Bioluminescence reaction in the firefly squid, *Watasenia scintillans*

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Each spring, in a far-off region of northern Japan, where the Noto Peninsula projects into the Sea of Japan forming Toyama Bay (maximum depth, ca. 1,200 meters), the deep-sea squid *Watasenia scintillans* (mantle length, ca. 6 cm; wet weight, ca. 9 gm), comes inshore to lay fertilized eggs. The migration takes place on a vast scale, involving hundreds of millions of squids. The migration is most notable in Toyama Bay. For nearly a hundred years the squid has served as a food source and a local fishery has developed around the squid in the small maritime city of Namerikawa. The squids are caught by setting nets in shallow water and in March-April; of this year, the recorded catch was 2,500 tons. The squid has been studied ever since the luminescence was first described by Watasé in 1905, for whom the squid is named. An early visitor was E. Newton Harvey, who made the long journey from Princeton and reported his observations in a paper in 1917. The squid has more than 800 minute luminous organs distributed over its ventral mantle, a row of 5 prominent organs lining the lower margin of its eye and a cluster of 3 tiny pigmented organs (less than 1 mm diam) on the tips of the 4th pair of arms. The ventral organs produce a steady glow of light, whereas the arm organs emit brilliant flashes of light (470 nm). The rhythmic flashing sometimes observed in the arm organs resembles that of a firefly flashing at night and so the squid is known in Japan as the “firefly squid.” The *Watasenia* reaction is due to a luciferin-luciferase reaction, involving a “soluble component”, “insoluble component,” ATP, Mg ions and molecular oxygen. The optimum pH is 8.8. If the arm organs are homogenized in Tris-HCl buffer, pH 8.3, and injected with ATP, a bright luminescence is observed. On centrifuging the homogenate, the supernatant is found to contain luciferin as a “soluble component,” whereas the pellet yields an unstable membrane-bound luciferase as “insoluble component.” Neither the “soluble component” nor the “insoluble component” gives light with ATP, except when reconstituted. There is an absolute requirement for molecular oxygen. Earlier work on structure determination, total chemical synthesis and measurement of light-emitting activity has shown that the luciferin is coelenterazine disulfate. Based on results with *Watasenia* and other luminescent systems, a hypothetical scheme for the *Watasenia* reaction is proposed involving: (1) a base/luciferase-catalyzed enolization of the C-3 keto oxygen of coelenterazine disulfate, (2) an adenylatation of the enol group by ATP forming the intermediate adenyl colenterazine disulfate, (3) removal of AMP and the addition of molecular oxygen to the C-2 carbon forming a dioxetanone intermediate and (4) spontaneous cleavage of the dioxetanone ring yielding carbon dioxide, coelenteramide disulfate and 60 kcal/mol of energy required for the blue light emission. The probable light emitter in the reaction is the excited state amide anion of coelenteramide bound to luciferase.