CONTROL OF THE Ca^{2+} DEPENDENT LUMINESCENCE OF LUMISOMES BY MONOVALENT CATIONS

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SYMMARY. - A particulate system which emits light upon addition of calcium ions was obtained from the bioluminescent Anthozoan Cœlenterate Veretillum Cynomurum. The preparation has properties similar to Renilla lumisomes : a flash of light is produced with decay kinetics which are unaffected by dilution; light emission is triggered by hypotonic Ca^{2+} solutions but solutions of this ion in 0.6 M NaCl failed to induce bioluminescence. Various alkali cations (isotonic solutions) inhibited the Ca^{2+} induced bioluminescence with increasing efficiency in the order $K^+ < Rb^+ < Cs^+ < Na^+$ and the emission obtained in presence of 0.6 M KCl has the same intensity as in hypotonic solution. Among other chemicals tested, sucrose, tetraethylammonium and Tris were similar to Na^+ ions with respect to inhibition whereas choline chloride did not inhibit.

The discovery of lumisomes (1, 2) has been of considerable interest in the study of the bioluminescence of Anthozoan Cœlenterates. Lumisomes are vesicles isolated in vitro which, when exposed to hypotonic Ca^{2+} solutions, give rise to an emission of light with the same characteristics as the in vivo emission. As in the intact animal, the light emitted is green, in contrast with the simple luciferin-luciferase reaction which produces blue light (1, 2, 3, 4, 5). Lumisomes will also produce flashes whereas in the two-component reaction only continuous emission is observed (1, 2). Lumisomes are not simply packages of luciferase and luciferin but represent a more complex system in which structural factors also play a role. For example calcium triggering is explained by the existence of a protein which sequesters the luciferin, and releases it in presence of Ca^{2+} and luciferase (6). However this system, luciferase, luciferin and binding protein, has concentration dependent kinetics while the life-time of the lumisome flash is independent of concentration.

Lumisomes thus appear to be the cellular site of bioluminescence. Nevertheless, to trigger this bioluminescence in vitro, a lysis of the vesicles is necessary prior to addition of Ca^{2+} ions, which apparently do not cross the membrane. Thus a paradoxical situation has been described in which non physiological conditions are required to stimulate a system which has been defined as the physiological one.

In the present communication, the isolation of a crude particulate fraction from Veretillum cynomorium which has similar properties to those of Renilla reniformis lumisomes, but which can be stimulated in the absence of lysis is described.

MATERIALS AND METHODS

Preparation of crude lumisomes

The animals were captured by divers from the Arago Laboratory (Banyuls/mer - France). They were kept at 15° C for short periods of time, in artificial sea water.

The purification was adapted from Cormier et al (1). Four animals were kept for 30 to 60 min at the temperature of the aquarium in 600 ml of 0.01 M Tris-HCl buffer (pH 7.5) containing 0.55 M NaCl, 0.05 M $MgCl_2$ and 0.02 M Ethyleneglycol-2-(2-aminoethyl)-tetracetic acid (EGTA). They were then homogenized with a Waring blender set at full speed for 30 sec, in 100 ml of the same buffer cooled to 4° C. The homogenate was filtered through Mira-cloth and the residue again homogenized in 50 ml of buffer and filtered. The combined filtrates were centrifuged at 500 g for 10 min, the supernatant saved and the pellet extracted repetitively with 50 ml fractions of the same buffer in a Potter homogenizer until solubilization of most of the activity. The pooled active 500 g supernatants were centrifuged at 20,000 g for 20 min. The pellet was resuspended in 50 ml of 0.01 M Tris-HCl buffer (pH 7.5), 0.02 M EGTA and 0.6 M NaCl (buffer A) by manuel operation with a Potter homogenizer. This fraction was washed twice with the same buffer and finally resuspended in 20 ml of buffer.

Assay of lumisomes

Lumisomes were assayed by measuring the maximal intensity

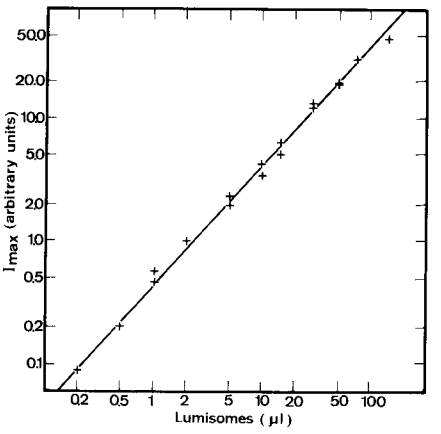

Fig. 1 Intensity of the flash (Imax) as a function of lumisome concentration. Crude lumisomes were suspended in buffer B and diluted in the same buffer. They were assayed as described in the experimental section.

(Imax) of the flash of light obtained by addition of hypotonic Ca^{2+}. The standard assay mixture contained 0.35 ml of 0.01 M Tris-HCl (pH 7.5), 0.25 mM EGTA, 0.6 M NaCl (buffer B) and 0.05 ml of crude lumisomes in a scintillation vial, to which 1.6 ml of 0.01 M Tris-HCl (pH 7.5) containing 3 mM $CaCl_2$ was added with a syringe. Light emission was measured with a photomultiplier and the results expressed in arbitrary units.

RESULTS

As earlier described for lumisomes from Renilla and other species (1), the particulate fraction obtained from Veretillum emitted a flash of light when hypotonic Ca^{2+} solutions were added. The kinetics of emission ($k \sim 2.3$ sec^{-1}) were not altered by dilution and the flash height (Imax) was proportional to lumisome concentration over a 1,000 fold dilution range (fig. 1).

Intact Renilla lumisomes were only slightly stimulated by addition of Ca^{2+} solutions containing 0.6 M NaCl and thus isotonic to sea water (1). In the same way, the flash of light obtained by triggering the Veretillum particles by Ca^{2+} ions was greatly impaired in presence of high concentrations of NaCl. The effect of NaCl concentration is shown in fig. 2. Since NaCl does

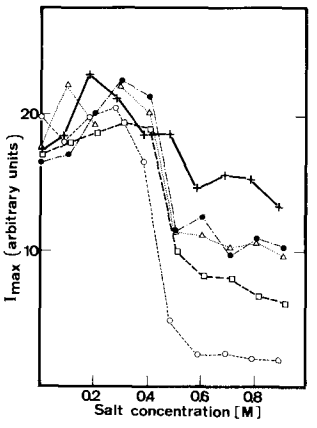

Fig. 2 Effect of alkali chlorides on lumisome bioluminescence. Crude lumisomes (50 µl in buffer A) were added to 0.35 ml of 0.01 M Tris-HCl (pH 7.5), 0.25 mM EGTA and 0.6 M NaCl (curve 2, 3, 4, 5) or KCl (curve 1). $CaCl_2$ (3 mM), Tris-HCl pH 7.5 (0.01 M) and various salts were injected :
curve 1 : (+ —— +) KCl ; curve 2 : (●—·—●) NH_4Cl ;
curve 3 : (Δ...Δ) Rb Cl ; curve 4 : (□—— —□) CsCl ;
curve 5 : (O---O) NaCl. The concentrations given in the figure are the final concentrations of the salt after injection, neglecting the NaCl or the KCl present in the cuvette before injection.

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not inhibit the soluble system (6), this result suggests that Ca^{2+} ions do not enter intact particles rapidly. However, different results were obtained when KCl was substituted for NaCl. Isotonic and hypertonic solutions of Ca^{2+} in KCl were nearly as active as hypotonic ones (fig. 2). In presence of 1 M KCl, the maximal intensity of the flash was 75 % of that observed in hypotonic solutions. The pseudo first order rate constant for decay from maximum intensity was slightly decreased ($k \sim 1.5\ sec^{-1}$), resulting in comparable total light emission. When Ca^{2+} ions were injected with isotonic mixtures of NaCl and KCl ($Na^+ + K^+ = 0.6$ M), the intensity of the light emission (Imax) increased with the ratio K^+/Na^+, thus indicating that the nature of the cation and not the ionic strength was involved (Table I).

The intensity of the flash as a function of calcium concentration is shown in fig. 3. Potassium ions did not affect the ability of calcium ions to trigger lumisome bioluminescence. As with hypotonic solutions a concentration of 0.15 mM Ca^{2+} induced half the maximal response. On the contrary, sodium ions altered Ca^{2+} efficiency, but it may be noted that this effect is partly reversed at high concentrations (10 mM) of the latter ion. In fact, the

TABLE I

Sodium concentration (M)	Potassium concentration (M)	Imax
0.6	0	5.2
0.5	0.1	6.8
0.4	0.2	8.25
0.3	0.3	10.2
0.2	0.4	14.1
0.12	0.48	15.2

Crude lumisomes (50 µl in buffer B) were added to 0.35 ml of buffer B and assayed by injection of 1.6 ml of solutions containing $CaCl_2$ (3 mM), Tris-HCl pH 7.5 (0.01 M) and NaCl and/or KCl. The concentrations given in the table are the final concentrations after injection.

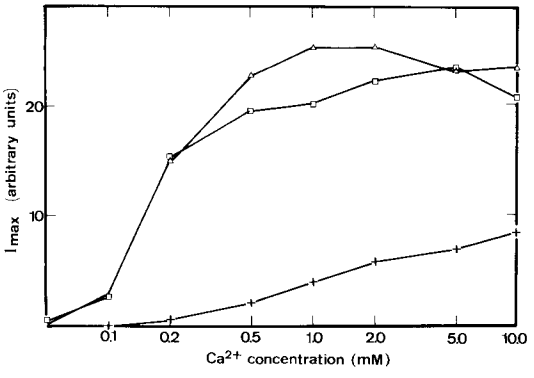

Fig. 3 Effect of Ca^{2+} concentration. Crude lumisomes (50 µl in buffer B) were added to 0.35 ml of a type B buffer with either 0.6 M NaCl (curve 1, 2) or 0.6 KCl (curve 3). $CaCl_2$ at the final concentration given in the figure was injected in 1.6 ml of 0.01 M Tris-HCl buffer (pH 7.5) (curve 1 : □), or in Tris buffer containing 0.6 M NaCl (curve 2 : +) or 0.6 M KCl (curve 3 : Δ).

kinetics of the light emission obtained under those conditions were complex since, in addition to the flash, a slow reaction which increased with Ca^{2+} concentration, was observed. The present results show that an element of the lumisomes, possibly the membrane, discriminates between Na^+ and K^+ ions. The presence of the former ions restrict the entry of Ca^{2+}, which penetrates

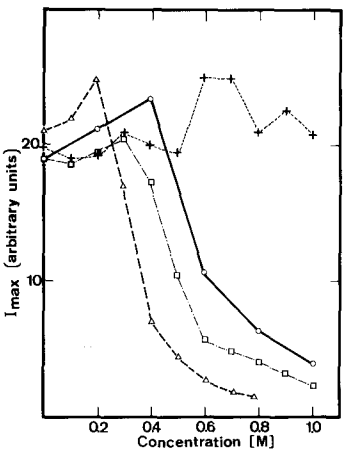

Fig. 4 Effect of various chemicals on lumisome bioluminescence. Crude lumisomes (50 µl in buffer A) were assayed as described in the experimental section. The compound tested was added with the Ca^{2+} ions and the concentration given in the figure is its final concentration after injection. Choline chloride (+ .... +) ; □—□ (Tris-HCl) ; Δ—Δ (Tetraethylammonium) ; O—O (sucrose).

the particles mostly by a concentration dependent leakage mechanism, whereas the rate of transport of the same Ca^{2+} ions is too fast to be followed by the bioluminescent flash when they are added in presence of potassium ions.

The effect of other monovalent cations has been examined. Among the alkali metals, Ca^{2+} transport was depressed with increasing efficiency in order : $K^+ < Rb^+ < Cs^+ < Na^+$ (fig. 2). Ammonium ion was equivalent to the rubidium cation. To account for selective processes, not only ionic radii should be considered but also the energies of solvation of the metal ion and of the ligand and the energy required to change the conformation of the ligand to a complexed configuration (7). Several systems having the same selectivity have been described (8, 9).

Since this system discriminates among the various alkali metals which have similar properties, it was of interest to test solutions of unrelated chemicals, charged or uncharged, isotonic to sea water (fig. 4). Surprisingly enough, choline chloride (up to 1 M solutions) did not inhibit the light emission induced by Ca^{2+} ions, though choline ion is not related to and much bigger than potassium ion. The flash height (Imax) decreased only at higher concentrations :

for 1.5 M, a 75 % inhibition was observed. In contrast, Tris-(hydroxymethy)-amino-methane, tetraethylammonium ions and sucrose behave like Na^+ ions and did inhibit the Ca^{2+} triggered light emission of lumisomes. To obtain 50 % inhibition, 0.5 M Tris, 0.35 M Tetraethylammonium and 0.6 M sucrose were required under the experimental conditions used. Under the same conditions, about 0.45 M NaCl produced the same inhibition. The fact that sucrose is an uncharged molecule and that Tris is only partially dissociated at pH 7.5 may explain the somewhat higher values obtained with those two components.

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