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Induction of Metamorphosis in the Symbiotic Scyphozoan *Cassiopea andromeda*: Role of Marine Bacteria and of Biochemicals

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Abstract

In the scyphozoan *Cassiopea andromeda* the polyp stage develops either from planula larvae (resulting from sexual reproduction of the scyphomedusae) which settle on a suitable substrate and then transform into scyphopolyps, or from asexually formed buds which emerge from the calyx of such polyps, detach, settle, and in turn metamorphose into scyphistomae. The series of metamorphic events in planula larvae is documented by SEM-pictures; photomicrographs of live specimens illustrate bud formation and bud metamorphosis. Planulae and buds do not undergo metamorphosis autonomously; they strictly require exogenous inducers (Wolk et al., *Roux' Archives Devel. Biol.* **194:** 487–490, 1985). Laboratory experiments demonstrated that such inducers can be released by species of marine bacteria. We show that growing *Vibrio alginolyticus* bacteria produce peptides which induce bud metamorphosis by degrading collagen by means of an extracellular collagenase. It is assumed that this mechanisms could also operate in the natural environment. Apart from various peptide fractions from bacterial or enzymatic hydrolysates of collagens and caseins, an increasing number of oligopeptides with known chemical structure was found to induce metamorphosis. These findings suggest that not only one specific, but a number of different metamorphic inducers might be present in the sea. Active oligopeptides contain 3 to 11 amino acids. The hexapeptide Z-Gly-Pro-Gly-Gly-Pro-Ala induced bud metamorphosis within

*Dedicated to Prof. Dr. Menachem Rahat, The Hebrew University of Jerusalem, on the occasion of his 60th birthday

24 hr at 1.2×10^{-5}M. Using this hexapeptide, dose- and time-dependence of the metamorphic reaction of buds was investigated. The assumption was substantiated that information, which was received by the buds during a series of intermittent treatments with inducer, is accumulated, and that metamorphosis occurs when a threshold value is reached. The reaction of buds to the simultaneous application of two different biologically active peptides was tested. Induction of metamorphosis by the heptapeptide β-casomorphin 1–7 could be strongly delayed when the related but inactive compound β-casomorphin 1–6 or the N-substituted morphine naloxone was applied prior to or concomitant with the inducer. Since induction of metamorphosis in *Cassiopea* causes irreversible settlement of planulae or buds on a substrate, the following questions were discussed: does the morphogenic signal of the inducer(s) simply trigger attachment at random, or is the signal also directive for the site of settlement, or are additional cues required for the selection of a "suitable substrate"?

Keywords: Scyphozoa, *Cassiopea andromeda*, scyphopolyp, planula larvae, asexual buds, induction of metamorphosis, *Vibrio cholerae, Vibrio alginolyticus*, extracellular collagenase, collagen, collagen peptides, casein peptides, β-casomorphins, opiate receptor, oligopeptides, substrate selection, chemo-attractant, environmental cues

Abbreviations: Antibiotics-containing seawater (ABS)

1. Introduction

Several studies bearing on the appearance of the polyp stage in species of the symbiotic rhizostome genus *Cassiopea* have been presented in the last three decades. Gohar and Eisawy (1960b) described transformation of planula larvae into scyphistomae in *Cassiopea andromeda*, whereas Curtis and Cowden (1971) investigated polyp development from vegetative buds in *C. xamachana*; both these studies emphasized "suitable substrate" as the prerequisite for settlement of larvae and buds and subsequent metamorphosis into scyphopolyps. The endosymbiotic algae (*Symbiodinium microadriaticum*) which are known to be involved in the control of medusa formation, and which are transferred from the polyp to the sexually reproducing generation (Hofmann and Kremer, 1981, for review), apparently play no role in metamorphosis (see Rahat and Hofmann, 1987, for discussion). Hofmann et al. (1978) and Neumann (1979) stated that buds as well as planulae proved unable to undergo polyp formation when maintained in sterile or antibiotics containing seawater from the North Sea. These authors proposed that marine bacteria are involved in producing soluble, metamorphosis inducing substances. They isolated physiologically active fractions from the supernatant of suspension cultures of the marine bacterium *Vibrio alginolyticus*.

Recent studies, performed under more stringent conditions, have unanimously proven that both planula larvae and buds do not metamorphose when cultivated under axenic conditions in artificial seawater made up only of anorganic salts (Wolk et al., 1985; Fitt et al., 1987). Exogenous inducers are required to compensate for the obvious lack of an intrinsic trigger of morphogenesis. Several studies accumulated evidence that natural inducers (which are obviously present in seawater from the Red Sea) can be replaced by a variety of soluble oligopeptides, proteins, or glycoproteins (Hofmann et al., 1984; Brand, 1984; Naust, 1985; Fitt and Hofmann, 1985; Wolk et al., 1985; Fitt et al., 1987). Strikingly, none of the compounds eliciting metamorphosis in planulae of the thoroughly investigated hydroid *Hydractinia echinata* or in veliger larvae of the mollusc *Haliotis rufescens* was effective in *Cassiopea andromeda* (see Hofmann et al., 1978; Fitt et al., 1987, for references). In the present paper we first refer to metamorphosis inducing substances of bacterial origin. We analyse in particular peptide fractions which result from collagen degradation by the exoenzymes of growing *Vibrio-alginolyticus*-bacteria. We also describe bioactive products obtained by enzymatic hydrolysis of bovine collagen and bovine casein by research grade enzymes. Qualitative results of the screening of those compounds for their capacity to induce metamorphosis are summarized. We further report on experiments in which dose- and time dependence of the morphogenetic response of buds to selected peptides were assayed. We also include data from competition and inhibition studies which led to speculate on receptor mediated transmission of morphogenetic signals. Ecophysiological aspects of bud and planula metamorphosis in *C. andromeda* are discussed.

2. Materials and Methods

Planula larvae were obtained from brooding medusae of *Cassiopea andromeda* in Elat (Israel) and maintained in autoclaved Red Sea seawater or in artificial seawater with or without the addition of antibiotics (see below) during transport to the laboratory. Planulae were assayed in solutions of various compounds made up with autoclaved natural or artificial seawater at a salinity of 41°/oo.

Asexual buds were collected from cultures of *Cassiopea andromeda* scyphopolyps and maintained in sterile natural seawater from the North Sea (at 30°/oo) to which 100μg/ml of each penicilline-G potassium salt, neomycine sulfate and streptomycine sulfate were added (antibiotics-containing seawater).

Assays were run either in sterile glass-petri dishes or in 24-well Nunclon tissue culture plates (Nunc, Denmark) with 2.4 ml wells. Metamorphosing planulae were staged according to the descriptions by Gohar and Eisawy (1960b). Bud metamorphosis was staged according to the drawings given to Fig. 1; these are based on Curtis and Cowden (1971). In experiments with buds, all those buds which at least had reached or depassed stage B as shown in Fig. 1a were considered to metamorphose.

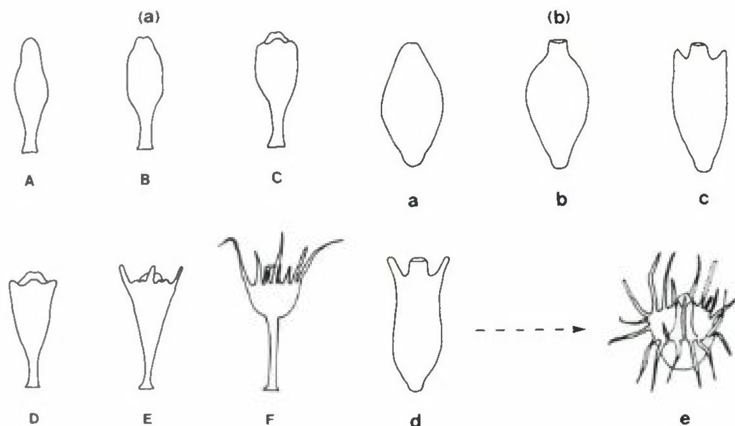


Figure 1. *Cassiopea andromeda.* (a) A–F: Metamorphosis of vegetative buds into polyps in the presence of suitable substrate or of chemical inducers; (b) a–e: Development of buds maintained in sterile medium without inducers. Stages according to Curtis and Cowden (1971). (From: Hofmann et al., *Mar. Biol.* **47**: 161–176 (1978), with permission by Springer-Verlag).

Vibrio alginolyticus were obtained form the American Type Culture Collection (Rockville), strain no. 17749 and culture were maintained on tryptic soy agar (Difco, Detroit). Aerated suspension cultures were run with 200 mg of tryptic soy broth dissolved in 500 ml North Sea seawater at pH 7.6. Density of cultures was read at 460 nm with a Spectronic 2000 Bausch and Lomb photometer and titers were determined using tryptic soy agar plates. Collagen digestion was carried out as devised by Welton and Woods (1975). Constituents: 1 mg/ml Type I bovine collagen (Serva, Heidelberg) in 0.1 M Tris-HCl buffer containing 0.002 M $CaCl_2$ and 2.34% NaCl at pH 7.6. After autoclaving, this medium was inoculated with 0.03 ml per ml of a *Vibrio alginolyticus*-overnight culture and then incubated at 34°C for up to 96 hr.

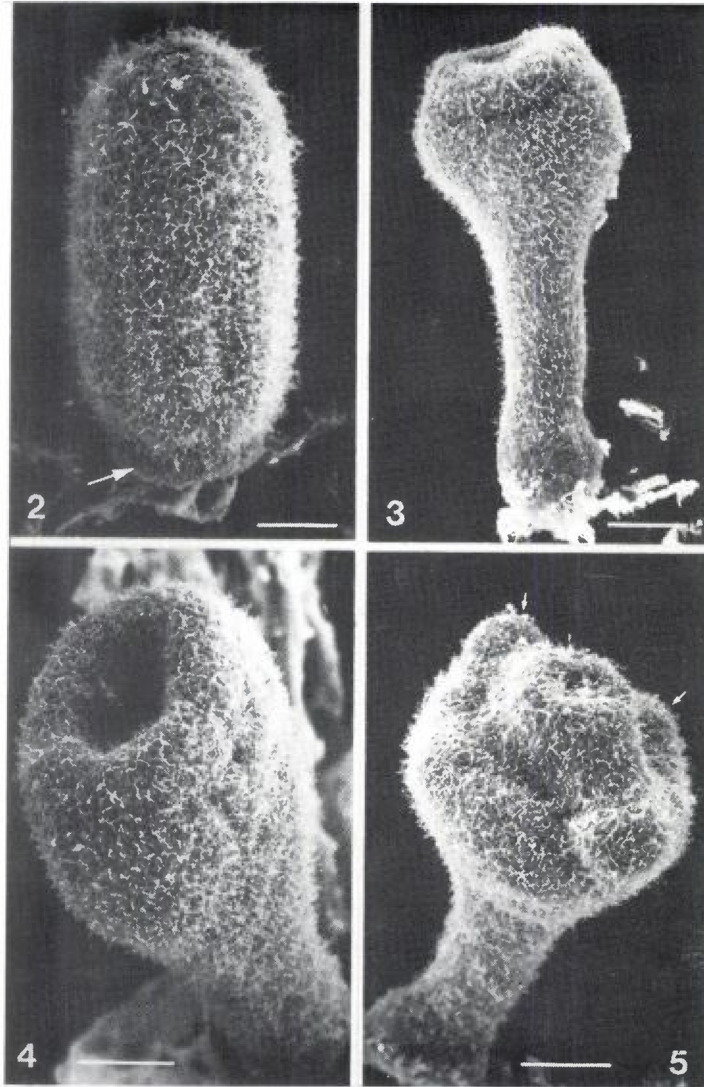
To stop the reaction 10^{-3}M EDTA was added, and bacteria were separated by centrifugation and sterile filtration. Following dialysis against distilled water the material was lyophilized and further separated and desalted by gel filtration (Sephadex G50, G25). Each step was accompanied by bioassays. Collagen degradation was performed at almost identical conditions with collagenases from *Achromobacter iophagus* (Boehringer, Mannheim) and *Clostridium histolyticum* (Serva, Heidelberg). The same chromatographic methods were used to separate the commercial collagen hydrolysate Polypep (Sigma, Munich). Naloxone and the peptide Ala-Pro-Gly were supplied by Sigma (Munich), all other peptides by Serva (Heidelberg).

All substances were dissolved in ABS and filtered through Millipore units before buds were added. Further details of the assay are given in the results and discussion section. Photomicrographs of live specimens were taken with a camera mounted on a dissection microscope. Planula larvae were prepared for scanning electron microscopy according to routine methods. Glutaraldehyde and OsO_4 were used for fixation and postfixation, 0.1 M Sörensen-buffer which contained 0.45 M sucrose was applied in all instances, and pH was adjusted to 7.5 to 7.7. Following dehydration and critical point-drying, the specimens were sputtered and then viewed with an ISI Super IIIa scanning electron microscope.

3. Results and Discussion

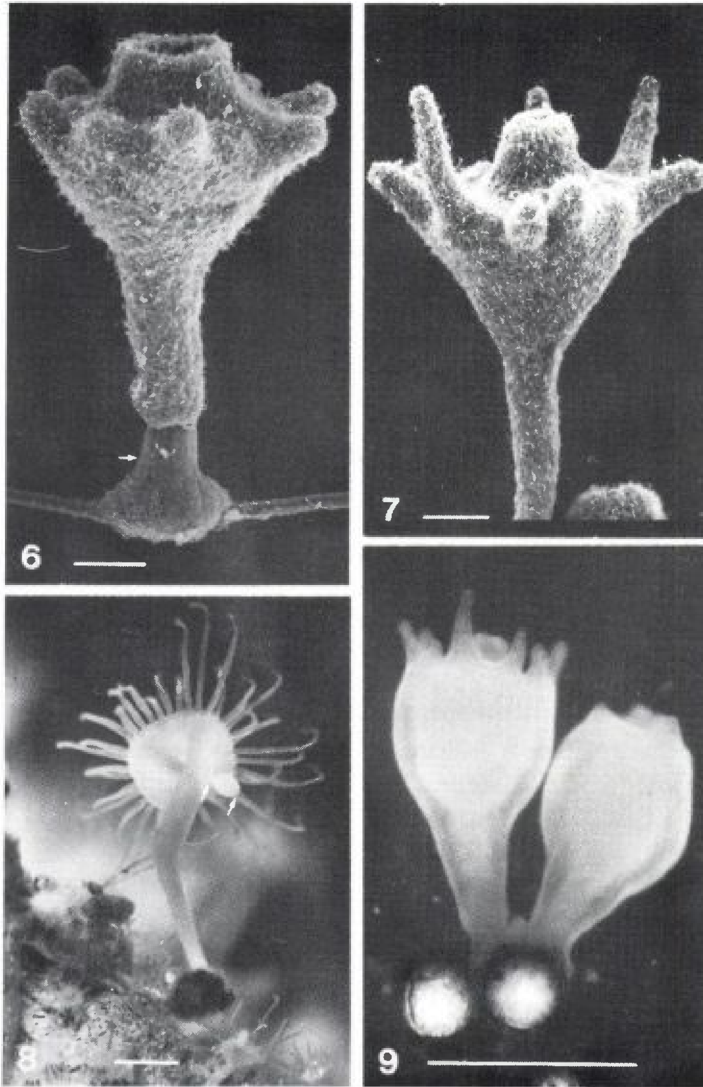
Development of scyphopolyps

As illustrated by Figs. 2–9, two different pathways lead to formation of the polyp stage within the metagenetic life-cycle of *Cassiopea andromeda*: medusae reproduce sexually and fertilized eggs develop into planula larvae which settle and then transform into sessile polyps. Some weeks later such polyps eventually start producing ciliated, spindle-shaped buds from one or more perradial sites at the lower part of the calyx. Such buds, after detaching from the adult polyps, in turn, settle and give rise to young polyps. In either case scyphistoma formation involves a series of metamorphic events. These normally begin with settlement on and irreversible attachment to a substrate of the previously mobile buds and larvae. Thereafter a pedal disc is formed and segregated from a slender, contractile stalk, and from a calyx with central hypostome and marginal tentacle *anlagen*. In the presence of suitable substrates buds may transform into polyps within 24 hr, but planula larvae reach this stage only after about 3 days. Vital marking and ^{3}H-thymidine incorporation studies indicate that on the cellular level, morphallaxis plays



Figures 2–9. *Cassiopea andromeda*. Development of scyphopolyps from planula larvae and vegetative buds.

(2) Planula settling on substrate particles. Note absence of cilia at the basal region (arrow). (3) Lateral view of early metamorphic stage of planula: segregation of peduncle-, stalk- and calyx-*anlagen*. (4) Oral view of early metamorphic stage showing developing coelenteric opening. (5) Metamorphosing planula with marginal tentacle rudiments (arrows) and central hypostome *anlage*.



(6) Young polyp stage, developing from a planula settling on a cellulose fibre. Note non-ciliated basal region of the stalk (arrow) which at later stages is secreting a protective perisarc. (7) Upper stalk and calyx region of young polyp with more elongated tentacle *anlagen* and distinct hypostome. Note cilia present on the majority of the cells. Individuals represented in Figs. 2–7 correspond to stages 2, 5, 7 and 11 of planula metamorphosis, as described by Gohar and Eisawy (1960b). (8) Scyphopolyp with bud emerging from the lower part of the calyx (arrows). (9) Vegetative buds metamorphosing on an *Artemia salina*-cyst. Stages D and E of bud metamorphosis according to Curtis and Cowden (1971). Scale bars: 50μm in Figs. 2–7, 500μm in Figs. 8 and 9.

an important role in bud metamorphosis (Hofmann, Hofmann and Manitz, unpublished observations).

Role of marine bacteria in induction of bud and planula metamorphosis

Marine bacteria could influence larvae and buds in three principal ways: (1) by establishing microbial films with particular surface properties to which the individuals could be exposed upon contact, (2) by secreting biologically active, metabolic products into the surrounding medium, (3) by modifying or degrading of organic substrates by means of exogenous enzymes, thereby leading to the release of soluble compounds. Though influences of microbial films cannot be ruled out at present, experimental proof has only been obtained for the existence of the other two pathways. Wolk et al. (1985) have shown that preparations of the 84,000 Dalton enterotoxic protein from the marine bacterium *Vibrio cholerae* (commonly referred to as cholera toxin) induces metamorphosis in larvae and buds of *C. andromeda*, acting at concentrations of 10 to 20μg/ml. Cholera toxin is a soluble, *secretory* bacterial protein.

Other experiments in our laboratory have shown that bacteria of the related species *Vibrio alginolyticus*, which are known to degrade the scleroprotein collagen by means of an inducible, extracellular collagenase, thereby release numerous soluble peptides. Some of the glycine-, proline- and hydroxyproline-rich peptides of such bacterial collagen digests were found to induce bud metamorphosis. In a typical experiment the supernatant of the filtered and centrifuged bacterial digest of 1 g of bovine type I collagen obtained after a 24 hr incubation with *Vibrio alginolyticus*-cells at 34°C, finally yielded 800 mg of lyophilized, biologically active material. In the bioassay 1 mg/ml of such partially digested collagen induced metamorphosis in 100% of the buds within 24 hr, whereas controls exposed to undigested collagen did not show any development. The fractions collected following separation by Sephadex G 50 gel chromatography were pooled according to the elution diagram which was read at 280 nm. Seven pools were obtained with apparent molecular weights ranging as follows (Table 1):

Table 1. Properties of pooled fractions from bacterially degraded collagen

Pool	Apparent molecular weight	Biological activity (bud metamorphosis assay)
A	>26 300 D	–
B	26 300 – 9 500	+
C	9 500 – 2 100	+
D	<2 100	–
E and F	not determined	–

Serial dilution experiments showed that the desalted material from pool C was more active than pool B material. Peptide fractions which induced bud metamorphosis were also obtained after incubation of bovine type I collagen with research grade collagenase from *Achromobacter iophagus* and *Clostridium histolyticum*, each enzyme providing a particular pattern of hydrolysates, corresponding to the different cleavage sites of the two endopeptidases at the collagen polypeptide chains. The commercial product Polypep (which is a collagen hydrolysate-preparation for use in histology, distributed by Sigma, Munich) was also found to contain metamorphosis inducing fractions. We assume that the mechanism of production of metamorphosis inducing factors by *Vibrio alginolyticus*, which was observed in the laboratory experiments, could also operate in the natural environment. The reasons are as follows: collagens of several types are the most abundant proteins in animal tissues and residues, and bacteria from the *Vibrio*-group (including *V. alginolyticus*) constitute a major part of the marine bacterial population. We expect that other species of microorganisms can be shown to release biologically active compounds as well. But, at the same time, we cannot exclude other biogenic sources of metamorphosis inducing compounds.

Biochemicals inducing metamorphosis

Not only collagen peptides but also soluble peptide fractions from enzymatic hydrolysates of the milk protein casein were observed to induce bud or planula metamorphosis (Hofmann et al., 1984; Naust, 1985; Fitt and Hofmann, 1985). Since it is known that complete proteolysis of collagen yields characteristic tripeptides of the type Gly-Pro-X, frequently Gly-Pro-Ala and Gly-Pro-Hyp (Harrington and Hippel, 1961), and

Table 2. *Cassiopea andromeda*: biochemicals inducing metamorphosis in buds and planula larvae

Chemicals	Buds	Planulae
Fractions from bacterial or enzymatic hydrolysates of bovine collagen	+	n.d.
Peptide fractions from enzymatic bovine casein hydrolysates	+	+
β-casomorphins		
1-3 Tyr-Pro-Phe	–	n.d.
1-4 Tyr-Pro-Phe-Pro	–	–
1-5 Tyr-Pro-Phe-Pro-Gly	+	+
1-5* Tyr-*Ala*-Phe-Pro-Gly	+	n.d.
1-5* Tyr-*Ala*-Phe-Pro-*Met*	+	n.d.
1-5*-Pro-Phe-Pro-Gly-amide	–	n.d.
1-6 Tyr-Pro-Phe-Pro-Gly-Pro	–	–
1-7 Tyr-Pro-Phe-Pro-Gly-Pro-Ile	+	+
Other oligopeptides		
Ala-Pro-Gly	+	+
Gly-Pro-Ala	+	+
Gly-Pro-Hyp	–	n.d.
Gly-Gly-Ala	–	n.d.
Gly-His-Gly	–	n.d.
Gly-Pro-Arg-Pro	n.d.	–
Gly-Gly-Pro-Ala	+	+
Gly-Gly-Asp-Ala	–	n.d.
Gly-Gly-His-Ala	–	n.d.
Z-Gly-Pro-Gly-Gly-Pro-Ala	+	+
Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2	–	n.d.
Glp-Gly-Leu-Pro-Pro-Gly-Pro-Pro-Ile-Pro-Pro	+	n.d.
Proteins and glycoproteins		
Choleratoxin (from *Vibrio cholerae*)	+	+
Thyroid stimulating hormone (TSH, bovine)	+	+

1-5*: modified β-casomorphin 1-5, modification in italics;

n.d.: no data; Z-: carbobenzoxy- ;

Compiled from Brand, 1984; Naust, 1985; Fitt and Hofmann, 1985, and unpublished results.

since cleavage of the β-casein chain by the caseinolytic activity of the *Achromobacter*-collagenase specifically leads to amino acid sequences beginning with Gly-Pro- or Ala-Pro-, we tested a variety of synthetic peptides with

similar amino acid configurations at the aminoterminal and/or carboxyterminal part of the molecules, and also compounds with different primary structure. The qualitative results of experiments performed on buds and planula larvae are summarized in Table 2. As already suggested by the collagen-experiments they show that there is not only one, very specific compound which induces metamorphosis in *Cassiopea andromeda*, but that a variety of peptides, containing 3 to 11 amino acids, are able to trigger the *entire* sequence of morphogenetic events which lead to polyp formation. We emphasize that we found among these the tripeptide Gly-Pro-Ala, which is a constituent of collagen digests (see above) and further that β-casomorphin 1-7 represents a peptide comprising amino acid nos. 60 to 66 of the β-casein molecule. A number of other active compounds is clearly structurally related to these two peptides. When comparing the primary structure of active and inactive peptides it becomes obvious that the presence of a proline residue next to the carboxyterminal amino acid is an essential, but not a sufficient prerequisite of an active compound. The additional stereochemical requirements cannot be defined yet. The specific activity of the various compounds determined at saturation conditions (concentrations at which 100% of buds metamorphose within 24 hr) varies considerably (Table 3):

Table 3. Specific activity of some metamorphosis inducing peptides

Compound tested	Concentration M incubation for	100% of the buds metamorphosed after
Z-Gly-Pro-Gly-Gly-Pro-Ala	1.2×10^{-5}	24 hr
β-casomorphin 1-7	1.9×10^{-4}	24 hr
β-casomorphin 1-5	5.7×10^{-4}	24 hr
Gly-Pro-Ala		48 hr

*toxic effects prevailed when used above this concentration

In structurally related peptides (e.g. with the sequence Gly-Pro-Ala at the carboxy-terminus, and in β-casomorphins) the molecules with the longer amino acid chain have a higher specific activity than the shorter peptides.

Kinetic studies

Figure 10 shows a clear dose-dependence of the metamorphic reaction when buds were exposed for up to 96 hr to different concentrations of the peptide Z-Gly-Pro-Gly-Gly-Pro-Ala. Whereas all buds reacted within 24 hr at or above 7μg/ml of the compound, at lower concentrations this or even a lower percentage of metamorphosed individuals was obtained only after prolonged incubation.

Using a constant concentration of 8μg/ml of the peptide, 6 hr was found to be the minimal period of incubation that yielded 100% metamorphosis. The time course of metamorphic events was investigated in buds which were incubated for 24 hr in 8μg/ml of Z-Gly-Pro-Gly-Gly-Pro-Ala. The observations were noted in terms of the stages depicted in Fig. 1a and are listed in Table 4.

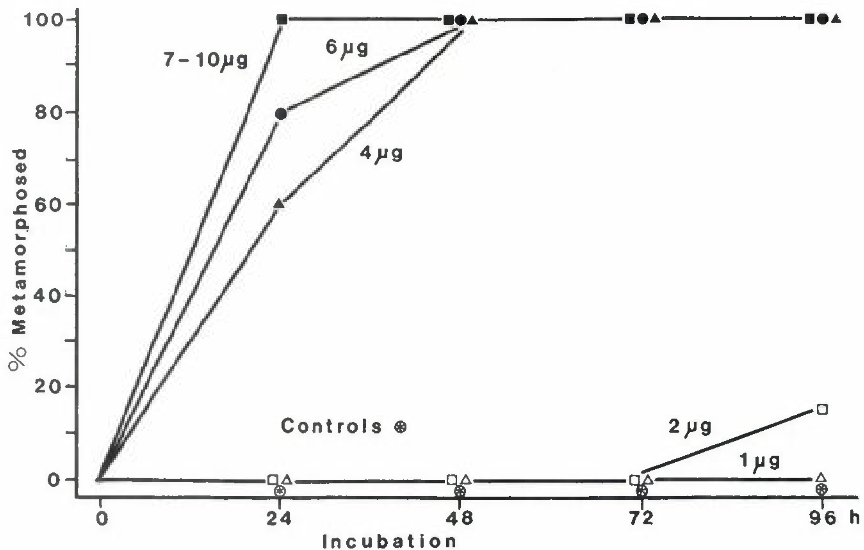


Figure 10. *Cassiopea andromeda.* Percent metamorphosis of buds treated with various concentrations of the peptide Z-Gly-Pro-Gly-Gly-Pro-Ala in ABS for up to 96 hr. Concentrations noted in μg/ml. Controls were maintained in ABS. n = 80 to 100 for each data point.

Table 4. *Cassiopea andromeda.* Time course of metamorphosis of buds induced by the peptide Z-Gly-Pro-Gly-Gly-Pro-Ala (8μg/ml).

Stage of metamorphosis	% of buds of the respective stages recorded after various incubations periods (h)					
	1–5 hr	6 hr	8 hr	10 hr	18 hr	20–24 hr
A[x]	–	–	–	–	–	–
B	–	100	20	15	–	–
C	–	–	80	85	–	–
D	–	–	–	–	–	–
E	–	–	–	–	5	–
F	–	–	–	–	95	100

[x] Stage A (see Fig. 1) was not considered. Individuals at this initial stage of metamorphosis frequently detach and resume swimming when touched or otherwise disturbed. n = 100.

When such experiments were performed at lower concentrations of the inducer (i.e. at 6 or 4μg/ml) metamorphosis started later and proceeded more slowly than at higher concentrations (see also Fig. 10).

In order to test whether buds can store signals received during treatment with metamorphosis inducing peptides, a series of experiments were performed in which individuals were intermittently exposed to the drug. Total incubation periods of up to 10 hr were subdivided into units of 1, 2, 3, 4 and 5 hr; each unit was followed by 2 washings and by a period of 24 hr during which the buds were maintained without the inducer. Following the last period of treatment with the inducer the buds were kept in ABS and observed for 48 hr. The results suggest that the information received during the successive units of treatment is stored and that buds undergo metamorphosis when a certain threshold value is reached. The effects of intermittent exposures to the drug are only approximately additive. Whereas continuous incubation for 7 hr (at 8μg/ml) caused all buds of a batch to metamorphose within 24 hr, the same percentage of transformed buds was obtained by intermittent treatment only after 8 to 10 hr total incubation time. Buds treated eight times for 1 hr however, showed 100% metamorphosis within 48 hr. These observations substantiate the assumption advanced by Fitt and Hofmann (1985) that buds and larvae of *Cassiopea andromeda* are able to accumulate the inducer (or its product) over time, and thus are not dependent

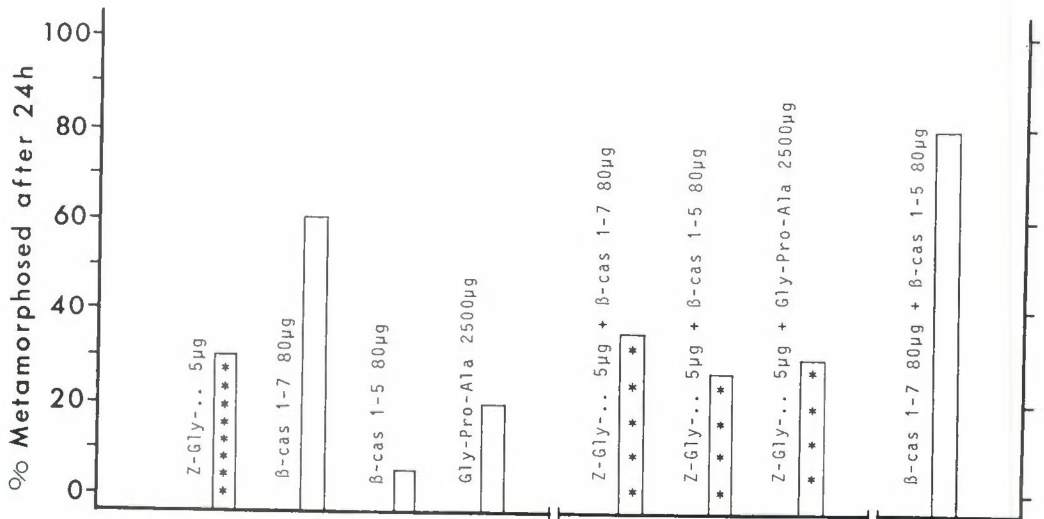


Figure 11. *Cassiopea andromeda.* Percent metamorphosis of buds after exposure to one inducing peptide (left part of the histogram), or to mixtures of two different inducers (right part of the histogram). Abbreviations: Z-Gly- : Z-Gly-Pro-Gly-Gly-Pro-Ala; β-cas 1-7: β-casomorphin 1-7; β-cas 1-5: β-casomorphin 1-5. Concentration inducated in μg/ml ABS. n = 40 in each test. Asterisks emphasize the experiments in which Z-Gly- was used.

upon a continuous and strong pulse of the inducer.

Most probably, buds or larvae encounter simultaneously several different inducing compounds in the environment. To study possible acceleration or inhibitory effects when different peptides hit buds at the same time, assays were run in which two inducers were mixed at concentrations, each of which *alone* elicited less than 100% of metamorphoses within 24 hr.

In Fig. 11 results of tests are presented in which the peptide Z-Gly-Pro-Gly-Gly-Pro-Ala was administered together with each of 3 other peptides, and in which β-casomorphin 1-7 was combined with the related β-casomorphin 1-5.

Two different categories of results were obtained. The effects of β-casomorphin 1-7 was enhanced upon the suboptimal amount of β-casomorphin 1-5. In the other experiments, however, no significant interaction with Z-Gly-Pro-Gly-Gly-Pro-Ala was observed; the rate of metamorphosis was always in the range obtained with this hexapeptide as the only inducer. From these and a number of other experiments (details not shown), it appears that in such experiments the peptide with the higher specific activity determined the rate of metamorphosis.

Studies with inhibitors are most important tools in the analysis of physiological mechanisms. With respect to metamorphosis induction in *Cassiopea andromeda*, information on inhibitory substances was scarce. Using fractions from enzymatic casein digests as the inducing agent, metamorphosis could be prevented when 80μg/ml of colchicine were applied concomitant with the inducer. The ED of the inhibitor was extrapolated to be at 3×10^{-2}M. Actinomycin D applied at 1μg/ml (ED_{50}, extrapolated, was at about 3×10^{-7}M) also completely inhibited bud metamorphosis (Hofmann and Fenners, unpublished results). However, we suspect that the observed inhibitory effects result from very general and unspecific interactions of the drugs with the cytoskeletal elements or the DNA-transcription apparatus rather than from specific interference with the system involved in the transmission of the morphogenetic signals.

A more specific type of interaction presumably accounts for the inhibitory effects which were observed when the non inducing β-casomorphin 1-6 (at concentrations of 60 to 400μg/ml) was applied to buds simultaneously with the inducer β-casomorphin 1-7 (100μg/ml). Whereas all of the controls in β-casomorphin 1-7 metamorphosed within 24 hr, morphogenesis was strongly delayed for 24 to 48 hr in the experiments. However, upon longer exposure, most of the buds entered metamorphosis.

Polyp formation could also be delayed when buds were preincubated with the inactive compound for 48 hr, then rinsed twice in ABS and thereafter incubated with the inducer. After 72 hr (instead of 24 hr in the controls) all of the buds were metamorphosed. It is important to note that the N-substituted morphine compound naloxone also interferes with the metamorphosis-inducing action of β-casomorphin 1-7 in *Cassiopea*-buds. Naloxone *per se* does not trigger metamorphosis, and increasingly toxic effects occur only when applied above 80 to 100μg/ml for prolonged periods of time. Polyp development was delayed when buds were incubated in naloxone (5 to 100μg/ml)prior to or concomitant with the application of the inducer β-casomorphin 1-7 (100μg/ml). As in the experiments using the inactive β-casomorphin 1-6, buds took up to 72 hr instead of 24 hr to enter metamorphosis. Intriguingly, neither β-casomorphin 1-6 nor naloxone exerted clear dose-dependent effects. Furthermore both these compounds were ineffective when administered prior to or simultaneously with the inducer Z-Gly-Pro-Gly-Gly-Pro-Ala.

It is tempting to discuss the results of the aforementioned experiments with inhibitors in terms of competition for binding sites of receptors involved in the

transduction of morphogenetic signals. The opioid action of β-casomorphin 1-7 in the guinea pig ileum muscle assay was found to be specifically antagonized by naloxone, the site of action being the predominant opiate receptor of the organ, the μ-receptor (Henschen et al., 1981). The use of radioactively labelled agonists and antagonists will enable us to test the validity of the concept of receptor-mediated induction of metamorphosis in *Cassiopea andromeda.*

Ecological aspects

Cassiopea-polyps are essentially sessile organisms, and in the initial phase of metamorphosis, larvae and buds normally settle on and irreversibly attach to a substrate. Assuming that secretory products of bacteria and soluble substances resulting from bacterial degradation of biogenic substrates play the alleged, important role in induction of metamorphosis, we must ask how this settlement and subsequent polyp formation is brought about. (1) Do these interorganismic messengers (chemicals) *just permit* settlement and metamorphosis *at random*? Or (2) are these chemical signals at the same time *chemo-attractants* and cause settlement next to their source? Can we speculate that the signals are even *indicative* for sites of settlement *which are advantageous for the Cassiopea-polyp generation*? (3) We have to ask further: do other types of organisms provide *additional cues* which, in the presence of inducer molecules, lead to *select* a particular substrate to settle on? Because eco-physiological data are completely lacking, we cannot present conclusive evidence for chemotaxis or substrate selection in *Cassiopea andromeda.* Only some preliminary findings indicate that larvae settle on some species of marine algae but not on others.

The thigmotactic "*touch-and-go*" swimming behaviour of buds observed in the laboratory leads to frequent contacts with the wall and the bottom of the dish. In the presence of metamorphosis-inducing substances, in an early reaction to the stimulus, buds and planulae start secreting mucus from the presumptive basal portion of the organism. They thereby "glue" to all sorts of materials: glass, plastics, cellulose fibres, other organic matter, etc, and then complete metamorphosis. Some metamorphose without prior attachment to a substratum hanging upside down from the surface pellicle. These observations show that in homogenous solutions of inducing compounds, even in the absence of specific environmental cues, behavioral traits of buds and larvae cause the majority of the individuals to attach in a seemingly random manner to a substrate and to transform into sessile polyps.

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