Bioluminescence of Coelenterates: Chemiluminescent Model Compounds

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Summary The chemiluminescence spectra of compounds related to the luciferins from certain coelenterates have been recorded, and a common pathway to the different bioluminescent emissions is indicated.

THE study of the chemiluminescence of model compounds has been strikingly successful in indicating both the mechanism and products of the *in vivo* bioluminescence in systems involving discrete luciferins.[1] However, a new class of bioluminescent reaction, in which a separate luciferin is not always found, has appeared recently. This requires only a 'photoprotein'[2] and in certain cases does not need oxygen. Nevertheless biochemical investigation of the coelenterates Obelia, Pelagia, Mnemiopsis, Campanularia Clytia, Phialidium, Lovenella, Ptilosarcus, Diphyes, Renilla, and Aequorea strongly suggest a common chemical mechanism and part structure.

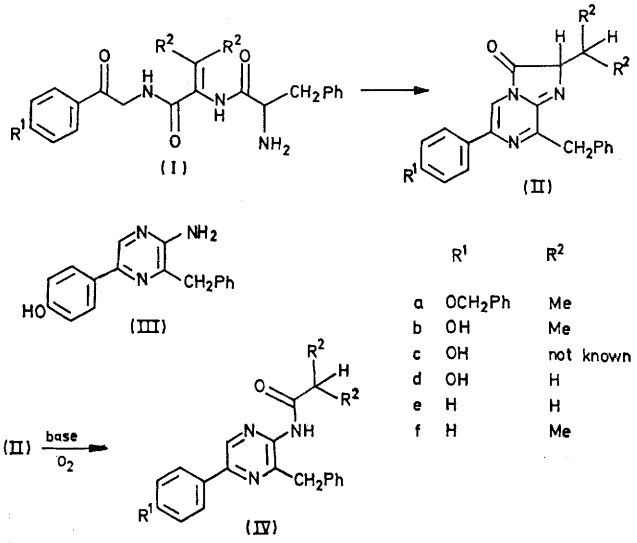
TABLE 1

	Compound	λ_{max}/nm of chemiluminescence	Compound	λ_{max}/nm of fluorescence
1	(IIa)	466[a]	(IVa)	474[a]
2	(IIb)	412[b]	(IVb)	418[b]
3		474[c]		414[c]
4		475*d*		414[d]
5		—		473[a]
6		497[a]		523[a]
7		505[c]		523[c]
8	(IIe)	447[a]	(IVe)	456[a]
9	(IIf)	453[a]	(IVf)	461[a]

[a] Potassium t-butoxide–Me_2SO. [b] Diglyme–acetate buffer pH 5·5. [c] Me_4NOH (two concentrations)–Me_2SO. [d] Potassium phthalimide–Me_2SO.

The isolation of (III) (described as a 'chromophore') from the photoprotein from Aequorea[4] and a part structure for Renilla luciferin[5] (IIc) in conjunction with our previous biosynthetic suggestion and synthesis[6] prompts us to make the prediction that the luminescent systems of all of the organisms named above may be derived from a modified tripeptide. In accordance with this prediction we have synthesised, from modified tyrosylvalinylphenylalanine, the essential structure required in these two cases and now report a comparison between its chemiluminescence and the known bioluminescence. The chemiluminescence of related compounds is also reported.

Cyclisation of (I; $R^1 = OCH_2Ph, R^2 = Me$) in K_2CO_3–DMF gave (IIa), isolated as the hydrochloride; (IIb) was obtained by debenzylation in CF_3CO_2H–HBr. Bright chemiluminescence, with a typical quantum yield of $3{\cdot}5 \times 10^{-4}$, gave the emission maxima shown (see Table 1) under a variety of conditions. A striking feature of the light emission is the range of colour obtainable from a single substrate (potentially from 414 to 525 nm). This range (Table 1) can be obtained by a combination of all of the ionised and unionised forms of the hydroxy and amide groups in the excited product (IV) isolated from the reaction in good yield, and fully characterised. No other suitably fluorescent product is formed. Thus different protein environments will result in bioluminescence characteristic



	R^1	R^2
a	OCH_2Ph	Me
b	OH	Me
c	OH	not known
d	OH	H
e	H	H
f	H	Me

of each species (Table 2). The mechanism in a chemically related (but biologically distant) system, Cypridina, has already been indicated by us[1] and confirmed *in vivo*,[7] generating an analogous excited product by, we assume, a similar path.

TABLE 2

Organism	Bioluminescence λ_{max}/nm
Cypridina hilgendorfii ..	460
Aequorea aequorea	465
Renilla reniformis	485
Obelia geniculata	475

Undoubtedly, important differences in the biochemistry of each of the organisms mentioned will emerge, but it seems clear to us that a combination of the cyclisation of different tripeptides and the different excited states possible in the products may in future account for the bioluminescence of a large variety of marine organisms. In this connection we note that (IIb) when tested with Renilla luciferase gave light. The efficiency was low (0·3% of the natural luciferin of an as yet partially unknown structure) and it is probable that better results would be obtained if the middle amino-acid were less hydrophobic than the valine used by us in the synthesis.

The results in Table 1 are interpreted on the basis that the compounds (IV) (formed in very high yield) are the emitting species. In the cases (IIa), (IIe), and (IIf) only two possible forms of (IV) exist—the neutral amide and the corresponding anion. Since the amides fluoresce at around 380—400 nm clearly the anion is the excited product.

The discrepancy in wavelength between fluorescence and chemiluminescence (entries 1,8, and 9) is a result of the method of measurement, and a correction can be applied which leads to an exact match of these spectra. With (IIb) a dianion is possible and the position of λ_{max} both in chemiluminescence and fluorescence is strongly dependent on base. Discussion of the progression in wavelength must await our full paper, but certain bases (*e.g.* potassium phthalimide) which do not ionise the amide product, catalyse the chemiluminescence. From the results (entry 4) it is clear that the amide is formed as anion (λ_{max} 475 nm) and emits a photon before it can protonate, since the medium is insufficiently basic to deprotonate the excited amide (λ_{max} 414 nm). For these reasons a match of chemiluminescence and fluorescence wavelength is not always obtainable in the same solution, although a common wavelength can always be found by adjusting the base strength in either separate solution. There are implications in these observations for the mechanism and it is obvious that important details remain to be added to the outline mechanisms at present available. All new compounds reported gave satisfactory spectra and analyses.

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