Proc. Natl. Acad. Sci. USA
Vol. 78, No. 11, pp. 6719–6723, November 1981
Biochemistry

K^+/Na^+-triggered bioluminescence in the oceanic squid *Symplectoteuthis oualaniensis*

(luminescent granules/membrane-bound bioluminescence/triggering by monovalent cations)

FREDERICK I. TSUJI*†‡ AND GARY B. LEISMAN*

*Marine Biology Research Division A002, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093; †Department of Biological Sciences, University of Southern California, Los Angeles, California 90007; and ‡Veterans Administration Medical Center, Brentwood, Los Angeles, California 90073

Communicated by Martin D. Kamen, July 24, 1981

ABSTRACT **A distinctive type of luminescent system present in the large dorsal luminous organ of the oceanic squid *Symplectoteuthis oualaniensis* is described. The organ produces an intense blue flash of light followed by a rapid decay in light intensity. Luminescence originates from numerous oval granules present in the luminous organ. The essential light-emitting components are membrane bound. Intact granules or washed homogenates of the granules are triggered to emit light by monovalent cations such as, in decreasing order of effectiveness, potassium, rubidium, sodium, cesium, ammonium, and lithium. Calcium, magnesium, and strontium ions do not trigger light emission. Analysis of the kinetics of the decay of light intensity suggests that two light-emitting components are involved, one decaying faster than the other. The light-emitting reaction has an absolute requirement for molecular oxygen. The optimum KCl or NaCl concentration is ≈0.6 M and the optimum pH is ≈7.8. A free sulfhydryl group is essential for activity.**

Molecular mechanisms of bioluminescence have been described in a wide range of marine organisms—e.g., bacteria, dinoflagellates, crustaceans, coelenterates, and fishes, but understanding the mechanism in cephalopods has remained an unsolved problem. The principal reason has been the inability to obtain suitable active extracts for biochemical studies. Goto and coworkers (1–4) have investigated this problem by determining the structures of compounds isolated from the luminous organs and liver of the Japanese firefly squid *Watasenia scintillans* and relating the structures to the luciferins and oxyluciferins of other marine organisms. Because the bioluminescence reactions of many such organisms involve common chemical mechanisms and oxidative products, it is possible to assign a structure to the putative luciferin in the reaction if the structure of the oxidized product (i.e., oxyluciferin) is known. By using this approach, these workers have proposed a sulfated form of an imidazopyrazine compound, structurally similar to *Renilla* luciferin and the light-emitting chromophore of aequorin, as the "luciferin" or substrate in the *Watasenia* reaction.

Symplectoteuthis oualaniensis is an oceanic squid common to the western Pacific and Indian Oceans (5, 6). The squid possesses a large oval organ (major axis, ≈2 cm) on the anterodorsal surface of the mantle that emits a bright flash of blue light when stimulated. The organ is yellow and consists of numerous ovate [0.4–1.1 mm (major axis) × 0.3–0.5 mm (minor axis)] photogenic granules. This paper describes some properties of this membrane-bound system, which emits light when triggered by potassium and other monovalent cations. A brief report has been published elsewhere (7).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Squid Specimens. Specimens of *S. oualaniensis*, mantle length 12–22 cm, were caught by hand jigging under night light from a ship off the coast of Oahu, Hawaii. The animals were kept in tanks supplied with running sea water. The dorsal luminous organ was excised with a pair of scissors and used immediately or stored at −20°C for later use or for air shipment in dry ice to La Jolla, California.

Chemicals. Chemicals were purchased from the following sources and were of the best grade available: Sigma—Tris, KCl, NaCl, acetylcholine chloride, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, equine Tris ATP, cyclic Tris AMP, dithioerythritol, 2-mercaptoethanol, *N*-ethylmaleimide, iodoacetamide, *p*-chloromercuribenzoic acid, 5,5'-dithiobis(2-nitrobenzoic acid), DL-cysteine·HCl, L-cysteine·HCl, reduced glutathione, NADH, NADPH, horseradish peroxidase, bovine catalase, gramicidin, valinomycin, dimethyl sulfoxide, and bacterial protease (type VII); Matheson, Coleman and Bell—LiCl, RbCl, and CsCl; Mallinckrodt—NH_4Cl, $SrCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, KNO_3, KOAc, EDTA, and KSCN; Calbiochem—dithiothreitol, and 2-(*N*-morpholino)ethanesulfonic acid; Baker—KI; Aldrich—ethanolamine·HCl; Schwarz/Mann—guanidine·HCl and urea; Worthington—α-chymotrypsin and trypsin; Matheson—UHP argon (99.999%) and L. and V. Industrial Supply—oxygen (99.5%). The following were received as gifts: nigericin from Robert J. Hosley, Eli Lilly; superoxide dismutase (dialyzed and lyophilized from Tris·HCl, pH 8.4) from H. M. Steinman, Albert Einstein College of Medicine; and tetrodotoxin from T. Goto, Nagoya University. Glass-distilled water was used throughout and chemicals were dissolved in buffer immediately before use. Oxygen in the argon was removed by passing it over hot copper filings in a heated furnace tube (Sargent–Welch).

Preparation of Test Suspension and Incubation Mixture. Test suspensions and incubation mixtures were prepared in 0.05 M Tris·HCl, pH 7.20 or 7.60, with or without 1 mM dithioerythritol. The excised luminous organ was rinsed with cold buffer, and then the photogenic granules were teased from the mantle tissue with a scalpel. The granules were rinsed with cold buffer, centrifuged at 8730 × *g* for 15 sec in a Beckman Microfuge, and homogenized in an all-glass homogenizer in buffer at 0°C. The homogenate was centrifuged at 8730 × *g* for 15 sec and the supernatant was decanted. The pellet was suspended in cold buffer and centrifuged as before. The final pellet was suspended in 4.5 ml of cold buffer or 4.5 ml of a buffer mixture containing the compound to be tested; these were designated test suspen-

Abbreviations: *I*, light intensity; I_0, initial maximal *I*; L.U., light units.

sion and incubation mixture, respectively. In tests involving more than one chemical, the final pellet was suspended in 4.5 ml of cold buffer, this solution was divided into 0.5-ml aliquots and centrifuged, and each pellet was then suspended in 0.5 ml of buffer containing the test compound. One hundred microliters of test suspension or incubation mixture was immediately transferred to a reaction vial (chloride meter, Kimble), the vial was placed in the photometer, and 200 μl of cation solution was injected with a hypodermic syringe into the vial to trigger the light reaction. The initial maximal light intensity triggered by 1.0 M KCl, determined periodically, served as a control reading. All test suspensions and incubation mixtures were kept in an ice bath.

Light Intensity Measurements. Light intensities were measured by using a Mitchell–Hastings photomultiplier photometer (8), calibrated with a standard light source (9), and recorded with a Hewlett–Packard 7101 BM strip chart recorder equipped with an event marker pen. Results are presented as relationships to light intensity (I) and initial maximal light intensity (I_0), measured in light units (L.U., 6.42×10^8 quanta/sec).

Bioluminescence Spectral Measurement. The emission spectrum was measured with a Perkin–Elmer MPF-44A fluorescence spectrophotometer using a Hamamatsu R777 multialkali photomultiplier with a relatively flat sensitivity response at 400–600 nm.

RESULTS

Examination of the Luminous Organ and Homogenate. The organ exhibited a yellow fluorescence in near-UV light. Microscopic examination of the homogenate and test suspension showed similar enclosed fibrilla- or vesicle-like particles distributed uniformly throughout the field. The particles were 2 to 3 μm in diameter × 10–100 μm long, and clusters of them rotated plane-polarized light.

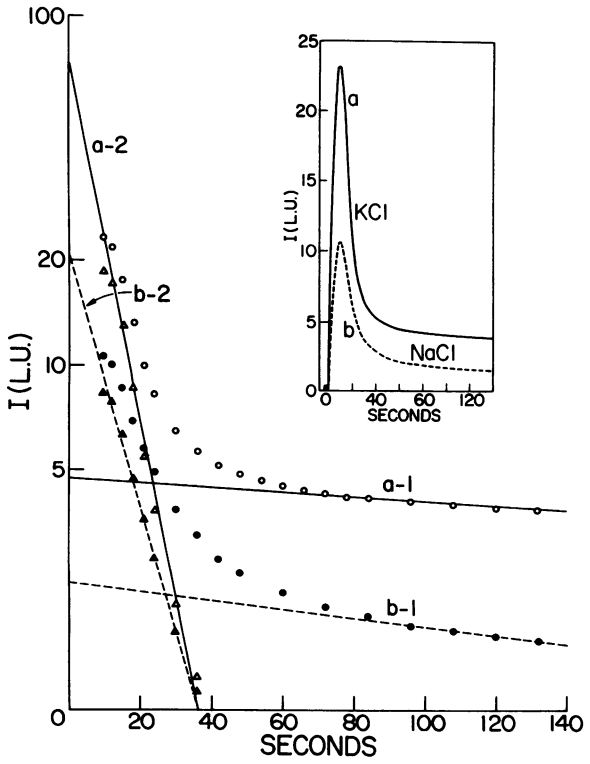

FIG. 1. Decay of light intensity after injection of 100 μl of 1.0 M KCl or NaCl (in 0.05 M Tris·HCl, pH 7.20) into vial containing a single photogenic granule. ○ and △, KCl; ● and ▲, NaCl. k: Curve a-1, 1.6 $msec^{-1}$; a-2, 114 $msec^{-1}$; b-1, 2.4 $msec^{-1}$; b-2, 83 $msec^{-1}$.

Table 1. Effect of cations on initial maximal light intensity of test suspension

Suspension	Cation	Final concentration, M	Relative I_0
1	K^+	0.67	100
	Rb^+	0.67	81
	Na^+	0.67	69
	Cs^+	0.67	60
	Li^+	0.67	13
	NH_4^+	0.67	26
2	K^+	0.67	100
	Ca^{2+}	0.67	0
	Mg^{2+}	0.67	1
	Sr^{2+}	0.67	2

Two hundred microliters of 1.0 M salt (chloride) solution was injected into 100 μl of test suspension. Salt solutions and test suspension were prepared in 0.05 M Tris·HCl, pH 7.20/1 mM dithioerythritol.

Triggering of Light Emission by Monovalent Cations. Single photogenic granules, test suspensions, and supernatants were examined on board ship and in the La Jolla laboratory with identical results. Fig. 1 *Inset* shows tracings of the decay of light intensity after injection of 1.0 M KCl or NaCl into reaction vials containing single photogenic granules; also shown are first-order plots of the decay of light intensity with time. A marked deviation from linearity is observed. However, if slow exponential decay (curves a-1 and b-1) is assumed and these data are extrapolated to zero time and subtracted from the total decay, then fast exponential decay curves (a-2 and b-2) can be plotted. A first-order decay constant was calculated for each curve. Test suspensions triggered with 1.0 M KCl or NaCl also gave similar biphasic decay curves, but with larger decay constants, whereas all supernatants tested were inactive.

Table 1 shows the effectiveness of various cations in triggering light emission from test suspensions. Monovalent ions, especially potassium, were effective and divalent ions were ineffective in triggering light emission. Ethanolamine gave a result similar to that of NH_4Cl. Subsequent injection of KCl into mixtures previously injected with KCl, NH_4Cl, $CaCl_2$, $MgCl_2$, or $SrCl_2$ gave little or no light; similar injection into suspensions previously injected with KCl and then washed with fresh buffer again gave little or no light. Guanidine hydrochloride and urea did not trigger light emission.

Emission Spectrum. Fig. 2 shows the bioluminescence emission spectrum for the KCl-triggered reaction. The emission extends from ≈400 nm to 600 nm, with a peak at 456 nm and a half-height band width of 60 nm.

Effect of Anions. When the potassium salts of various anions (200 μl of 1.0 M salt in 0.05 M Tris·HCl, pH 7.60) were injected into test suspensions (100 μl in 0.05 M Tris·HCl, pH 7.60/1 mM dithioerythritol), the following relative I_0 values were obtained: KCl, 100; KOAc, 51; KNO_3, 27; KI, 0; KSCN, 0.

Effect of KCl and NaCl Concentrations. The optimal concentration for triggering light emission was 0.6 M (final) for both KCl and NaCl (Fig. 3). At <0.1 M, very little light was triggered, whereas at >0.6 M, there was a progressive decrease in light intensity.

Effect of pH. The pH–activity curves for the KCl- and NaCl-triggered bioluminescence are given in Fig. 4. The relationship between final pH and I_0 was a bell-shaped curve with a maximum at ≈7.8.

Dependence on Molecular Oxygen. All test suspensions emitted a low level of light (Fig. 5*A*). However, immediately

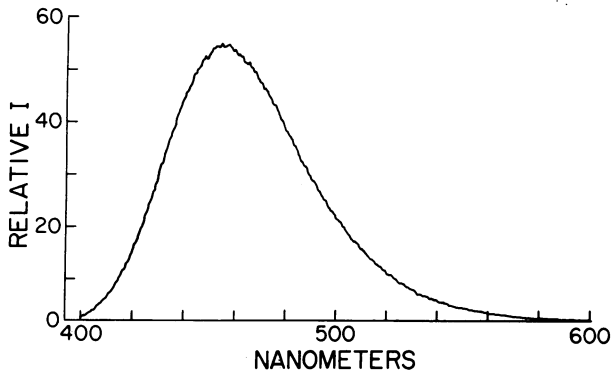

FIG. 2. Emission spectrum of KCl-triggered bioluminescence. One milliliter of 1.0 M KCl was mixed with 1 ml of test suspension, both in 0.05 M Tris·HCl, pH 7.60/1 mM dithioerythritol. Recording was started 6 min after mixing using a slit width of 15 nm and wavelength scan speed of 120 nm/min. The curve is not corrected for photomultiplier sensitivity or grating efficiency.

after we began to flush a suspension with argon, the light intensity decreased, reaching zero in ≈4 min. The subsequent addition of 1.0 M KCl to the suspension did not trigger light emission. However, when oxygen was admitted to the suspension, there was a bright flash of light, followed by a long-lasting decay (Fig. 5*B*). If the flow of argon was resumed while the light intensity was decaying (not shown), the light intensity was reduced to about one-third the intensity of the original low level in ≈10 min, when leveling off occurred.

Effect of Reducing Agents, EDTA, and Sulfhydryl-Modifying Agents. Addition of a reducing agent increased I_0 (Table 2) and extended the useful life of the suspension from an average duration of 3 hr to ≈6 hr. Similar results were obtained with EDTA and ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid. The most effective agent was dithioerythritol or dithiothreitol, but dithioerythritol in increasing concentrations inhibited activity. Thus, in some experiments, 1 mM dithioerythritol was added to the Tris·HCl buffer.

Of four sulfhydryl-modifying agents tested, only *p*-chloromercuribenzoic acid inhibited K^+-stimulable activity completely (Table 2). The inhibition was readily reversed by washing and resuspending the pellet in 10 mM dithioerythritol.

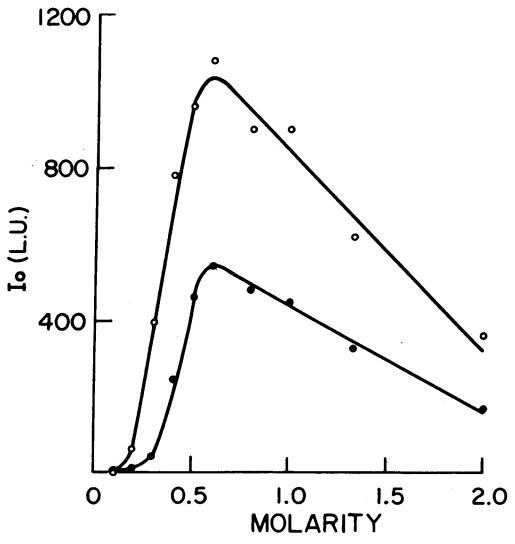

FIG. 3. Relationship between final salt concentration and I_0 for KCl- and NaCl-triggered light emission. One hundred microliters of test suspension was injected with 200 μl of salt solution, both in 0.05 M Tris·HCl, pH 7.20. ○, KCl; ●, NaCl.

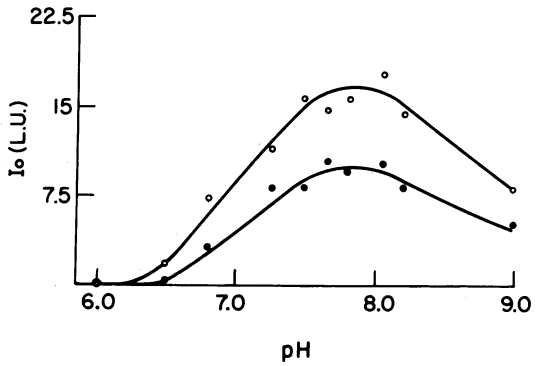

FIG. 4. Relationship between final pH and I_0 values for KCl- and NaCl-triggered light emission. One hundred microliters of test suspension (in 0.01 M Tris·HCl, pH 7.20) was mixed with 100 μl of pH buffer and then 200 μl of 1.0 M KCl or NaCl (in glass-distilled water) was injected into the mixture. pH buffers: 2-(*N*-morpholino)ethanesulfonic acid (Mes)/Tris, 0.1 M in Mes at pH 6.00, 6.50, 6.80, and 7.25; Tris/Mes, 0.1 M in Tris at pH 7.48, 7.65, 7.78, 8.05, 8.25, and 9.00. ○, KCl; ●, NaCl.

Effects of Other Agents. Ionophores and KCl (values in parentheses are final concentrations) were injected into test suspensions in 0.05 M Tris·HCl, pH 7.60/1 mM dithioerythritol in the following order: ionophore, 0.1 M KCl (0.04 M), 2.1 M KCl (0.60 M). Ionophores used were gramicidin (0.19 μM), valinomycin (0.67 μM), and nigericin (0.67 μM), first dissolved in dimethyl sulfoxide and then diluted with 0.05 M Tris·HCl, pH 7.60/1 mM dithioerythritol. Injection of the ionophores and 0.1 M KCl gave no light, whereas subsequent injection of 2.1 M KCl triggered light emission at I_0 values equal to that of the control. Similarly, neither injection of ATP (1 mM), cyclic AMP (1 mM), NADH (3.1 mM), NADPH (2.4 mM), or acetylcholine chloride (1 mM) nor prior addition of catalase (250 μg/ml), horseradish peroxidase (270 μg/ml), or superoxide dismutase (255 μg/ml) to test suspensions had any effect on KCl-triggered light emission. Test suspensions incubated with α-chymotrypsin (1.7 μg/0.42 mg of wet homogenate in 0.08 M Tris·HCl, pH 8.0/0.1 M $CaCl_2$) showed 50% loss of KCl-stimulable activity in 1 min, whereas incubation with trypsin (1.2 μg/0.42 mg of wet homogenate in 0.05 M Tris·HCl, pH 8.0/0.012 M $CaCl_2$) showed 73% loss of activity in 1.5 min. Similar results were obtained with bacterial protease. Finally, test suspensions (0.05 M Tris·HCl, pH 7.20/1 mM dithioerythritol) incubated for 1 hr with tetrodotoxin (11.5 μg/ml in 0.05 M Tris·HCl, pH 7.20/1 mM dithioerythritol) gave the following relative I_0 values when injected with 1.0 M NaCl or KCl: control, 100; NaCl, 111; KCl, 85.

DISCUSSION

The *Symplectoteuthis* light-emitting reaction appears to represent a type of oxygen-dependent bioluminescence system in which the essential light-emitting components are membrane bound and potassium or sodium ions are required to trigger the reaction. Such ion-triggered bioluminescence has been observed previously in the soluble systems of coelenterates and in the dinoflagellate *Gonyaulax polyedra*. Thus, in coelenterates, triggering of light emission is by calcium ions acting on a photoprotein—e.g., aequorin (11, 12); in *Gonyaulax*, it is by protons acting on a luciferin-binding protein that releases luciferin for reaction with luciferase (13); and in the coelenterate *Renilla reniformis* it is by calcium ions acting on a luciferin-binding protein to release luciferin (14). Molecular oxygen is

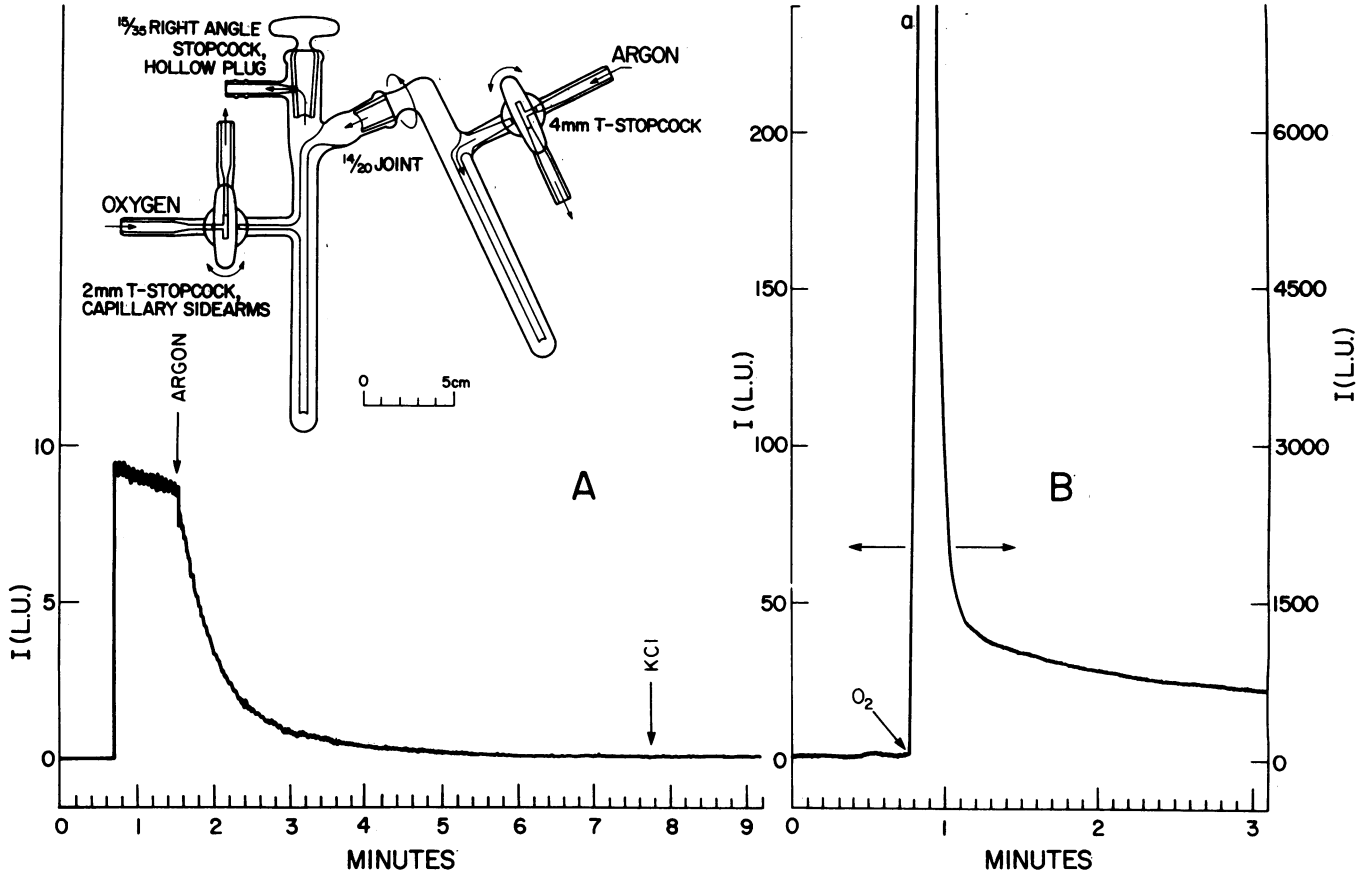

FIG. 5. Effect on KCl-triggered bioluminescence of flushing the test suspension with argon. (*A*) Result of flushing a test suspension with argon and subsequent addition of 1.0 M KCl. (*B*) Result of admitting oxygen to such a suspension. The buffer used was 0.05 M Tris·HCl, pH 7.20. (*Inset*) Diagram of the mixing A tube, adapted from Harvey (10). The left and right arms of the A tube contained 1.0 ml of test suspension and 1.0 ml of 1.0 M KCl, respectively. The bottom of the left arm was seated above the window of the photomultiplier. The connections were (*i*) a T stopcock on the right arm to the outlet tubing of the furnace tube, (*ii*) inlet tubing of the furnace tube to the needle valve of the pressure gauge on the argon tank, and (*iii*) a T stopcock on the left arm to the needle valve on the oxygen tank. All connections were via flexible copper tubing, brass Swagelok fittings, and Sealstex cement (Arthur H. Thomas). All experiments were carried out under light-tight conditions in a darkroom. When we began to flush the A tube with argon, the right-angle stopcock was closed and the stopcock on the left arm was opened to the atmosphere to clear its capillary of air, after which the T stopcock on the left arm was closed and the right-angle stopcock was opened; then, when KCl was being transferred from the right to the left arm, the right arm was rotated upward ≈120° with the argon flowing, thus forcing the KCl into the left arm. To introduce oxygen into the left arm, the stopcock on the right arm was closed and that on the left arm was opened (the right-angle stopcock remained open).

required for luminescence in the *Gonyaulax* and *Renilla* reactions but not in the photoprotein reaction. Further, the systems in *Symplectoteuthis*, the closely related squid *Ommastrephes pteropus* (15), and *W. scintillans* (1) all appear to resemble each other in that the light-emitting components are membrane bound.

Examination of the kinetics of the decay of light intensity in intact photogenic granules (Fig. 1) and homogenates of *Symplectoteuthis* triggered by KCl suggests that two light-emitting components are involved (7). One component has a fast, and the other has a slow, rate of decay. The same reaction appears to be occurring, whether it is in granules or homogenates and whether it is triggered by potassium or sodium ions (Figs. 1, 3, and 4). The fast decay may be due to accumulation of an intermediate that is triggered by potassium or sodium ions. Biphasic kinetics with first-order characteristics have also been observed in the bioluminescence system of the boring clam *Pholas dactylus* (16, 17). An oxygen-bound intermediate appears to be formed during the *Symplectoteuthis* reaction when a suspension is incubated with KCl under oxygen-free conditions and then mixed with molecular oxygen. This is suggested by the fact that the resulting light emission is extremely difficult to extinguish by argon flushing compared with the relative ease with which the original low-level light is extinguished.

Measurement of the *Symplectoteuthis* emission spectrum (Fig. 2) was carried out under several different conditions, but the spectrum did not show any significant change even when it was measured 1.5 min after mixing (λ_{max}, 455 nm) or when the slit was narrowed to 5 nm (λ_{max}, 458 nm). Interestingly enough, the *Symplectoteuthis* emission spectrum shows a large discrepancy from that of *O. pteropus*, which has a peak at 474 nm (15), and from the fluorescence emission spectrum of *Watasenia* oxyluciferin, the presumptive emitter, which has a peak at 400 nm (1). Further, the *Symplectoteuthis* spectrum shows little resemblance to the *in vitro* bioluminescence emission spectrum of *Pholas*, which has a peak at 505 nm (16).

Another interesting property of the *Symplectoteuthis* system is the high salt concentration (0.6 M) required to trigger maximal light emission (Fig. 3). Thus, it is of interest that the *Pholas* reaction also requires a high NaCl concentration (0.5 M) for optimal light emission (18). In contrast, homogenates of the *O. pteropus* light organ are insensitive to injections of cations, anions, and cofactors (15). The *Symplectoteuthis* system differs to an even greater extent from the well-studied molluscan system

Table 2. Effects of reducing agents, EDTA, and sulfhydryl-modifying agents on I_0 of test suspension

Suspension	Addition	Final concentration, mM	Incubation time, min	Relative I_0
1	None	—	—	100
	2-Mercaptoethanol	10	80	289
	Dithioerythritol	10	90	520
	Dithiothreitol	10	100	453
2	None	—	—	100
	Dithioerythritol	1	60	300
	Dithioerythritol	3	60	229
	Dithioerythritol	9	60	156
3	None	—	—	100
	EDTA	1	85	675
	EDTA/dithioerythritol	1/1	90	1136
	EDTA/dithioerythritol	1/10	95	619
4	None	—	—	100
	L-Cysteine	1	60	149
	DL-Cysteine	1	60	137
	Glutathione	1	60	205
	Dithioerythritol	1	60	252
5	None	—	—	100
	N-Ethylmaleimide	10	60	100
	5,5'-Dithiobis(2-nitrobenzoic acid)	1	60	100
	Iodoacetamide	10	60	72
	p-Chloromercuribenzoic acid (A)	1	1	0
	Suspension A/wash/resuspension in buffer (B)	—	—	4
	Suspension B/resuspension in buffer/dithioerythritol*	10	1	42

Test suspensions and incubation mixtures were prepared in 0.05 M Tris·HCl, pH 7.20.

* After assay, suspension B was centrifuged and the pellet was resuspended in 0.05 M Tris·HCl, pH 7.20/10 mM dithioerythritol.

of *Latia neritoides*, whose reaction involves a luciferin that is an enol formate, a luciferase, a purple protein cofactor, and molecular oxygen and has an emission peak at 536 nm (19).

The increase in KCl-triggered light emission in the presence of EDTA (Table 2) is probably due to the removal of divalent cations that inhibit the triggering mechanism or activate contaminating peptidases that digest the active component. The high and long-lasting activity obtained by adding dithioerythritol to test suspensions and the rapid inhibition by *p*-chloromercuribenzoic acid and its reversal by dithioerythritol (Table 2) suggest that a free sulfhydryl group is essential to the light-emitting reaction.

We thank Dr. Richard E. Young, University of Hawaii, for arranging laboratory space on board the R/V *Kana Keoki* and for helpful discussions on squid bioluminescence. This work was supported in part by research grants from the National Science Foundation.

1. Goto, T., Iio, H., Inoue, S. & Kakoi, H. (1974) *Tetrahedron Lett.*, 2321–2324.
2. Inoue, S., Sugiura, S., Kakoi, H., Hasizume, K., Goto, T. & Iio, H. (1975) *Chem. Lett.*, 141–144.
3. Inoue, S., Kakoi, H. & Goto, T. (1976) *Tetrahedron Lett.*, 2971–2974.
4. Inoue, S., Taguchi, H., Murata, M., Kakoi, H. & Goto, T. (1977) *Chem. Lett.*, 259–262.
5. Clarke, M. R. (1966) *Adv. Mar. Biol.* **4**, 91–300.
6. Young, R. E. (1975) *Comp. Biochem. Physiol.* **52B**, 141–143.
7. Leisman, G. & Tsuji, F. I. (1981) in *Bioluminescence and Chemiluminescence, Basic Chemistry and Analytical Applications*, eds. De Luca, M. & McElroy, W. D. (Academic, New York), pp. 709–714.
8. Mitchell, G. W. & Hastings, J. W. (1971) *Anal. Biochem.* **39**, 243–250.
9. Hastings, J. W. & Weber, G. (1963) *J. Opt. Soc. Am.* **53**, 1410–1415.
10. Harvey, E. N. (1926) *Biol. Bull.* **51**, 89–97.
11. Johnson, F. H. & Shimomura, O. (1978) *Methods Enzymol.* **57**, 271–291.
12. Morin, J. G. & Hastings, J. W. (1971) *J. Cell. Physiol.* **77**, 305–311.
13. Hastings, J. W. (1978) in *Bioluminescence in Action*, ed. Herring, P. J. (Academic, New York), pp. 129–170.
14. Cormier, M. J. (1978) *Methods Enzymol.* **57**, 237–244.
15. Girsch, S. J., Herring, P. J. & McCapra, F. (1976) *J. Mar. Biol. Assoc. U. K.* **56**, 707–722.
16. Henry, J. P., Isambert, M. F. & Michelson, A. M. (1970) *Biochim. Biophys. Acta* **205**, 437–450.
17. Michelson, A. M. (1978) *Methods Enzymol.* **57**, 385–406.
18. Henry, J. P. & Michelson, A. M. (1970) *Biochim. Biophys. Acta* **205**, 451–458.
19. Shimomura, O., Johnson, F. H. & Kohama, Y. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2086–2089.