Biochimica et Biophysica Acta, 314 (1973) 104–109

BBA 46569

SIMILARITIES IN THE BIOLUMINESCENCE FROM THE *PENNATULACEA**

JOHN E. WAMPLER[a], YASHWANT D. KARKHANIS[a], JAMES G. MORIN[b] and MILTON J. CORMIER[a]

[a]*Department of Biochemistry, University of Georgia, Athens, Ga. 30601 (U.S.A.) and* [b]*Department of Biology, University of California Los Angeles, Calif. 90024 (U.S.A.)*

(Received February 9th, 1973)

SUMMARY

1. The *in vivo* bioluminescence spectra of six species of cnidarians from the subclass *Alcyonaria*, order *Pennatulacea*, have been examined. They are all strikingly similar having as a predominant feature a narrow green emission peak with a maximum at 19640 cm^{-1} (509 nm). Each of these spectra is structured with a shoulder in the region of 18500 cm^{-1} (540 nm) and in some cases another shoulder in the 21000-cm^{-1} (475-nm) region.

2. A protein bound green-fluorescent chromophore has been isolated from each of these animals. The fluorescence spectra of these chromophores are identical. Their peak position, 19640 cm^{-1} (509 nm), and narrow structured character strongly suggest that they represent the emitter responsible for the corresponding parts of the bioluminescence spectra.

3. The data indicate that in each of these Pennatulids, the overall bioluminescence spectrum can be explained as the combination of two emitting species, the fluorescence of the green-fluorescent, protein-bound chromophore and the broad blue fluorescence typical of the *in vitro* reactions.

INTRODUCTION

In recent years investigations into the physiology and biochemistry of coelenterate bioluminescence have yielded several important results. Spectral similarities between diverse species have been noted[1–3], and these similarities have extended into the biochemistry of the reactions involved[2–5]. We recently reported[5] that the components required for luminescence in *Renilla* were also found in a number of bioluminescent coelenterates, *e.g. Aequorea forskalea, Obelia geniculata, Cavernularia obesa, Ptilosarcus guerneyi, Stylatula elongata, Acanthoptilum gracile, Parazoanthus lucificum*, and *Mnemiopsis leidyi*. Depending on the organism the similarities include one or more of the following: luciferyl sulfate, luciferin sulfokinase, and luciferase. One of the more interesting aspects of coelenterate bioluminescence is the possible involvement of energy transfer between the excited state product and the characteristic acceptor chromophore which has been investigated by Morin and Hastings[2] and Wampler *et al.*[6,7].

* This is Contribution No. 266 from the University of Georgia Marine Institute, Sapelo Island, Ga., U.S.A.

This paper deals with a detailed spectroscopic investigation using specialized instrumentation to study some species within the subclass *Alcyonaria*, order *Pennatulacea*. It is shown that of the species examined, without exception, the spectral envelope can be easily described as the combination of emissions from two emitting chromophores, one having a broad blue structureless emission band and the other a narrow structured band characterized by a peak with a maximum at 19640 cm^{-1} (509 nm).

METHODS AND MATERIALS

Renilla mülleri were obtained from Gulf Specimens, Incorporated (Panacea, Fla., U.S.A.). *Renilla reniformis* were obtained from the University of Georgia Marine Institute, Sapelo Island, Georgia. *Renilla köllikeri*, *Stylatula elongata*, *Acanthoptilum gracile*, and *Ptilosarcus guernyi* were obtained by one of us (J.M.) from the California coast.

The *in vivo* bioluminescence of these animals was routinely measured by quick freezing the animal with dry ice and observing the relatively long lived luminescence upon thawing. This technique gave reproducible results and identical results to those obtained by mechanical, chemical, and electrical stimulation. The spectra were determined using a rapid scanning, computerized spectrofluorimeter system[8] and the techniques previously described[6]. Fluorescence spectra were determined by using the same instrument.

It should be pointed out that until recently[6] measurements of high resolution *in vivo* spectra have not been made primarily due to limitations in instrumentation. The spectra reported here are averages of five or more scans taken with a cycle time of 15 s per scan. Each individual scan is in fact a 32-sample average and the spectra are represented by 500 data points. The spectra reported were plotted by the spectrofluorimeter system[8] directly and thus the actual noise is shown. The spectra are corrected for both emission variations and the spectral sensitivity of the analyzing system. All of the spectral features discussed in this manuscript are well outside of the accuracy and precision limitations of the data reported.

The green fluorescent proteins were partially purified according to the preparation procedure of Karkhanis and Cormier[9].

RESULTS AND DISCUSSION

In our examinations of the *in vivo* bioluminescence of several bioluminescent pennatulids, we have found a striking similarity in the emission spectra. The representative spectra in Fig. 1 illustrate this point. In analyzing this data we want to focus attention on three parts of the spectra shown: (1) the blue portion above 20500 cm^{-1} (490 nm) here seen as slight contributions to the total spectrum for *A. gracile*, *P. guerneyi*, and *R. mülleri*, (2) the structured emission band with a narrow primary band peaking at 19640 cm^{-1} (509 nm), and (3) the details of the structure giving the shoulder in the 18500-cm^{-1} (540–570 nm) region. As has been pointed out in our previous study of *R. reniformis* bioluminescence[6], the entire band can be analyzed as a combination of two emissions, (a) the emission characteristic of the bioluminescence product (the broad blue component) and (b) the emission characteristic of the fluores-

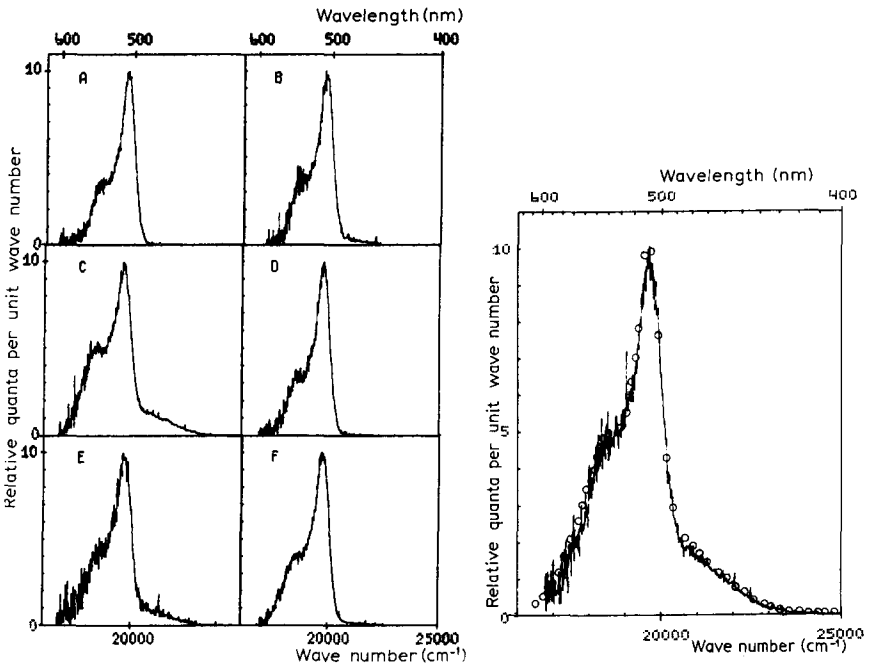

Fig. 1. *In vivo* bioluminescence of live *Pennatulacea*. (A) *S. elongata*. (B) *A. gracile*. (C) *P. guerneyi*. (D) *R. köllikeri*. (E) *R. mülleri*. (F) *R. reniformis*.

Fig. 2. *In vivo* bioluminescence ofa *R. mülleri* (solid line). The open circles are a computer fit using 52% emission of the fluorescence of the isolated green fluorescent protein and 48% emission of the *in vitro* bioluminescence from the reaction of purified luciferin catalyzed by purified luciferase.

cence of a protein bound, green fluorescent chromophore which is responsible for both the narrow peak at 509 nm (19640 cm^{-1}) and the shoulder in the 18500-cm^{-1} (540–570-nm) region. Studies of the spectroscopy of the green chromophore indicated[6] that the peak at 19640 cm^{-1} (509 nm) and the shoulder at 18500 cm^{-1} (540 nm) are vibrational bands of the same electronic transition. A cursory examination of the spectrum of each of the Pennatulids (Fig. 1) studied here, again reveals the similarity of each to *R. reniformis*. Furthermore, a closer examination reveals that for most of these animals two kinds of variation are seen when they are compared with each other. First, there is the variation of the blue component in its contribution to the overall spectrum, the most extreme difference shown is between *S. elongata* (A) and *P. guerneyi* (C). Secondly, there are differences in the details of the spectral shape in the 18500-cm^{-1} (540-nm) region, which are well illustrated in comparing *R. köllikeri* (D) to *P. guerneyi* (C).

As was pointed out previously[6] a variation in the contribution of the blue component (440–490 nm) was seen when the spectra of individual colonies of *R. reniformis* were compared, but the details of the shape of the rest of the band remained constant. A 10-fold variation in the ratio of these two emission components was seen when comparing different animals. This variation is well outside of the deviation of measurements on a single animal ($\pm 10\%$). Detailed examinations of a large number

of *R. mülleri* colonies indicate that a few have a major contribution from both emitting species. Fig. 2 is an example of such a spectrum. However, even in this case the total spectral envelope can be accounted for by the combination of the blue product emission characteristic of the *in vitro* reaction and the green fluorescent protein emission. To illustrate this, Fig. 2 shows a computer fit (open circles) for the appropriate combination of these two emission spectra to match the *in vivo* spectrum.

In considering the variability of this ratio (I_{509}/I_{470}) we felt it might be useful to examine whether these differences also extend to individual polyps within a single colony. While we were not able to examine the emission spectrum of a single polyp, we did examine the emission from small isolated sections of the colony. In *Renilla* the colony grows by radial expansion so that the outer most polyps are the youngest and the inner most, those near the peduncle, are the oldest. Fig. 3 shows the spectra obtained from various parts of a single colony of *R. mülleri*. In no case were there any obvious differences in the spectral shape within a single colony. These observations have been verified in an examination of both *R. köllikeri* and *P. guerneyi*.

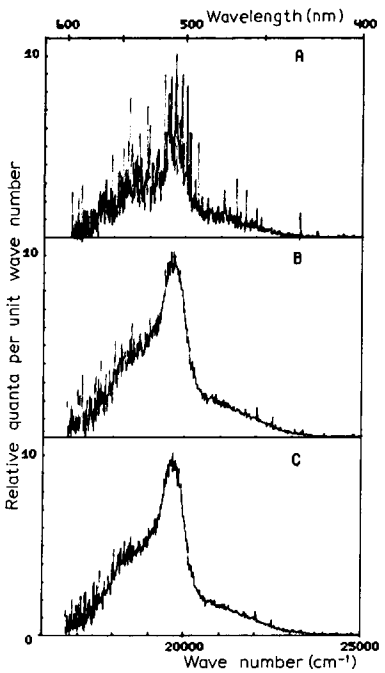

Fig. 3. A spectral comparison of the *in vivo* bioluminescence for different sections of the same colony of *R. mülleri*. (A) Section from edge of colony. (B) Midsection of colony. (C) Center section of colony.

While the minor differences in the details of the structure of the 18 500-cm^{-1} (540-nm) shoulder were readily apparent in comparing the spectra from different species (see Figs 1C and 1D), there was no significant variation of the I_{509}/I_{540} ratio within the populations examined for individual colonies of a particular species. That is for 15 different *R. mülleri*, 22 *R. reniformis* and seven *P. guerneyi* the standard deviation of the I_{509}/I_{540} ratio was about 10% and not significantly different from

the deviation in measurements on a single animal. This then is in contrast to the above results for the blue component.

The reasons for the variation in the ratio of shoulder to peak height (I_{509}/I_{540}) between species were not readily obvious. Since the 18500-cm^{-1} (540-nm) shoulder is thought to arise from the vibrational structure in the green chromophore emission[6], several explanations were tenable. First, the differences seen could be due to actual chemical differences between the chromophores from different species, or, second, the chromophore might be the same but have spectra which are perturbed by virtue of the particular protein environment in which it is found. Finally the observed differences might simply represent an inner filter effect due to the presence of various pigments in the photocytes or surrounding cells of these animals. The first two explanations appear unlikely since the isolated green fluorescent proteins of each of these animals have identical fluorescence spectra as shown in Fig. 4. This observation has also been shown to extend to *Aequorea forskalea*[5]. While an inner filter effect has not been directly demonstrated it is of interest to note that the species exhibiting the most obvious variation in structure in the 18500-cm^{-1} (540-nm) region, *P. guerneyi* (Fig. 1C), is also the most highly colored species examined (bright orange).

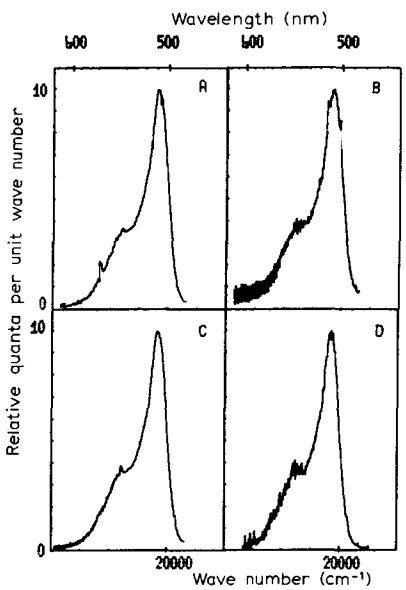

Fig. 4. Fluorescence emission spectra of green fluorescent proteins isolated from (A) *S. elongata*, (B) *A. gracile*, (C) *P. guerneyi*, and (D) *R. köllikeri*. These spectra were determined using excitation at 470 nm.

Thus we conclude that for the species of *Pennatulacea* examined, the spectra of bioluminescence observed are extremely similar to the *R. reniformis* spectrum. The similarity extends to the resolution of the spectra into contributions from two emission components: a broad, structureless blue emission and a narrow, structured green emission. For *R. reniformis* and *R. mülleri*, previous studies[6] indicate that the sources of these emissions in each are the excited states of the bioluminescence reaction

product and the green fluorescent, protein bound chromophore. In addition the green-fluorescent proteins isolated from the other species described in this paper fit into this scheme, *i.e.* they exhibit the same spectral shape as that from *Renilla.*

The recent demonstration of interspecies bioluminescent cross reactions between *Renilla* and other coelenterates[5] and the broad blue, non-structured nature of their *in vitro* spectra is also consistent with the concept of energy transfer as a general phenomena among many bioluminescent cnidarians[3,7]. Transfer would then explain the predominance of the green, narrow, structured component in the *in vivo* spectra described here.

ACKNOWLEDGEMENTS

This work was supported in part by the National Science Foundation, the National Institutes of Health, and the U.S. Atomic Energy Commission. M.J.C. is a Career Development Awardee, No. 1-K3-6M-3331-07, of the U.S. Public Health Service.

REFERENCES

1 Morin, J. G. and Hastings, J. W. (1969) *Biol. Bull.* 137, 402
2 Morin, J. G. and Hastings, J. W. (1971) *J. Cell. Physiol.* 77, 305
3 Morin, J. G. and Hastings, J. W. (1971) *J. Cell. Physiol.* 77, 313
4 Hori, K., Nakano, Y. and Cormier, M. J. (1972) *Biochim. Biophys. Acta* 256, 638
5 Cormier, M. J., Hori, K., Karkhanis, Y. D., Anderson, J. M., Wampler, J. E., Morin, J. G. and Hastings, J. W. (1973) *J. Cell. Physiol.* 81, 291
6 Wampler, J. E., Hori, K., Lee, J. W. and Cormier, M. J. (1971) *Biochemistry* 10, 2903
7 Wampler, J. E., Karkhanis, Y. D., Hori, K. and Cormier, M. J. (1972) *Fed. Proc.* 31, 1133
8 Wampler, J. E. and DeSa, R. J. (1971) *Appl. Spectrosc.* 25, 623
9 Karkhanis, Y. D. and Cormier, M. J. (1971) *Biochemistry* 10, 317