tris-HCl, pH 7.6). (e) Chromatography of the active fractions of d (29–42 ml) on Bio-Gel P-2 (column size,  $1.3 \times 47$  cm; total volume, 40 ml; eluent, 0.1 M NaCl/0.01 M Tris-HCl, pH 7.6). Db (dextran blue), void volume; salt, NaCl position;  $\square$ , location of head-activator activity.

methanol in 5 mM ammonium bicarbonate were 11.2 min and 10.5 min, respectively. From 200 kg of sea anemones, we obtained 20 nmol (20  $\mu$ g) of the pure head activator with a 20% yield, which would correspond to a  $2 \times 10^9$ -fold enrichment.

The head activator was located in the various column fractions by determining the biological activity. In later steps, when the head-activator peptide was free from other protein or peptide contaminants, we usually located it first by hydrolyzing a small sample of all of the fractions and looking for the characteristic head-activator pattern of the dansylated amino acids on thin-layer plates; 10 pmol were sufficient for that purpose. In a second step, we determined the biological activity. By this method we found, especially by rechromatography of HPLC fractions, that a second peptide sometimes appeared slightly ahead of the biologically active head-activator peptide (Fig. 2c), which had the same amino acid pattern but was not active.

**Hydra.** From 3 kg of hydra, the head activator was extracted and purified as described, starting with the 250-ml Sephadex G-10 column (Fig. 1b) as the first gel chromatography step. The head activators from hydra and sea anemones were eluted from all columns, including the HPLC columns described in Fig. 2, at identical positions. From 3 kg of hydra, we isolated 0.5  $\mu$ g (500 pmol) of the pure head activator.

**Sequence Analysis. Amino acid composition.** The head activator was hydrolyzed and dansylated, and the amino acids were separated by two-dimensional thin-layer chromatography on polyamide plates (9). The head activator contains nine amino acids: Gly, Glu, Ile, Leu, Lys, Phe, Pro, Ser, Val (Fig. 3). Because a quantitative evaluation is difficult on such plates, we separated the dansylated amino acids by HPLC on two serially connected Lichrosorb RP-8 and RP-18 columns, using both absorption at 254 nm and fluorescence with excitation at 365 nm and with emission at 451 nm for detection. Fig. 4a shows the separation of an equimolar mixture of the nine amino acids (50 pmol each) contained in the head activator, and Fig. 4b shows the head-activator hydrolysate. Both from the relative fluorescence and from the absorption at 254 nm we can deduce that only two amino acids occur twice, namely Gly and Pro. The same analysis was found for the active head-activator peptide from *Anthopleura*, for the inactive peptide of Fig. 2c, and for the peptide from hydra. Thus, the head activator is composed of the following amino acids: Gly (2), Glu (1), Ile (1), Leu (1),

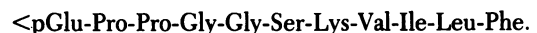
Lys (1), Phe (1), Pro (2), Ser (1), and Val (1).

**Amino terminus.** The active head activator was found to lack a free amino terminus. Acid hydrolysis of the dansylated peptide yielded monosubstituted lysine, indicating that only the  $\epsilon$ -amino group of the lysine was available for dansylation. The second peptide from Fig. 2c had a free amino terminus—namely, glutamic acid—and, therefore, was named Glu peptide or Glu head activator. This peptide could be converted quantitatively into the active head activator by treatment overnight with anhydrous trifluoroacetic acid (Fig. 5). From this we concluded that the active head activator had <pGlu as an end group.

**Amino-terminal sequence.** The amino-terminal sequence of the Glu peptide was determined by the consecutive version of the dansyl Edman procedure (10) to be Glu-Pro-Pro-Gly-Gly. A further degradation was impossible due to the fact that too many reaction products accumulated that could not be removed by organic solvent extraction because it also removed the residual head-activator peptide.

**Carboxy-terminal sequence.** The head activator was degraded partially by carboxypeptidase A, B, and Y, indicating that the carboxy end is open. By using carboxypeptidase Y, the Glu peptide could be degraded almost completely within 2 hr, whereas only Phe, Leu, and Ile were cleaved from the <pGlu peptide. Sequential digestion products of the Glu peptide with carboxypeptidase Y are shown in Fig. 6, establishing the carboxy-terminal sequence as -Lys-Val-Ile-Leu-Phe. A further analysis with this enzyme was impossible because all remaining amino acids more or less appeared simultaneously.

**Sequence analysis of fragments.** The head activator was unaffected by chymotrypsin and by pyroglutamate aminopeptidase. It was cleaved by trypsin and by an endopeptidase isolated from *Astacus fluviatilis* (11) called "low molecular weight protease." This endopeptidase was available in very pure form, worked perfectly at a dilution of 1  $\mu$ g/ml, and cleaved peptides preferentially at the amino side of Ala, Thr, Ser, and (less pronounced) Gly (11). Incubation of the <pGlu head activator for 1 hr resulted in Gly being cleaved out. A subsequent end group analysis showed Ser to be the amino acid adjacent to the cut and to Gly, and a subsequent kinetic digestion with aminopeptidase M revealed the sequential appearance of Ser, Lys, Val followed immediately by Ile, Leu, Phe (Table 1). From these data we deduce the following sequence for the biologically active head activator from *Anthopleura*:



The resulting molecular weight of 1142 is in excellent agreement with earlier physicochemical determinations (1).

The head activator from hydra was cleaved by trypsin and by the low molecular weight protease, yielding fragments with

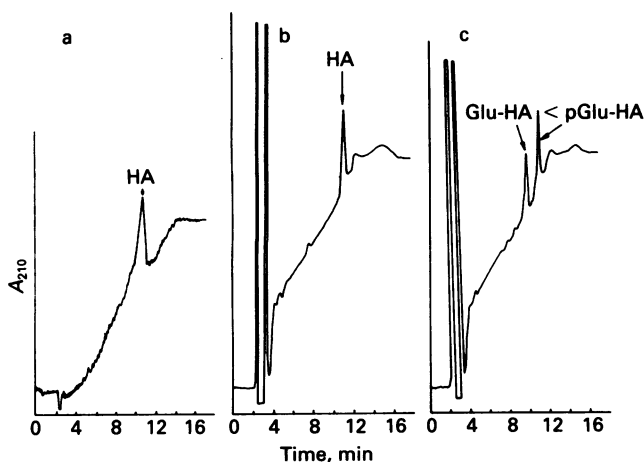


FIG. 2. Chromatography of the head activator (HA) on a Li-Chrosorb RP-8 HPLC column (column size, 250  $\times$  4 mm; particle size, 7  $\mu$ m; flow-rate, 1 ml/min) with a gradient from 20% to 40% acetonitrile in 0.1% trifluoroacetic acid (a) and from 40% to 60% methanol in 5 mM ammonium bicarbonate (b) as mobile phase. Gradient time was 10 min; in each case, 250 pmol of the head activator was applied. (c) Separation of the Glu from the <pGlu head activator under conditions as in a.

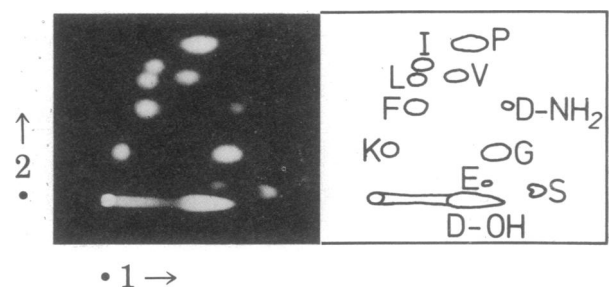


FIG. 3. Separation by thin-layer chromatography of the dansylated amino acids after total hydrolysis; 10 pmol of the head-activator hydrolysate was applied to the plate. The solvents used were 1.5% formic acid in the first dimension and benzene/acetic acid, 9:1 (vol/vol), in the second (9). Amino acid residues are indicated by the one-letter code.

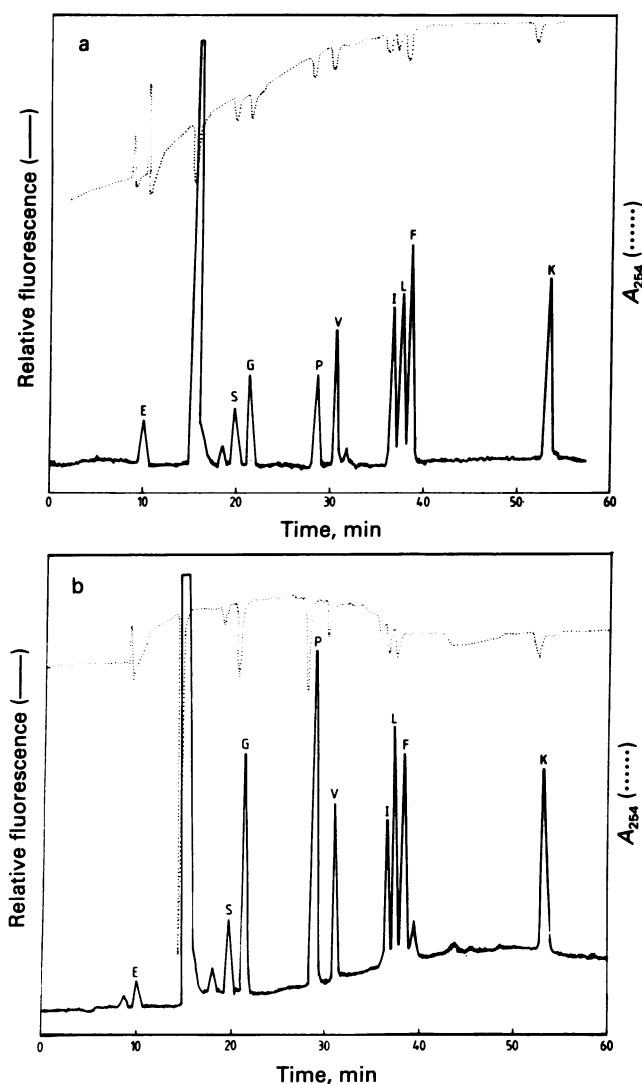


FIG. 4. Separation of dansylated amino acids on two serially connected LiChrosorb RP-8 and RP-18 columns (column size,  $250 \times 4$  mm; particle size,  $7 \mu\text{m}$ ; flow rate, 1 ml/min; mobile phase, 25-min gradient from 29% to 57% methanol in 0.01 M  $\text{Na}_2\text{HPO}_4$ , pH 7.1). (a) Fifty picomoles of the nine amino acids contained in the head activator was dansylated and applied to the column. (b) Dansylated hydrolysate of 50 pmol of the head activator. The amino acid residues are indicated by the one-letter code.

amino acid compositions identical to those of fragments of the peptide from sea anemones. Digestion with carboxypeptidase Y confirmed the carboxy-terminal sequence.

## DISCUSSION

The isolation of morphogenetic substances is difficult for the following reasons. (i) The assays for such substances are delicate, require biological skill, and are extremely laborious. (ii) As regulatory molecules, morphogens act at very low concentrations. This means that sources are scarce and that elaborate and extensive purification procedures are required. It is the famous search for a needle in a haystack. Removing the haystack is a quasi industrial-scale enterprise, requiring facilities and hands not always easily available. (iii) The needle is then present in microquantities, demanding the knowledge of and accessibility to the most recent advances and equipment for microanalysis.

In spite of these difficulties, and with a lot of patience and some luck, we succeeded in isolating such a morphogenetic substance from hydra. Hydra is a good model system to study mor-

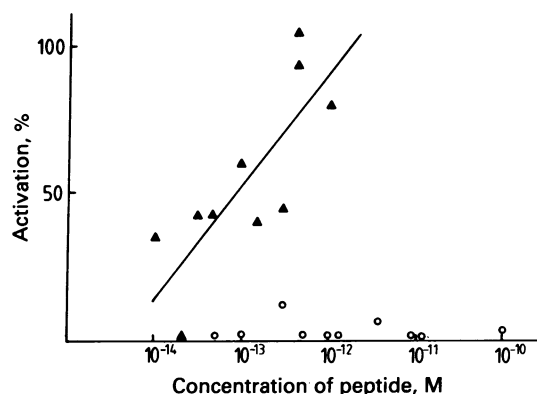


FIG. 5. Biological activity of the Glu head activator assayed before (○), and after (▲) treatment with anhydrous trifluoroacetic acid.

phogenesis because it is simple and easily amenable to experimental manipulations (2). Thus, it was relatively easy to develop an assay for a substance required for head induction (12). This head inducer or head activator was found to occur also in other coelenterates (8), which was fortunate because they are  $10^4$  times larger than hydra and abundant in the ocean. From 200 kg of *Anthopleura elegantissima*, we isolated 20  $\mu\text{g}$  of the pure head activator. This required a  $10^9$ -fold enrichment, and our overall yield was approximately 20%. The purification was complicated by the fact that the head activator, probably due to its very hydrophobic carboxy end, is a very sticky molecule. In an impure state, it adsorbed to other molecules (and eluted with them); in a pure state, it adsorbed to glassware, other surfaces, and to itself.

One problem in the final purification was that the head activator was not detectable with conventional methods. Because it contains only one aromatic residue, phenylalanine, the UV absorption is minimal: 10 nmol/ml would give an absorbance at 256 nm of 0.003. Detection by attaching a chromo- or fluorophore was either not sensitive enough or destroyed the biological, enzymatic, and chemical reactivity of the molecule. The first improvement came with the introduction of HPLC methods. For the first time, we could visualize the head activator as a physical entity, not a ghost, and also saw that it was pure.

A qualitative amino acid analysis revealed that the head activator contained nine different amino acid residues. For a quantitative evaluation, we again made use of HPLC to separate the dansylated amino acids and monitor their appearance by means of a fluorescence detector; 50 pmol could easily be visualized by this method. We found that two amino acids occur twice in the head-activator peptide, proline and glycine, resulting in the following amino acid composition: Gly (2), Glu (1), Ile (1), Leu (1), Lys (1), Phe (1), Pro (2), Ser (1), and Val (1).

The sequence analysis of the head activator was complicated by the fact that the amino terminus, which should be open for a chemical stepwise degradation, was blocked by  $\text{c-pGlu}$ . All

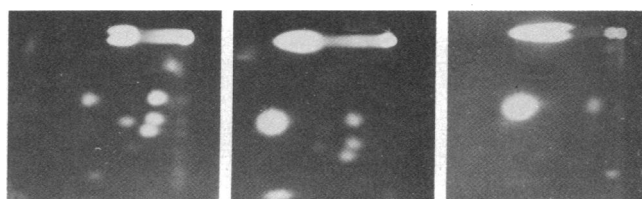


FIG. 6. Kinetics of the digestion of the Glu head activator by carboxypeptidase Y. After 2 min, only Phe is visible (Leu very weak); after 10 min, Phe, Leu, and Ile are equally strong (Val very weak); and after 30 min, Lys (bis-Lys) appears in addition to Phe, Leu, Ile, and Val.

of our attempts to open the pyroglutamyl ring intentionally either chemically or enzymatically were futile. Either we opened the ring and hydrolyzed other peptide bonds simultaneously or we found no effect. Fortunately, we discovered a peptide in some of our HPLC fractions that was identical with the head activator in its amino acid composition, had glutamic acid as amino-terminal amino acid, but was completely inactive in our biological assays. This peptide could be converted into the biologically active <pGlu head activator by treatment with anhydrous trifluoroacetic acid. This Glu peptide seemed to be ideal for a chemical degradation. However, we found that after one degradation cycle, which also converts the  $\epsilon$ -amino group of the lysine into its hydantoin derivative, the peptide became so hydrophobic that it no longer stayed in the water phase but was extracted together with the water insoluble by-products of the Edman reaction into the organic phase. Omitting the extraction or using fragments allowed us to determine the sequence of the last five amino acids. For the rest of the sequence analysis, we had to use enzymes. Normally an enzyme-to-substrate ratio of 1:1000 is used for such a purpose. Because we did have little substrate and because enzymes cannot be diluted indefinitely, we ended up using an enzyme-to-substrate ratio of 1:1 or 1:10. A number of commercial enzymes were too impure to be used this way. Fortunately, we obtained a pure endopeptidase, isolated from *Astacus fluviatilis*, from R. Zwillig (11). This enzyme could be used at a concentration of 1  $\mu$ g/ml, and it cleaved our peptide at a convenient site. In combination with various carboxypeptidases, aminopeptidase M, and Edman degradation, we finally arrived at the following sequence for the head activator: <pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe (Table 1). This sequence is consistent with enzyme specificities. Chymotrypsin, which cleaves preferentially after aromatic amino acids, was not able to destroy the head activator. Pyroglutamate amino peptidase also had no effect, which was confusing in the beginning because the enzyme should be able to remove the <pGlu. However, the enzyme does not function if the <pGlu is followed by Pro, which was the case with the head activator. The biological activity was destroyed by trypsin, which cleaved the peptide after Lys and yielded one fragment containing Glu-Pro-Gly-Ser-Lys and another with Val-Ile-Leu-Phe. The endopeptidase from *Astacus fluviatilis* cleaves preferentially in front of Ala, Thr, Ser, and (less frequently) Gly (11). We found that the head activator was cleaved twice by this enzyme, in front of Ser and Gly. A surprising finding was that the Glu peptide was a better substrate for carboxypeptidase Y than the <pGlu analogue. Whereas the Glu peptide was digested

almost to the last amino acid (except Glu), only the last three carboxy-terminal amino acids could be removed from the <pGlu peptide. This may indicate that the <pGlu head activator has a specific structure that is destroyed by opening the pyroglutamyl ring. Our data are consistent with the notion that all amino acids in the head activator are unsubstituted and are in the L configuration.

The peptide analyzed was isolated from sea anemones. We also isolated 500 pmol of the head activator from hydra and repeated some of the key experiments with this peptide. The findings (i) that the hydra peptide shows the same chromatographic behavior in all systems used, including such strange ones as Sephadex G-10 purchased before and after 1979 and precise ones such as HPLC under different conditions, (ii) that it has the same amino acid composition, and (iii) that it is degraded by the same set of enzymes are taken as evidence that the two peptides are identical.

The head activator is produced in hydra by nerve cells. We have shown earlier that a head-activator-like peptide can be isolated from mammalian hypothalamus and intestine (13). These peptides are identical in their sequence to the head activator from hydra (14). The sequence of the head activator is not identical with any of the sequences described so far for other neuropeptides. There is some minor sequence homology at the amino end of the molecule with bradykinins, given by the sequence X-Pro-Pro-Gly-X-Ser-X (15). Whether this has any functional implication remains to be elucidated.

Meanwhile, we have confirmed the structure of the head activator by chemical synthesis (16) and could show that the synthetic product is chemically and biologically indistinguishable from the native head activator from hydra.

We thank all those who helped with the isolation of the peptide. In particular we want to thank Beate Zachmann and Elke Schilling for their excellent technical assistance during the later part of this work. We are indebted to Dr. R. Zwillig for his generous gift of purified protease and thank Drs. C. J. P. Grimmelikhuijzen and Chr. Birr for discussion. This work was started at the Max-Planck-Institute (Tübingen), continued at the European Molecular Biology Laboratory (Heidelberg), and finished in our present location. We acknowledge the contribution of these institutions. This work is supported by the Deutsche Forschungsgemeinschaft; H.C.S. is a recipient of a Heisenberg Fellowship.

Table 1. Sequence analysis of the head activator

Method	Amino acid sequence
Edman	<u>Glu-Pro-Pro-Gly-Gly-</u>
Carboxy-peptidase Y	<u>-Lys-Val-Ile-Leu-Phe</u>
LM <sub>1</sub> P*	<u>-Gly-</u>
LM <sub>1</sub> P and end group	<u>-Gly-Ser-</u>
LM <sub>1</sub> P and Edman	<u>Glu-Pro-Pro-Gly-</u>
LM <sub>1</sub> P and aminopeptidase M	<u>-Gly-Ser-Lys-Val-Ile-Leu-Phe</u>

\* LM<sub>1</sub>P, low molecular weight protease, an endopeptidase isolated from *Astacus fluviatilis* (11).

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