Effect of a Disulfide Reducing Agent on Coelenterate Nematocyst Capsules

Recent work has revealed the presence of a single hydroxyproline-rich, collagen-like protein linked by disulfide bonds in the microbasic mastigophore nematocyst capsule of a sea anemone[1]. Subsequently, other workers have confirmed this in a different species of sea anemone[2]. Since the presence in protein of the imino acid hydroxyproline is diagnostic of collagens, and since collagens were thought to be devoid of disulfide bonds, the above discovery is of considerable interest. Because of this and because of the great complexity and diversity of these intracellular organelles, the present study was undertaken to determine how widespread was this phenomenon among the various classes of coelenterates.

The coelenterates used were collected in Kaneohe Bay, Oahu, Hawaii. 5 species of corals, 4 sea anemones, 1 zoanthid, 1 scyphozoan and 1 hydroid were examined. The various nematocyst types were identified by a key prepared from a variety of sources, but mainly based on WERNER[3].

Excised tentacle smear preparations were prepared by conventional means. To these preparations were added 3 drops of $10^{-2}M$ dithioerythritol (DTE) prepared in *Tris* (hydroxy) methylaminomethane buffer at pH 9.1. Based on previous experiments of the authors, this combination of concentration and pH proved to be optimal in attacking disulfide linkages. The time required for dissolution of the various nematocyst capsules at 28 °C was then observed and recorded.

It was discovered that the nematocysts had to be discharged and free of surrounding mucus contamination before the DTE became effective. Most nematocysts that were either undischarged or partially discharged so that the shaft was blocking the opercular opening did not respond to the reducing agent, even after several hours' exposure. The first detectable effect of the DTE on the discharged nematocyst capsule was a slight buckling of the normally smooth capsule wall. The capsule wall soon appeared to become less dense microscopically. Solubilization appeared to start from the inner surface of the capsule wall, and to proceed outward. Before the capsule dissolved entirely, a thin membrane-like coat remained visible, but this eventually disappeared also. The everted shaft, thread and barbs did not dissolve during the period of observation, indicating a possible difference in the chemical composition of these structures.

It will be noted from the Table that, of the nematocysts examined from all 3 classes of coelenterates, most dissolved within 1–3 min, if they were to be affected by the DTE at all. For example, the stenoteles of the hydroid, *Pennaria tiarella*, the nematocysts of the scyphozoan, the zoanthid and all the sea anemones dissolved in 1–3 min. A solubilization time greater than 3 min for these groups generally indicated that the nematocysts were not going to be affected by the DTE under the experimental conditions.

The nematocysts of the corals seemed the most resistant to dissolution by DTE. Of the 4 corals tested,

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Effect of dithioerythritol (DTE) on nematocyst capsules and spirocysts

Species	Nematocyst type	Time for capsule to dissolve (min)	Comments
Class Hydrozoa			
(1) *Pennaria tiarella*	Large stenotele	2	
	Small stenotele	2	
Class Scyphozoa			
(1) *Cassiopeia* sp.	Holotrichous isorhiza	–	Undissolved after 18 min
	Microbasic homotrichous eurytele	1	
Class Anthozoa			
(0) Actiniaria (anemones)			
(1) *Boloceroides lilae*	Spirocysts	–	Undissolved after 30 min
	Microbasic amastigophore (discharged and undischarged)	1–2	
(2) *Macranthea cookei*	Microbasic-p-mastigophore	3	
(3) *Anthopleura* sp.	Microbasic mastigophore	2	
(4) *Aiptasia pulchella*	Microbasic amastigophore (acontia and tentacles)	1–2	
	Spirocysts	–	Undissolved after 30 min
(0) Zoanthidia			
(1) *Zoanthus sandwichensis*	Holotrichous isorhiza	2	
(0) Madreporaria (stony corals)			
(1) *Cyphastrea ocellina*	Holotrichous isorhiza	9	
	Microbasic mastigophore	10	
(2) *Porites compressa*	Microbasic mastigophore	3	
(3) *Tubastrea manni*	Microbasic-b-mastigophore	–	Undissolved after 10 min
(4) *Pocillopora meandrina*	Microbasic mastigophore	–	Partially dissolved after 15 min (?)
(5) *Fungia scutaria*	Atrichous isorhiza	–	Undissolved after 15 min
	Microbasic mastigophore	–	Undissolved after 30 min

only the microbasic mastigophore of *Porites compressa* dissolved within 3 min, whereas those of *Cyphastrea* required 9–10 min to dissolve. The other coral nematocysts examined remained undissolved after 10–30 min exposure to DTE. In all cases, the spirocysts remained unaffected by the DTE, thus providing evidence for the chemical differences between spirocysts and nematocysts proper[4]. Electron micrographs by one of us (R.N.M.) have revealed the distinct ultrastructural differences between anemone spirocysts and nematocysts.

The unfired nematocysts of the various coelenterates tested, with one notable exception, remained unaffected by the DTE in the present study. The single exception was the microbasic amastigophore of the swimming sea anemone *Boloceroides*; both the discharged and undischarged microbasic amastigophores dissolved in 1–2 min.

The fact that only 1 nematocyst type, the microbasic amastigophore of *Boloceroides*, dissolved in an undischarged state deserves further study in regard to the osmotic theory of nematocyst discharge. All the other undischarged and partially discharged nematocysts appeared to be totally unaffected by the DTE.

That some of the nematocysts examined required 9–10 min to dissolve, or did not dissolve at all during the period under examination, remains enigmatic. This was especially true for the coral nematocysts. One obvious possibility is that some coral nematocysts may differ chemically. However, it is also possible that the relatively large amounts of mucus liberated by the corals (compared to the other coelenterates examined) protected the nematocysts from dissolution by DTE in spite of steps taken to minimize this problem[5].

Zusammenfassung. Während sich die Mehrzahl der entladenen Nesselkapseln mehrerer hawaiischer Coelenteraten in Dithioerythritol (DTE) auflöst, sind die Spirozysten und die meisten unentladenen Nesselkapseln in diesem die Disulfidbindungen reduzierenden Reagens unlöslich.

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The Role of the Bacterial Cell Wall in the Development of the Lethal Effect of Colicin E1

The basic biosynthetical processes of sensitive bacterial cells – and consequently their growth and division – are blocked by colicins immediately after the contact[1,2]. In spite of that, this blockade is temporarily reversible; in a part of the cells, it is possible to abolish the inhibitive action of colicins for a limited time interval by trypsin[3] or a specific anti-colicin serum[4]. The first, reversible phase of the effect of colicins can thus be regarded as the bacteriostatic one; this gradually passes on to the irreversible phase representing the bactericidal effect.

Some previous results, with colicins E2 and G, suggested that their receptors could not be situated in the cell wall of a sensitive bacterium (as had been supposed), but in its cytoplasmic membrane[5]. In the present paper we thus tried to find out the role of the bacterial cell wall in the development of the effect of colicin E1 (produced by the strain *Shigella dispar* P 14); we followed the chronological dependence of the onset of the bactericidal effect on the degree of structural damage of the cell walls of sensitive bacteria.

Firstly, we compared the outset of the bactericidal effect of colicin E1 on intact bacterial cells and spheroplasts. Sensitive rods of the strain *Escherichia coli* B1 from the stationary phase of growth or glycine spheroplasts[6] of the same strain, were incubated in broth at 37 °C with an excess of colicin E1. After 1, $2^1/_2$ or 5 min, trypsin (0.25 mg/ml) was added, reactivating those elements, the inhibition of which had not yet reached the irreversible phase. (Neither the cells nor the spheroplasts themselves were influenced by trypsin.) A drop of each suspension was transferred to the surface of a little agar block mounted on a slide, covered with a coverslip and framed with paraffin; this preparation was then photographed in a phase-contrast microscope in 30 min intervals for 5–6 h. From the photographs the percentages of dividing rods or of dividing and regenerating spheroplasts, that is of elements exhibiting their viability, were calculated.

In control preparations without colicin, nearly 100% of rods and over 63% of spheroplasts divide, while only some 10^{-4}% of rods and still less spheroplasts survive the action of colicin E1 (without succeeding trypsin addition). The effect of trypsin reactivation is clearly visible from Table I: after all 3 exposures to colicin used, it is possible to reactivate the rods in a remarkably higher proportion than is the case with spheroplasts. So the bactericidal step of colicin E1 effect starts more readily in an absolute majority of spheroplasts, lacking the rigid murein cell wall layer, than in intact rods of the same strain; this layer evidently delays the onset of its irreversible, bactericidal effect.

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