Proline Control of the Feeding Reaction of *Cordylophora*

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ABSTRACT The colonial hydroid *Cordylophora* is a carnivore whose feeding is induced by substances released from captured prey. An active molecule, probably the only one, has been isolated from a fraction of the laboratory food of *Cordylophora*, brine shrimp larvae, and identified on paper chromatograms as the imino acid proline. Reagent proline induces the feeding reaction at 10^{-5} M. The reaction is specific in that only two α-imino acids very closely related to proline were found to possess significant activity: azetidine-2-carboxylic acid and pipecolic acid. The response to proline is inhibited by magnesium ions and enhanced by phosphate. Since previous studies have shown that the feeding reactions of *Hydra*, *Physalia*, and *Campanularia* are controlled by reduced glutathione, the phylogenetic implications of the proline control of feeding in *Cordylophora* are discussed. The feeding reactions of both *Cordylophora* and *Hydra* are also induced by proteases, suggesting similar mechanisms of induction in the two hydroids.

A diversity of organisms make use of chemicals emanating from their specific food either to seek out or to recognize that food (23). Most coelenterates, either as swimming medusae or sessile polyps, do not seek their food, but rather wait passively until a potential prey collides with their tentacles. If the prey has suitable properties (29), nematocysts on the tentacles puncture the cuticle or epidermis of the prey, simultaneously holding and poisoning it. Whether or not captured prey is eaten depends on a second reaction (6). If chemicals in the fluids released from the pierced prey elicit a response, the tentacles holding the prey contract to the mouth, the mouth opens, and the food is swallowed. It is this second reaction which is known as the feeding reaction.

Coelenterates of all major groups give a feeding reaction in response to tissue juices. Dilute meat extracts produce appropriate reactions in sea anemones (21), reef-building corals (1), and jellyfish (10). Members of the

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class Hydrozoa, and particularly hydra,[1] have been most thoroughly studied. Hydra normally eat only living prey, but Beutler (3) found that hydra will swallow gelatin fragments after the fragments have been soaked in fresh tissue juice. Ewer (6) found that fresh tissue juice could induce a feeding reaction in hydra even though no particulate matter was present.

In 1955 Loomis (16) demonstrated that the feeding reaction of *Hydra littoralis* is mediated by reduced glutathione released in the body fluids of captured prey. Glutathione induces a coordinated feeding reaction at concentrations as low as 10^{-5} M; the response is specific to the peptide backbone of glutathione (5, 14, 16). Lenhoff and Schneiderman (15) found that the feeding reactions of two other hydrozoans, the polymorphic Portuguese Man-of-war, *Physalia*, and the calyptoblastic colonial hydroid *Campanularia*, are also controlled by glutathione.

Cordylophora, a gymnoblastic colonial hydroid, has a chemically mediated feeding reaction, but one mediated by some compound other than glutathione. That compound has been found to be the α-imino acid proline.

MATERIALS AND METHODS

Cordylophora lacustris, clone A, was grown as described by Fulton (8, 9). The culture solution was CCS5 or CVD, which is CCS5 containing 1.5×10^{-4} M disodium ethylenediamine tetraacetate. Unless otherwise indicated, colonies had been starved for about 24 hours when used in an experiment.

For bioassays, fully grown hydranths with a short length of attached stem were removed from colonies. To 1 ml of test solution in a depression of a Pyrex spot plate, a drop of CVD containing two or more hydranths was added, and the reaction scored using a dissecting microscope. For most experiments, Phos-CCS5 was used as the bioassay environment. Phos-CCS5 was prepared daily by a 2:1:7 mixture of stock CCS5; 0.066 M phosphate buffer, pH 6.8; and demineralized water. Stock CCS5 contained NaCl, 14.6 gm; KCl, 0.372 gm; $CaCl_2 \cdot 2\ H_2O$, 3.68 gm; $MgSO_4 \cdot 7\ H_2O$, 6.16 gm in 1 liter of demineralized water. Phos-CCS5 had a pH of about 6.6, and was unstable.

In scoring the feeding reaction a complete reaction, including tentacle waving, oral cone bending, and finally mouth opening, was recorded as $++$; a reaction which involved tentacle and oral cone bending as $+$; waving tentacles only as $\pm$; and no response as $-$. Experience with *Cordylophora* permits scoring $+$ and particularly $++$ feeding reactions unequivocally and without confusing them with mouth opening or tentacle waving produced by unfavorable conditions.

Hydra littoralis was grown in BVT as described by Loomis and Lenhoff (17). Feeding reaction assays were performed in BVT on individuals starved for 1 day.

Two preparations of *Artemia*, designated juice and extract, were used to study the feeding reaction. *Artemia* juice was the supernatant fluid obtained by homogenizing

[1] As used here, the term hydra refers to any species while *Hydra* refers to *H. littoralis*.

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dense suspensions of nauplii in demineralized water in a Pyrex tissue grinder and centrifuging off the insoluble residue. Protein-free *Artemia* extract was prepared from the juice by adding trichloroacetic acid to the supernatant and chilling and centrifuging. The trichloroacetic acid was removed from the supernatant by repeated extractions with ethyl ether, and after removal of the ether *in vacuo* or with heating the extract was ready for use.

Ion exchange column chromatography was done on Dowex 1-x8 and Dowex 50W-x8 resins (both 100 to 200 mesh). The resins were washed successively on a Büchner funnel with water, 2 N HCl, water, 2 N NaOH, water, 2 N HCl, and demineralized water, and poured on to columns made of 20 mm Pyrex tubing. Material was allowed to flow through the columns by gravity, and the effluent collected manually in test tubes. The active fraction was concentrated by evaporation.

Ascending paper chromatography was accomplished on Whatman No. 1 paper using solvents prepared as described by Smith (26) and equilibrated in the jars for 24 hours before chromatograms were run. As a control for R_f values a mixture containing 2 mg per ml each of L-glycine, L-leucine, L-lysine·HCl, and L-proline was used. An aliquot of 0.002 ml of the known mixture and three 0.004 ml aliquots of the unknown mixture were spotted in a row on an 11 × 30 cm strip of paper. Solvent was allowed to ascend the strips, after which they were air-dried. A strip containing one known and one unknown application was cut off and treated with ninhydrin in acetone-pyridine (26). Other strips containing unknowns were cut up and eluted with Phos-CCS5 for bioassay. For satisfactory bioassays it was necessary (*a*) to adjust pH of eluants from butanol–acetic acid chromatograms, and (*b*) to extract residual phenol with slightly acidified ether from the phenol-ammonia chromatograms before elution.

RESULTS

Characterization of the Feeding Reaction Inducer

When *Cordylophora* are fed larvae or nauplii of the brine shrimp, *Artemia*, the nauplii captured are quickly paralyzed and the tentacles holding them curl in toward the mouth. The oral cone bends toward the nauplii, and on contact the mouth spreads around the prey and swallows it. This feeding reaction is similar to that observed in *Hydra*, except that in *Hydra* the mouth does not bend toward the food. *Cordylophora* tentacles capture miscellaneous objects, such as bits of dried agar or hair, but such objects are dropped without having elicited a feeding reaction.

Since glutathione induces a feeding reaction in other hydroids, numerous attempts were made to obtain a feeding reaction in *Cordylophora* using glutathione at concentrations from 10^{-2} to 10^{-7} M and hydranths starved up to a week. No definite response could be elicited, though waving of tentacles was frequently observed at higher glutathione concentrations. The same glutathione solutions induced a feeding reaction in *Hydra* (Table I).

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Evidence that *Cordylophora* has a chemically mediated feeding reaction was obtained when fresh *Artemia* juice induced a response in *Cordylophora* similar in all respects to that observed in hydranths fed living nauplii, even when the juice was diluted 10,000-fold. Hydranths in *Artemia* juice attempted to ingest any object they encountered, including the glass walls of their container or each other. The inducer, unlike the active compound for *Hydra*, was stable to oxidation and aging, and survived exhaustive acid hydrolysis (Table I). *Cordylophora* even ate ground beef and other meat fragments that did not induce any response in *Hydra*. Such meat fragments were only eaten if they were fed to *Cordylophora* as soon as put under water. If the fluid which

TABLE I

COMPARISON OF THE FEEDING REACTION INDUCERS IN *CORDYLOPHORA* AND *HYDRA*

A ++ indicates an unequivocal feeding reaction, and a – indicates no response to the test material. The responses were evaluated at several dilutions of each test material.

Test material	*Cordylophora*	*Hydra*
Reduced glutathione	–	++*
Fresh *Artemia* juice	++	++*
Fresh *Artemia* juice, treated with 0.3 per cent H_2O_2 for 12 min.	++	–*
Artemia juice, stored at room temperature for several hours	++	–*
Artemia juice, hydrolyzed in 6 N HCl, 48 hrs., 100°C	++	
Ground beef	++	–

* Confirming Loomis (16).

leaches out of the fragments was allowed to diffuse away, the hydranths held but did not swallow the fragments, giving further indication that a diffusible compound is involved.

BIOASSAY FOR THE INDUCER

Bioassays of inducer activity performed in CVD gave satisfactory results with *Artemia* juice and extract, but when fractionation of these mixtures was attempted, the results of bioassays became erratic. Therefore the bioassay environment was studied by observing the response of hydranths to serial dilutions of *Artemia* extract in various solutions.

Alterations of pH or buffer had a profound influence on the response to *Artemia* extract (Table II). Phosphate-buffered CCS5, pH below 7, permitted greater response to a given concentration of inducer than did tris- or bicarbonate-buffered CCS5, pH above 7. This was not entirely a pH effect, however, since phosphate-buffered solutions gave consistently better results

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than did unbuffered solutions or solutions buffered with histidine at a similar pH. Furthermore, dilution of the phosphate buffer fourfold reduced the activity 10- to 50-fold even though the pH during the assay remained constant at 6.6. Phosphate itself clearly enhances the feeding reaction induced by *Artemia* extract. These observations permitted the development of an assay solution (Phos-CCS5) which increased both the sensitivity and the reproducibility of bioassays of inducer activity.

It seemed possible that at least part of the enhancement of inducer activity by phosphate buffer might be due to complexing of the magnesium and calcium ions in CCS5 by phosphate ions. It was found, indeed, that removal of magnesium from unbuffered or regular (bicarbonate-buffered) CCS5

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TABLE II
INFLUENCE OF pH AND BUFFER ON THE FEEDING RESPONSE TO *ARTEMIA* EXTRACT

Unbuffered stock CCS5 was diluted in several buffers as indicated. *Artemia* extract was diluted in these solutions, and the activity bioassayed with *Cordylophora* starved for 1 day. Only the responses most often observed at a 1/1000 dilution of the extract are indicated in the table.

Buffer	pH	Response
Phosphate, 0.0066 M	5.8	++
None	6.0	+
Histidine, 0.005 M	6.4	±
Phosphate, 0.0066 M	6.6	++
Phosphate, 0.0017 M	6.6	+
Bicarbonate, 0.002 M	7.3	+
Tris*, 0.005 M	7.4	+
Tris, 0.005 M	8.0	±

* Tris(hydroxymethyl)aminomethane.

increased the activity about 10-fold over that observed with magnesium present (Table III). However, removal of magnesium from phosphate- or tris-buffered CCS5 did not enhance the activity of inducer in these solutions.

PROPERTIES OF THE INDUCER

Preliminary studies using *Artemia* juice as source of inducer showed that although the inducer did not survive ignition, it was stable to acid hydrolysis (Table I) and similar treatments. It was readily dialyzable and soluble in trichloroacetic acid, but insoluble in ether and other non-polar solvents. On small ion exchange columns, the active material in *Artemia* extract could be washed off the anion exchange resin Dowex 1 (chloride form) with water but had to be eluted from the cation exchange resin Dowex 50W (hydrogen form) with acid. When paper chromatograms were run in isopropanol-

formate or butanol-formate (24) the inducer moved on the paper and gave one active spot, suggesting that a single substance in *Artemia* extract has biological activity.

These experiments indicated that the feeding reaction inducer was probably a single molecule of low molecular weight, and in addition provided tools for its identification.

Isolation and Identification of the Inducer

The inducer in the trichloroacetic acid–soluble, ether-insoluble fraction of *Artemia* juice was separated from salts and further purified using ion exchange columns, and then identified by paper chromatography. An *Artemia* extract, which had activity at a 1/10,000 dilution, was loaded on a column of Dowex 1 (chloride form). The active material was washed through with water and

TABLE III
INFLUENCE OF MAGNESIUM IONS ON THE FEEDING RESPONSE TO *ARTEMIA* EXTRACT

The procedure was the same as that described in Table II. CCS5 contained 0.005 M $MgSO_4$.

Buffer	pH	Response	
		CCS5	Mg-free CCS5
None	6.0	+	++
Phosphate, 0.0066 M	6.6	++	++
Bicarbonate, 0.002 M	7.3	+	++*
Tris, 0.005 M	7.4	+	+

* Somewhat erratic.

concentrated by evaporation. This fraction was loaded on a Dowex 50W (hydrogen form) column, and washed with water and 0.1 N HCl, during which time no measurable activity came off. The active material was then eluted with 1.5 N HCl; most of the activity was concentrated in five tubes, which were pooled, concentrated, and brought to dryness over NaOH to remove HCl. The residue was dissolved in 0.3 ml of water.

The concentrate was spotted on paper along with a mixture of known substances containing glycine, leucine, lysine, and proline, selected to provide a check on the accuracy of the R_f determinations. Ascending chromatograms were run in three solvents, after which the papers were dried and residual solvent extracted. A strip containing a known and an unknown was treated with ninhydrin, and other unknown strips were cut into segments which were eluted and bioassayed in Phos-CCS5.

A tracing of the three chromatograms treated with ninhydrin is given in

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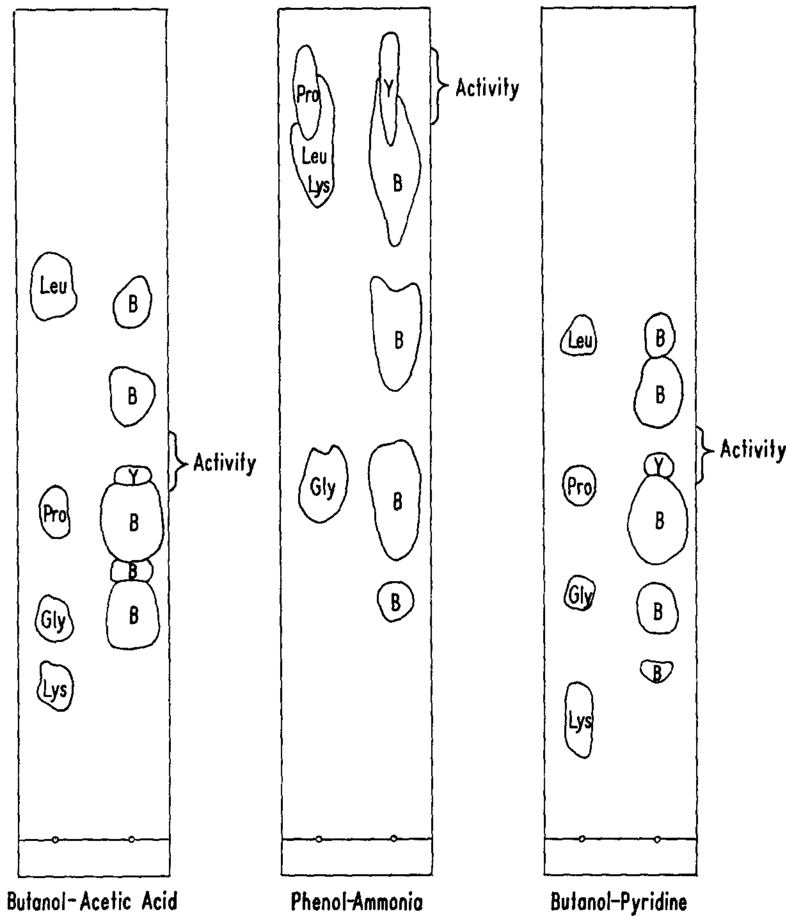

FIGURE 1. Tracings of the ninhydrin spots on the three ascending chromatograms described in the text. On each chromatogram, the strip to the left represents the known amino acids, that to the right the unknown sample. The braces indicate the areas on adjacent unknown strips from which inducer activity could be eluted. The solvents used are described in Smith (26); the actual size of the paper strips shown is 4 × 22 cm. Abbreviations: Gly, glycine; Leu, leucine; Lys, lysine·HCl; Pro, proline; B, blue; Y, yellow.

Fig. 1. The knowns gave R_f values corresponding closely to those in Smith (26). There were five to six ninhydrin-positive spots on the chromatograms of the unknown; one of these correlated perfectly with inducer activity. That spot had the R_f value of proline and gave the yellow ninhydrin color characteristic of proline.

This provisional identification of the inducer could be tested directly with the compound in question. Reagent L-proline in Phos-CCS5 induced a ++ feeding reaction at concentrations as low as 10^{-5} M (Table IV). In standard

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culture solutions, such as CVD or CCS5, which contain magnesium ions and do not contain phosphate, proline induced feeding reactions erratically at various concentrations. In experiments similar to those described in Tables II and III, proline has given results similar to those obtained with *Artemia* extract. Proline at low concentrations regularly induced a feeding reaction in phosphate-buffered CCS5 or in unbuffered CCS5 from which magnesium ions were omitted.

This simple procedure led to the tentative identification of the natural inducer as proline, an identification supported by the high biological activity of reagent proline.

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TABLE IV

INDUCTION OF THE *CORDYLOPHORA* FEEDING REACTION BY PROLINE

The following indicate the responses most regularly observed when dilutions of reagent L-proline in Phos-CCS5 were bioassayed with *Cordylophora* hydranths that had been starved for 1 day.

	Concentration of L-proline, M						
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	None
Response	++	++	++	++/+	+*	+*	–

* Occasional hydranths gave a ++ response

Specificity of Proline as the Cordylophora Feeding Reaction Inducer

A general survey for activity of amino acids revealed that though alanine and several others induced some tentacle waving at higher concentrations (*e.g.*, 10^{-3} M), only proline had the ability to induce a complete feeding reaction. A variety of structural analogues of proline have been examined for inducer activity. Serial dilutions of 0.01 M stock solutions were made in Phos-CCS5 (range 10^{-3} to 10^{-5} M). The structures of some of the compounds examined are illustrated in Fig. 2.

Two compounds, both closely related to proline, were moderately active. The recently discovered proline analogue with a 4-membered ring, azetidine-2-carboxylic acid (7),[2] had as much or slightly less activity than proline, and the analogue with a 6-membered ring, pipecolic acid, appeared about 10-fold less active than proline. Both of these have been found in nature, though only in plant tissues so far (7). One other substitution in the upper part of the ring (thioproline or 4-thiazolidinecarboxylic acid) reduced the activity 100-fold but apparently did not abolish it.

[2] L-Azetidine-2-carboxylic acid was generously provided by Dr. L. Fowden, 4-hydroxy-L-proline by Dr. A. Meister, and L-prolinamide·HCl and L-prolylbenzyl-ester·HCl by Dr. E. L. Smith. Other proline analogues were obtained from commercial sources.

Two commercial samples of hydroxyproline (isolated from nature) were also slightly active, but this activity (as that of thioproline) could be accounted for if the samples contained about 1 per cent proline. A sample of proline-free hydroxyproline was inactive at all concentrations tested, indicating that the slight activity of commercial hydroxyproline was indeed due to proline.

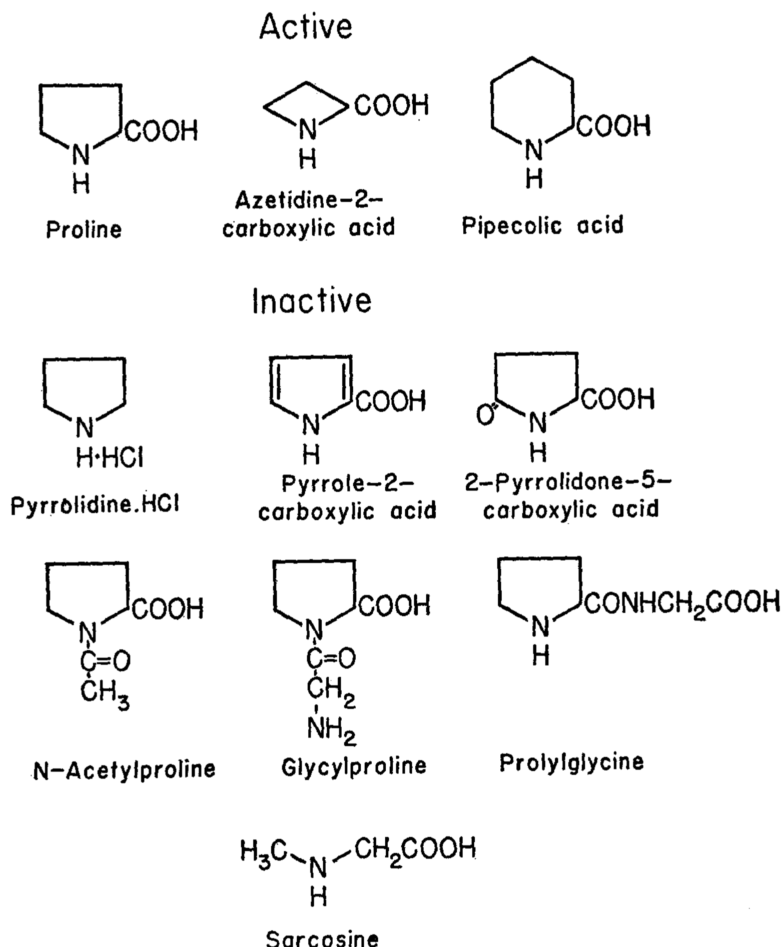

FIGURE 2. Formulae of some of the proline analogues tested for inducer activity.

All other proline analogues tested were inactive up to molar concentrations more than 100-fold greater than that needed for activity of proline. Proline peptides are probably not a source of inducer activity, as indicated by the experiments with prolylglycine and glycylproline. Removal (pyrrolidine) or substitution (prolinamide, prolylbenzyl ester, prolylglycine) on the carboxyl group destroyed activity, as did substitutions on the imino group (acetyl-

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proline, glycylproline). Substitution of either pyrrole or pyrrolidone rings for the pyrrolidine ring led to inactive analogues. Finally, sarcosine, a noncyclic α-imino acid, had no activity.

Of the inactive compounds, none acted as an antimetabolite of proline, even at 100-fold molar excess. It is possible that if substantially higher concentrations did not induce side effects, antimetabolite activity could be demonstrated, since inhibition indices of 100 or greater are not uncommon (28). No really effective proline antimetabolite is known for other systems (18).

TABLE V

ACTIVATION OF THE FEEDING REACTION BY PROTEASES

Cordylophora assays were performed in Phos-CCS5 at pH 6.6 with hydranths starved for 1 day; results for *H. littoralis* are from Lenhoff and Bovaird (13).

Test solution	*Hydra*	*Cordylophora*
10^{-5} M reduced glutathione	++	−
10^{-5} M L-proline	−	++
20 μg/ml papain	−	−
20 μg/ml papain + 10^{-3} M cysteine	++	++
10^{-3} M cysteine	−	−
100 μg/ml trypsin	++	++
100 μg/ml trypsin + 100 μg/ml trypsin inhibitor	−	−

Protease Activation of the Feeding Reaction

Recently Lenhoff and Bovaird (13) showed that certain proteolytic enzymes activate the feeding reaction of *Hydra littoralis* much as does glutathione. A variety of other enzymes and proteins are inactive in this respect. The observation of Lenhoff and Bovaird was readily extended to *Cordylophora*; the two proteases tested activated a feeding reaction indistinguishable from that induced by proline (Table V). The enzymes were dialyzed thoroughly using the procedure of Lenhoff and Bovaird (13) to remove glutathione, proline, or other small molecules. Papain itself induced no response in *Cordylophora* whereas when the protease was activated by brief incubation with 10^{-3} M cysteine, the active enzyme induced a good feeding reaction, including cannibalism and attempts to ingest the glass walls of the container. Cysteine itself possessed no activity.

Dialyzed trypsin also induced a good feeding reaction. In contrast, if the trypsin was incubated with crystalline soy-bean trypsin inhibitor (also

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dialyzed) for about 30 minutes before adding *Cordylophora*, the feeding reaction was almost completely suppressed.

Neither papain, trypsin, or soy-bean inhibitor had any deleterious effects on *Cordylophora*. Further, at least in the case of trypsin, it seems clear that the enzyme does not act by releasing proline from *Cordylophora*, since trypsin acts only at lysine and arginine bonds (*cf.* references 2, 19).

These trials represent two critical tests of protease activity: activation of papain by a reducing agent and inhibition of tryptic activity with soy-bean inhibitor. Both results suggest that the activity is the result of the proteolytic activity of the enzyme.

DISCUSSION

A conceptual picture of the feeding reaction of *Cordylophora* may be developed from these studies. Nematocysts pierce the body wall of a prey organism, leading to the release of body fluid containing the feeding reaction inducer, proline. In response to the proline, tentacles are brought to the mouth, and the mouth opens and swallows the prey. Under appropriate conditions, the same reaction may be induced by immersion of a hydranth in a proline solution.

The feeding reaction is undoubtedly the result of coordinated contraction and relaxation of muscle fibers in a *Cordylophora* hydranth. These muscle fibers have been carefully described by Pauly (22), and appear to be similar to those found in *Hydra* (20, 25). Fibers at the base of ectodermal cells run parallel to the long axis of the hydranth, and other fibers at the base of endodermal cells form a ring around the hydranth. The feeding reaction would appear to involve contraction of the longitudinal fibers in the ectoderm and relaxation of the endodermal fibers. The movements are highly coordinated; *e.g.*, bending of a tentacle toward the mouth must involve contraction of ectodermal fibers on only one side of the tentacle. Further, the response under the artificial condition of total immersion in a proline solution remains as coordinated as the response to gradients of inducer pouring out of prey organisms, indicating that the feeding reaction is coordinated by some factors other than localized exposure to inducer.

The conclusion that the normal feeding reaction of *Cordylophora* is caused by proline is based on the following evidence. Extracts of *Artemia* larvae and other organisms elicit a feeding reaction, and a small and stable molecule is involved in this response. Paper chromatograms of such tissue extracts yielded only a single spot capable of inducing the feeding reaction. The feeding reaction inducer was isolated from *Artemia* extract and identified on paper chromatograms as proline. Reagent proline induced the feeding reaction at

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low concentrations. Examination of the specificity of the reaction indicates that only certain imino acids are capable of eliciting the feeding reaction; the feeding reaction obtained in response to these imino acids is in all respects similar to that obtained with tissue extracts. Though no decisive test has been found to show that proline is the only component of tissue fluids capable of inducing the feeding reaction, the specificity to proline and closely related imino acids provides good evidence for this view.

On the basis of the survey of proline analogues, the requirements for inducer activity seem to be relatively simple and yet specific: a heterocyclic α-imino acid which is not substituted or unsaturated in such a way as to influence the recognition of the imino acid group. The inducer activity of α-imino acids having 4- , 5- , and 6-membered rings and probably also of thioproline suggests that the receptor recognizes primarily the imino acid region.

An interesting feature of the feeding reaction of *Cordylophora* as compared with that of *Hydra* lies in the marked differences between the two active molecules. There is little in the written structure of glutathione to suggest any possible similarity to proline, but glutathione may exist in solution in several cyclized forms (4, 27), one of which if correct introduces a heterocyclic α-imino acid into the molecule. Though *Cordylophora* probably could not recognize such a molecule because of the substituents, it is conceivable that some evolutionary relationship may lie in this observation.

For *Cordylophora*, and also for *Hydra* (12), ions in the aqueous environment have been found to influence the ability to respond to the appropriate inducer. In *Cordylophora*, phosphate enhances the response to proline, while magnesium ions inhibit the response. It is possible that these two effects may be related to each other, and possibly also to a requirement for calcium ions (*cf*. reference 12); these observations require further study.

It has been noted that the response of *Cordylophora* to proline in standard culture solutions is negligible compared to that in Phos-CCS5. Yet the same hydranths will gorge themselves on *Artemia* larvae or other suitable prey in the same standard culture solutions. The explanation of this paradox is probably that with captured prey, proline is being released as a component of the body fluid, containing a host of other substances, perhaps including phosphate and other substances which can sequester magnesium ions. Some of these substances may provide suitable conditions for the induction of the feeding reaction by proline. Many other factors may also influence the feeding reaction, including the mechanical factor of prey capture, and the factor of localized exposure to relatively high concentrations of body fluid.

The results of this study are provocative in relation to hydroid phylogeny. The three organisms whose feeding reactions are induced by glutathione are scattered throughout the class Hydrozoa: the gymnoblast *Hydra* and the

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calyptoblast *Campanularia* in different suborders, and *Physalia* in a different order altogether (*cf.* reference 11). The response of these diverse hydrozoans to glutathione has led to the suggestion that the glutathione mechanism was selected early in hydroid evolution (15). The fourth hydrozoan examined, *Cordylophora*, utilizes proline, but in other respects appears fairly closely related to *Hydra*. There are many possible explanations of the difference. *Cordylophora* might represent an offshoot from a main line of organisms responding to glutathione. Alternatively, coelenterates may utilize a diversity of mechanisms to recognize their prey, and the initial finding of three responding to glutathione might be fortuitous or due to glutathione being a particularly favorable mechanism of prey recognition (16), selected for repeatedly in the evolution of the Hydrozoa. The validity of such conjectures must await a comparative study of the feeding reactions of Hydrozoa.

The only requirement of a feeding reaction mechanism is that it provide for selection of suitable food. Although the glutathione mechanism serves to limit *Hydra* to living prey (16), size and availability of potential food and other considerations suggest that the major natural food of both *Hydra* and *Cordylophora* is probably small arthropods and to a lesser extent small worms. Proline, glutathione, or a variety of other small molecules would provide for the recognition of these food sources. From this point of view, it is difficult to see, if glutathione is the primitive mechanism of the Hydrozoa, why the proline feeding mechanism was ever selected for in the evolution of *Cordylophora*.

In a hydroid feeding reaction, the chemical permitting prey recognition comes from other organisms, so the *only* possibility for evolution would be the development of a chemoreceptor system capable of recognizing a suitable molecule already available. Proline and glutathione have been demonstrated to be "suitable molecules." The marked stereospecificity of the inducers for *Hydra* and *Cordylophora* suggests that the receptor might be a protein. Although the low molecular weight inducers for *Hydra* and *Cordylophora* are different a similar underlying mechanism is suggested by the fact that the feeding reactions of both hydroids can be induced by proteases. Perhaps proteases act non-specifically on the chemoreceptor of either hydroid to initiate the same chain of events which lead to a feeding reaction in specific response to glutathione or proline.

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