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H^+-DEPENDENT CONTRACTION OF THE TRITON-EXTRACTED TENTACLE OF THE DINOFLAGELLATE *NOCTILUCA MILIARIS*

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Summary

The intracellular conditions required for contraction in the food-gathering tentacle of a marine dinoflagellate *Noctiluca miliaris* were examined in the isolated tentacle, treated with Triton X-100. The tentacle flexed to its full extent when the pH of the reactivation medium was lowered to 4·0, and extended when it was raised again to its original value. Adenosine triphosphate (ATP) was not needed for the pH-dependent flexion–extension. The flexion was also produced by Ca^{2+}, but in a concentration range more than a thousand times higher than the effective H^+ concentration range for producing the flexion. It is concluded that movement of the tentacle in a live specimen of *Noctiluca* is controlled by membrane potential-regulated cytoplasmic pH.

Introduction

Movement of the food-gathering tentacle of a marine dinoflagellate, *Noctiluca miliaris*, is controlled by changes in membrane potential, termed the tentacle-regulating potentials (TRPs) (Eckert & Sibaoka, 1967). The TRPs are generated across the outer membrane, that is, the protoplasmic membrane which faces the external solution (Oami *et al.* 1988). In normal sea water, quick flexions of the tentacle are always accompanied by hyperpolarizing spikes of the TRPs (Hisada, 1957). The flexion, however, does not occur in the absence of external Ca^{2+}, even though the spike remains unchanged (Eckert & Sibaoka, 1967; Oami *et al.* 1988). When *Noctiluca* cells are stimulated to produce a bioluminescent flash, there is a concomitant flexion of the tentacle (Oami & Naitoh, 1987). The flash is triggered by a stimulus-evoked action potential (the flash-triggering action potential; FTP) (Eckert, 1965), which is generated across a protoplasmic membrane facing the intracellular flotation vacuole (the inner membrane) (Eckert & Sibaoka, 1968). The membrane current responsible for the FTP is carried by H^+. The cytoplasmic pH, therefore, may be temporarily lowered by the H^+-dependent FTP. The lowered pH, in turn, activates a H^+-sensitive flash mechanism (Nawata & Sibaoka, 1979). The pH of the flotation vacuole of *Noctiluca* is low enough

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(pH 3·5) to supply H^+ for generation of the FTP (Nawata & Sibaoka, 1976). In contrast to the flexion associated with the TRPs, the flash-associated flexion occurs even in low-Ca^{2+} sea water (Oami & Naitoh, 1987). These observations also implicate H^+ in the control of tentacle movement.

To investigate the mechanism by which the tentacle movements are controlled by membrane potential, we examined the effects of some chemicals, especially H^+, Ca^{2+} and ATP, on the isolated tentacle, treated with Triton X-100.

Materials and methods

Specimens of *Noctiluca miliaris*, cultured in artificial sea water (ASW) as previously described (Oami *et al.* 1988), were washed with cold ASW (0–1 °C) and kept in it for 10 min. They were then transferred to a cold extraction medium (0–1 °C), consisting of 0·02 % (by volume) Triton X-100, 10 mmol l^{-1} EDTA, 100 mmol l^{-1} KCl and 30 mmol l^{-1} Tris–maleate buffer (adjusted to pH 7·0 with NaOH) (Naitoh & Kaneko, 1972), and kept in this solution for 17 min. During the extraction, the cell bodies distorted, but the tentacles held their shape. They were then washed with a cold reference medium (0–1 °C), which consisted of 50 mmol l^{-1} KCl and 10 mmol l^{-1} Tris–maleate buffer (pH 7·0), and kept in this solution for several minutes before being tested. An extracted cell was mechanically agitated to detach the tentacle from the cell body. The detached tentacle (200–300 μm in length) was held with a suction pipet (20–30 μm in inner diameter; Fig. 1) by lowering hydrostatic pressure within the pipet. This operation was accomplished in an experimental vessel filled with reference medium (about 0·8 ml). A reactivation medium, which contained chemicals to be tested, was then introduced into the vessel. More than 95 % of the medium in the vessel was removed prior to introducing the new medium.

A magnified image of the extracted tentacle was recorded on videotape before and after introducing reactivation medium into the vessel. The degree of flexion of the extracted tentacle in each reactivation medium was measured after playback of the recorded images. All the experiments were performed at room temperature (18–23 °C).

Results

In the first series of experiments, the effects of pH on the extracted tentacles were examined. Each reactivation medium consisted of 50 mmol l^{-1} KCl and 10 mmol l^{-1} buffer. Tris–maleate buffer was employed for the pH range from 8·5 to 5·5, and citrate buffer for the range from 5·5 to 3·0.

As shown in Fig. 1, the extracted tentacle, which was more or less straight at pH 8·5 (A1, B1), flexed as the pH was lowered (A1–6, B1–4). Some tentacles coiled at pH levels below 5·5 (B5, B6). The flexed or coiled tentacle extended again when the pH was raised to its original value (A7, B7). This pH-controlled flexion–extension could be repeated more than 20 times.

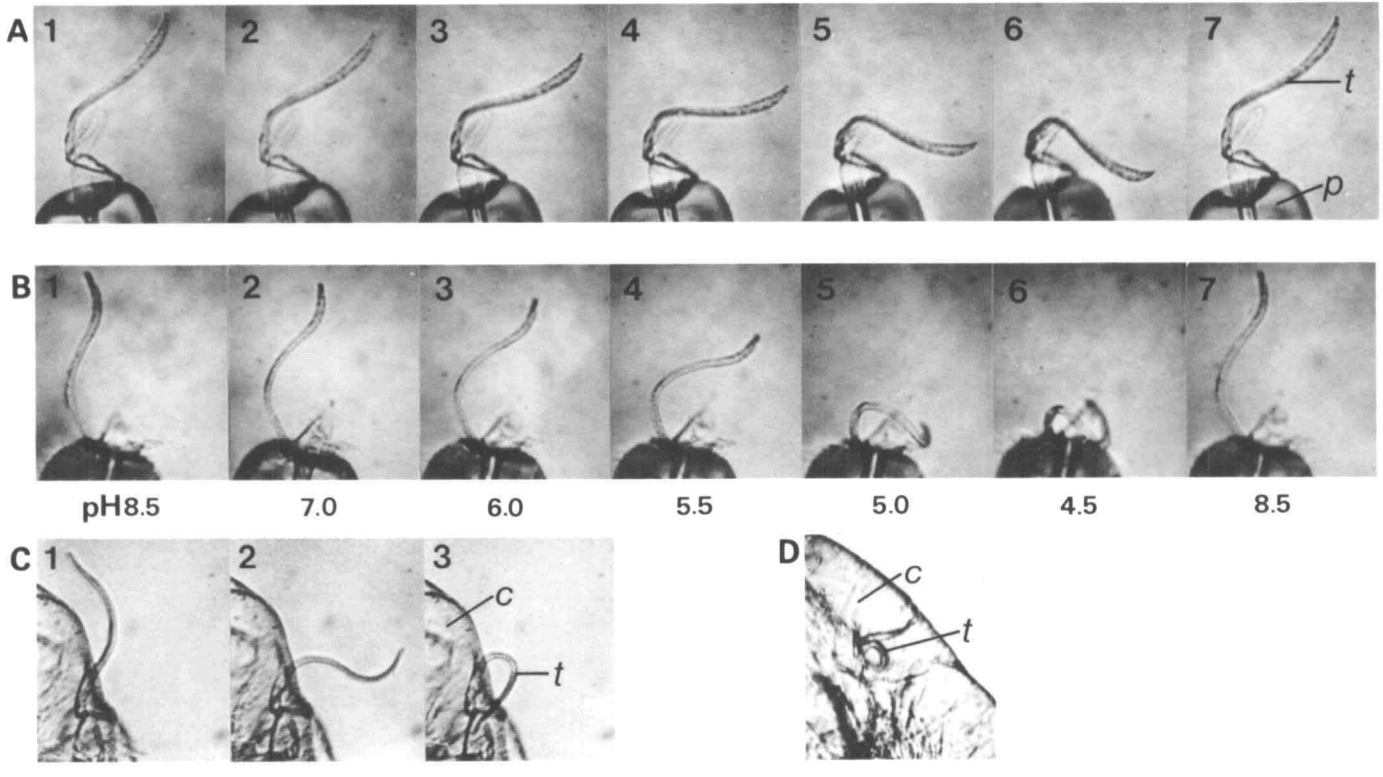

Fig. 1. (A, B). Two typical examples of motile responses to H^+ ions in Triton X-100-extracted food-gathering tentacles of the marine dinoflagellate *Noctiluca miliaris*. Photographs are magnified images of an extracted tentacle videorecorded 10–20 s after its subjection to various solutions with different pH values. An extracted tentacle (*t*) with its basal appendages was held at the tip of a suction pipet (*p*). After the pH of the reactivation medium had been lowered, the tentacle flexed in A1–6, whereas in B it flexed (1–4) and finally coiled (5, 6). The tentacle resumed its original shape when the pH was raised again (compare 1 with 7 in both A and B). (C) Photographs of a spontaneously moving tentacle of a live specimen of *Noctiluca* in artificial sea water (ASW). 1, extended; 2, half flexed; 3, completely flexed. *c*, cell body. (D) A photograph of a coiled tentacle of a live specimen in Ca^{2+}-rich ASW.

The extracted tentacle always flexed in the proximal region (the basal one-third to one-quarter) towards the cytostome. Moreover, the flexion–extension occurred in a single plane more or less perpendicular to the cell surface. These characteristics of the pH-controlled flexion–extension are very similar to those of the membrane potential-controlled movements of a live tentacle (compare Fig. 1A with 1C). Coiling was also seen in a live tentacle, especially when the specimen was in a Ca^{2+}-rich solution (Fig. 1D).

To examine the degree of the flexion quantitatively, we measured the distance between the position of the tip of the tentacle in the reference solution (pH 7·0) and that in each reactivation medium, and expressed it as a value relative to that distance for pH 5·5. About one-third of the extracted tentacles examined coiled when the pH was lower than 5·5. After they had coiled, measurement of the distance was impossible. We therefore used the distance at pH 5·5 as the reference for the flexion.

The extent of flexion increased as pH was lowered (Fig. 2); slightly over the range between pH 8·5 and 6·0, much more between pH 6·0 and 4·0. It became maximal at pH 4·0. The degree of flexion tended to decrease with further lowering of the pH.

In the next series of experiments, the effects of Ca^{2+} on the extracted tentacles were examined. Ca^{2+} concentration in the reactivation medium was changed by addition of $5\,mmol\,l^{-1}$ ethyleneglycol-bis(aminoethylether) tetraacetic acid (EGTA)-Ca^{2+} buffer to the medium for concentrations below $10^{-5}\,mol\,l^{-1}$ (Porzehl *et al.* 1964), and by addition of enough $CaCl_2$ for concentrations above $10^{-5}\,mol\,l^{-1}$. The pH of each reactivation medium was kept constant at 7·0 using $10\,mmol\,l^{-1}$ Tris–maleate buffer. When Ca^{2+} concentration was raised above $1\,mmol\,l^{-1}$, the tentacle flexed (Fig. 3). The degree of flexion increased with increasing Ca^{2+} concentration. The tentacle extended again when the Ca^{2+} concentration was lowered to its original lowest level. However, the extension was

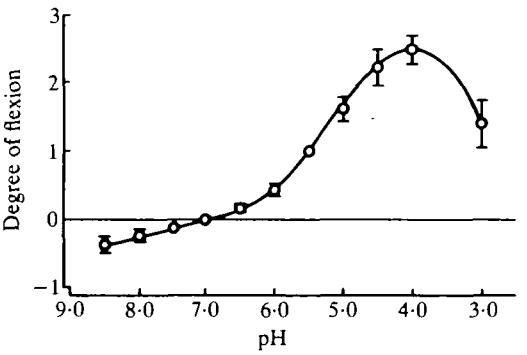

Fig. 2. The degree of flexion in the Triton X-100-extracted tentacles of *Noctiluca miliaris* as a function of pH of the reactivation medium in the absence of Ca^{2+}. The degree of flexion (ordinate) is described in the text. Each point is a mean ± its standard error of 6–9 measurements with different tentacles.

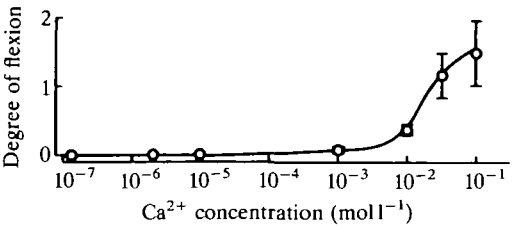

Fig. 3. The degree of flexion in the Triton X-100-extracted tentacles of *Noctiluca miliaris* as a function of Ca^{2+} concentration of the reactivation medium at pH 7·0. For the degree of flexion (ordinate) see text. Each point is a mean ± its standard error of five measurements with different tentacles.

incomplete, and the tentacle remained slightly flexed after the Ca^{2+} concentration had been lowered.

In the last series of experiments, the effects of some physiologically important ions and of ATP on the degree of flexion were examined at two different pH values. The results obtained are summarized in Table 1. At concentrations as high as 100 mmol l^{-1}, K^+ and Na^+ produced a slight flexion at pH 7·0, but they showed no effects at pH 5·5. Addition of more than 5 mmol l^{-1} Mg^{2+} to the reactivation medium always enhanced the degree at both pH values. Addition of ATP up to 10 mmol l^{-1} had no effect at either pH. Addition of ATP (0·1–5 mmol l^{-1}) together with Mg^{2+} (0·1–5 mmol l^{-1}) and Ca^{2+} (0·01–1 mmol l^{-1}) also had no effect.

Table 1. *Effects of some chemicals on Triton X-100-extracted and isolated tentacles of* Noctiluca miliaris *at two different pH values*

$[K^+]$ (mmol l^{-1})	$[Na^+]$ (mmol l^{-1})	$[Mg^{2+}]$ (mmol l^{-1})	Degree of flexion pH 7·0	pH 5·5
0	0	0	−0·34 ± 0·15	0·90 ± 0·12
50	0	0	0	1
100	0	0	0·39 ± 0·17	0·97 ± 0·04
0	50	0	0·00 ± 0·18	0·83 ± 0·13
0	100	0	0·21 ± 0·14	0·94 ± 0·09
50	0	5	0·33 ± 0·11	1·22 ± 0·04
50	0	10	0·59 ± 0·09	1·47 ± 0·12

The concentration of K^+ or Na^+ was changed by addition of KCl or NaCl to a 10 mmol l^{-1} buffer solution.

The concentration of Mg^{2+} was changed in a solution containing 50 mmol l^{-1} KCl and 10 mmol l^{-1} buffer.

50 mmol l^{-1} KCl solution at pH 7·0 is the reference solution.

The degree of flexion in the reference solution is regarded as 0, and that in 50 mmol l^{-1} KCl solution at pH 5·5 is regarded as 1.

The degree of flexion in each test solution is expressed as a value relative to the value at pH 5·5 (see the text).

Each value is the mean ± its standard error of five measurments with five different tentacles.

Substitution of KNO_3 for equimolar KCl in the reference solution did not affect the behavior of the extracted tentacle.

Discussion

Our findings clearly indicate that H^+ is the most effective ion in producing flexion of the extracted tentacle. The largest increase in the degree of flexion occurred at H^+ concentrations between 10^{-6} and 10^{-5} mol l^{-1}. It is interesting to note here that various kinds of cellular movement, such as contraction of skeletal muscle fibers of vertebrates (Hellam & Podolsky, 1969), reversed beating of cilia in ciliate protozoans (Naitoh & Kaneko, 1972) and cessation of cytoplasmic streaming in giant algal cells (Hayama *et al.* 1979), are under the control of bioelectric modulation of cytoplasmic Ca^{2+} concentration in a similar concentration range (10^{-7}–10^{-5} mol l^{-1}). Contraction of the stalk of the vorticellid protozoans is also controlled by intracellular Ca^{2+} in this concentration range (Amos, 1971; Amos *et al.* 1975).

The presence of an H^+-sensitive flexion mechanism in the tentacle explains our previous finding that a bioluminescent flash is always accompanied by a flexion of the tentacle (Oami & Naitoh, 1987): the increase in cytoplasmic H^+ concentration produced by the FTP (Nawata & Sibaoka, 1979) would activate the H^+-sensitive flash and the flexion mechanism simultaneously.

As mentioned previously, external Ca^{2+} is required for the TRP-associated flexion of the tentacle (Eckert & Sibaoka, 1967; Oami *et al.* 1988). The hyperpolarizing spike of the TRPs is assumed to drive external Ca^{2+} into the cytoplasm through calcium channels, which are activated by a slow depolarization preceding the spike (Oami *et al.* 1988). It is not feasible that the Ca^{2+} influx raises cytoplasmic Ca^{2+} concentration to over 100 mmol l^{-1} to activate the flexion mechanism directly (See Fig. 3). However, it is conceivable that a small increase in cytoplasmic Ca^{2+} concentration caused by the Ca^{2+} influx would activate a hypothetical H^+ transport system in the inner membrane, and the activated system would then facilitate the transport of H^+ from the vacuole into the cytoplasm. This increased cytoplasmic H^+ concentration would activate the flexion mechanism. Hypothetical mechanisms for the bioelectrical control of the movements of the tentacle are shown schematically in Fig. 4.

Tentacle flexion of *Noctiluca* resembles contraction of the stalk of *Zoothamnium* in its independence of ATP hydrolysis. According to Amos *et al.* (1975), contraction of the stalk is attributable to conformational changes in a contractile protein of the stalk, spasmin, which is caused by binding of Ca^{2+} to spasmin. Our findings suggest that flexion of the tentacle is produced by similar conformational changes in a hypothetical protein, elicited by H^+, not Ca^{2+}.

Various types of H^+-dependent cellular activities have been reported. For example, movement of bacterial flagella (Manson *et al.* 1977; Matsuura *et al.* 1977), production of ATP in mitochondria (Mitchell, 1961), actin polymerization in fertilized sea-urchin eggs (Johnson & Epel, 1976) and sol–gel conversion in the

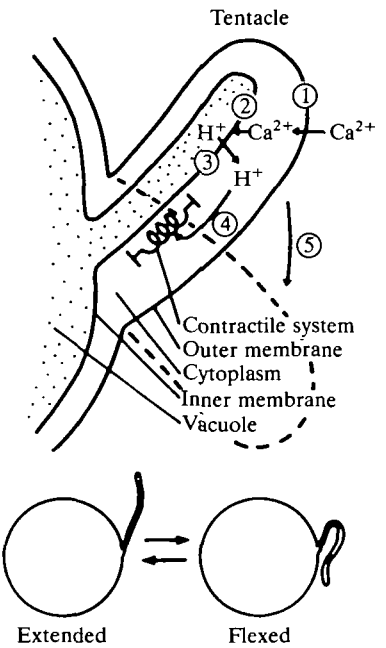

Fig. 4. Schematic representation of the proposed mechanism for bioelectrical control of movement of the food-gathering tentacle in a marine dinoflagellate *Noctiluca miliaris*. External Ca^{2+} is driven into the cytoplasm of the tentacle by a hyperpolarizing spike of the tentacle-regulating potentials (Eckert & Sibaoka, 1967; Oami *et al.* 1988) through Ca^{2+} channels activated by a slow depolarization preceding the spike (1). The resultant increase in cytoplasmic Ca^{2+} concentration activates a H^+ transport system in the inner membrane facing the flotation vacuole (2). The transport system conveys H^+ from vacuole to the cytoplasm (3). The resultant increase in cytoplasmic H^+ concentration activates the H^+-sensitive contractile system in the tentacle (4). The activation of the system results in flexion of the tentacle (5). The Ca^{2+} influx ceases when the hyperpolarizing spike terminates. Cytoplasmic Ca^{2+} concentration is decreased by pumping out or sequestering of Ca^{2+}. Thus the H^+ transport system is deactivated. Cytoplasmic H^+ concentration is decreased by pumping out of H^+ or by some other means (not yet identified). The contractile system is then deactivated. The tentacle extends owing to its elasticity. The lower insets show the positions of extended and flexed tentacles relative to the cell body.

protoplasm of amoeboid cells (Hellewell & Taylor, 1979; see also Nuccitelli & Heiple, 1982).

This is the first report of this exceptional motile system among eucaryotic cells. The system is independent of both ATP and Ca^{2+}; instead, it is regulated and energized by H^+.

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