Circular polarization observed in bioluminescence

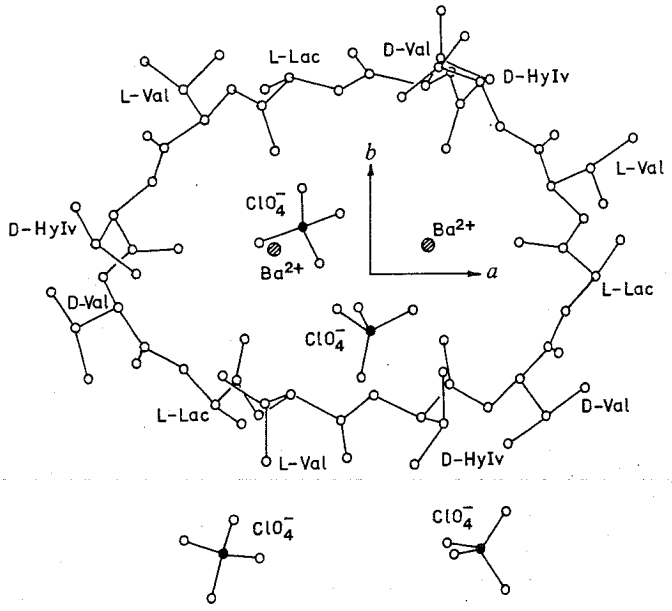

Fig. 2 The location of the valinomycin molecule, the barium ions and the perchlorate groups as viewed along the *c* axis in the crystals of a 1:2 valinomycin–barium perchlorate complex.

of valinomycin with Ba^{2+}. CD data on the titration of barium perchlorate with the ionophore in acetonitrile indicated valinomycin–Ba^{2+} complexes with 2:1, 1:1 and 1:2 stoichiometries, although the conformation of the molecule stabilized only at high salt concentrations (S.D. and K.R.K.E., in preparation). CD could not be used with barium thiocyanate because of the high UV absorption of the thiocyanate group. However, proton NMR indicated a stable 1:2 valinomycin–barium thiocyanate complex, although the titration curve based on NMR data for the thiocyanate salt was different from that for the perchlorate salt (S.D. *et al.*, in preparation). Thus, it seemed that valinomycin could form 1:2 complexes with barium perchlorate as well as barium thiocyanate and we decided to attempt X-ray crystallographic studies on these complexes.

Complexes (1:2) of valinomycin with barium perchlorate and barium thiocyanate were crystallized by slow evaporation of slightly aqueous acetonitrile solutions containing the respective components in molar proportions. The crystals were mounted in thin-walled glass capillaries together with the solvent. X-ray diffraction photographs showed that the valinomycin–barium perchlorate complex crystallizes in the orthorhombic space group $P2_12_12_1$ with $a = 28.304$ Å, $b = 16.938$ Å, $c = 19.543$ Å and $z = 4$, whereas the thiocyanate complex crystallizes in the monoclinic space group C2 with $a = 29.48$ Å, $b = 16.49$ Å, $c = 19.92$ Å, $\beta = 111.4°$ and $z = 4$. X-ray intensity data from both the crystals were collected on a computer controlled four-circle CAD-4 diffractometer using graphite monochromatized MoKα radiation to a maximum Bragg angle of 23°. The structure of the barium perchlorate complex was subsequently determined by the heavy atom method and refined by the block-diagonal structure factor least squares technique to an *R* value of 0.13 for 3,504 observed reflections ($I > 2\sigma(I)$). So far, the two barium ions and all the non-hydrogen atoms belonging to the valinomycin molecule, the four perchlorate groups and four water molecules have been located.

The location of the barium ions, the valinomycin molecule and the perchlorate ions in the crystal structure is shown in Fig. 2. The main-chain dihedral angles[4] in the valinomycin molecule correspond approximately to those for an extended chain. The molecule can be described as an extended depsipeptide chain, with no internal hydrogen bond, wound in the form of an ellipse with the barium ions located at the foci. Three consecutive amide carbonyl groups coordinate to one of the barium ions whereas the remaining three consecutive amide carbonyl groups coordinate to the other barium ion. The coordination of the cation is completed by perchlorate and water oxygens. The conformation of valinomycin observed in the structure is totally different from those found in the crystal structures of uncomplexed valinomycin[5,6] and its potassium complex[7]. It is also different from the conformations unambiguously characterized so far in solution studies[1–3].

We thank the University Grants Commission and the Department of Science and Technology, India, for financial assistance, and Dr A. M. Shaik for help with data collection. The atomic coordinates have been deposited in the Crystallographic Data Bank, University Chemical Laboratory, Lensfield Road, Cambridge, UK.

Received 3 March; accepted 5 June 1980.

1. Ovchinnikov, Yu. A., Ivanov, V. T. & Shkrob, A. M. in *Membrane Active Complexes* (Elsevier, Amsterdam, 1974).
2. Pressman, B. C. *A. Rev. Biochem.* **45**, 501–530 (1976).
3. Ovchinnikov, Yu. A. *Eur. J. Biochem.* **94**, 321–336 (1979).
4. Ramachandran, G. N. & Sasisekharan, V. in *Advances in Protein Chemistry* (eds Anfinson, C. B. *et al.*) 284–437 (Academic, New York, 1968).
5. Karle, I. L. *J. Am. chem. Soc.* **97**, 4379–4386 (1975).
6. Smith, G. D. *et al. J. Am. chem. Soc.* **97**, 7242–7247 (1975).
7. Neupert-Laves, K. & Dobler, M. *Helv. chim. Acta* **58**, 432–442 (1975).

Circular polarization observed in bioluminescence

Hans Wynberg*, E. W. Meijer*, J. C. Hummelen*, H. P. J. M. Dekkers†, P. H. Schippers† & A. D. Carlson‡

* Department of Organic Chemistry, University of Groningen, The Netherlands
† Department of Theoretical Organic Chemistry, University of Leiden, The Netherlands
‡ Department of Neurobiology & Behavior, State University of New York at Stony Brook, Stony Brook, New York 11790

While investigating circular polarization in luminescence[1,2], and having found it in chemiluminescence[3,4], we have studied bioluminescence because it is such a widespread and dramatic natural phenomenon[5,6]. We report here that left and right lanterns of live larvae of the fireflies, *Photuris lucicrescens* and *Photuris versicolor*, emit circularly polarized light of opposite sense.

Firefly larvae (Fig. 1) were gathered in the US and sent by airmail to The Netherlands. They were alive up to and during most of the experiments. In our first attempts to detect polarization of luminescence, we measured the total light emission from both lanterns of the larvae. The results were puzzling and disappointing; vanishingly small circular polarization was observed, with only a few exceptions. To our surprise, we noted—fortuitously—that measurements of the light emission from the left or right lanterns separately gave more constant and encouraging results. During the latter experiments one important fact was established. By shifting and rotating the lanterns through many angles and positions with respect to the optical axis of the apparatus, we found that orientational artefacts could not be the origin of the circular polarization subsequently measured. Whereas in these early experiments we used excised lanterns rigidly mounted in a cell, in the later experiments we preferred to attach entire larvae with fine wire to a holder. The holder was positioned in front of a diaphragm so that one lantern only was fixed onto the optical axis of the apparatus. Continuous bioluminescence, lasting from several minutes to an hour, was achieved by feeding the larvae with a concentrated solution of racemic amphetamine hydrochloride in water. Luminescence usually began 5–30 min after the addition of amphetamine. Measurements were then made immediately.

The circular polarization of luminescence was measured as the anisotropy factor or g_{lum} factor $(I_L - I_R)/\frac{1}{2}(I_L + I_R)$. The difference in the numbers of left and right circularly polarized

0028–0836/80/320641—02$01.00

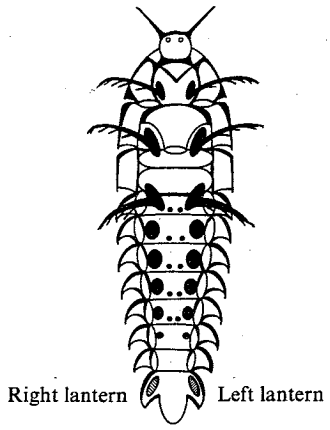

Fig. 1 Sketch of the firefly larva (ventral view). The shaded spots at the bottom represent the lanterns.

photons emitted, $I_L - I_R$, was modulated at a frequency of 50 kHz and detected as alternating photocurrent; the average luminescence $\frac{1}{2}(I_L + I_R)$ was measured as direct photocurrent[1,2,7–9]. Measurements of circular polarization of bioluminescence (CPBL) of 16 firefly larvae lanterns are shown in Fig. 2, revealing that the left and right lanterns emit polarized light of opposite sense. Within each group there is a considerable variation in the total light emission and values of g for the individual lanterns. In these experiments, the construction of the sample holder prevented a detailed investigation of orientational effects. However, on the basis of results of earlier experiments, we believe that neither position effects nor those due to linear polarization could have decisively affected our result.

What is the origin of the CPBL, and what is the origin of the difference in handedness shown by the left and right lanterns? According to the accepted mechanism of firefly bioluminescence mediated by luciferin, an achiral emitting state, III* (see Fig. 3), is involved[5,10–13]. This achiral excited state, by itself, offers no possibility of explaining the CPBL. It may be argued however, that the true excited state consists of a complex between the achiral III* and the enzyme luciferase. Such a chiral excited state complex should, in principle, be capable of emitting circularly

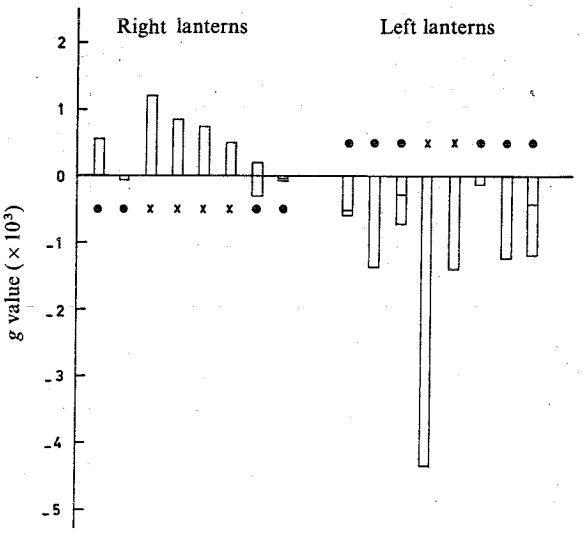

Fig. 2 Results of measurements of circular polarization of bioluminescence of firefly larvae lanterns with sufficiently high emission intensity. The height of each bar represents the g value for an individual lantern of *Photuris lucicrescens* (X) or *Photuris versicolor* (●). Superimposed bars refer to the effect of orientational changes (see text). The circular polarization is measured at the peak of the emission band at a wavelength of 540 nm using a spectral bandwidth of 40 nm. The r.m.s. noise level corresponds with $g \leq 5 \times 10^{-4}$.

polarized light[3,4]. A second explanation would invoke a circularly dichroic medium capable of partially absorbing the emitted light. Neither of these two modes can explain why the left and right lanterns emit polarized light of opposite sense, unless enantiomeric enzymes, active sites or membranes are proposed. In the absence of any evidence for enantiomeric structures, we are forced to conclude that the CPBL of opposite sense must, at least in part, be based on a macroscopic phenomenon rather than on molecular chirality.

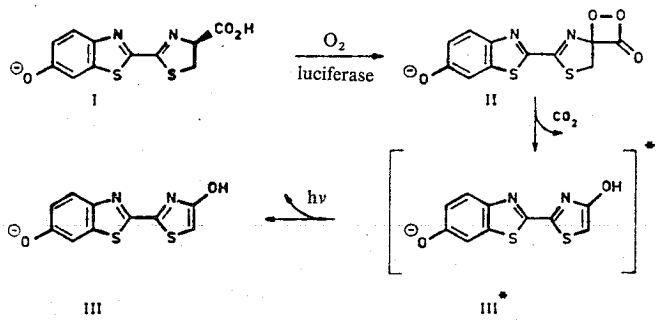

Fig. 3 Schematic mechanism of luciferin/luciferase bioluminescence. Oxygenation of luciferin (I) yields the intermediate II which in turn releases CO_2, resulting in the production of III (or, by H^+-abstraction, the enol dianion) in an electronically excited state. This state emits a quantum of light as it goes to its electronic ground state.

We propose the following hypothesis. The light from a bioluminescent emitter may be (partially) plane polarized due to anisotropy of an absorbing medium or, more likely, by inhomogeneous formation of excited states due to local molecular organization within a photocyte[14]. This plane polarized light will become elliptically polarized when it passes through a linearly birefringent medium. Orientated biopolymers can serve as such a medium. On a macroscopic scale the larvae, like many living organisms, have a symmetry plane dividing the lanterns. In this sense the lanterns are enantiomeric, even though their constituent molecules are of the same chirality; they may be viewed as macroscopic meso-structures. It is reasonable to assume that this macroscopic mirror image relationship holds on the level of the membrane structure and orientation of the emitters. This will result in circularly polarized light of opposite sense. The emitters from one lantern emit partially plane polarized light making angle $+X°$ with the fast axis of its linear birefringent medium. The result is ellipticity of one sense $(+Y)$. The mirror image relationship of the emitters in the other lantern results in partially plane polarized light with angle $-X°$ with respect to the fast axis of its linear birefringent medium. This must result in ellipticity of opposite sense $(-Y)$. We may expect, then, that the meso-orientated lanterns in any bioluminescent organism will emit circularly polarized light of opposite handedness, provided the chirality on the molecular level does not exceed the effect. The results described above suggest experiments dealing with the linear polarization of firefly larvae bioluminescence. Such experiments are planned.

Received 29 February; accepted 13 June 1980.

1. Dekkers, H. P. J. M. & Closs, L. E. *J. Am. chem. Soc.* **98**, 2210–2219 (1976).
2. Emeis, C. A. & Oosterhoff, L. J. *Chem. Phys. Lett.* **1**, 129–132 (1967).
3. Wynberg, H. & Numan, H. *J. Am. chem. Soc.* **99**, 603–605 (1977).
4. Wynberg, H., Numan, H. & Dekkers, H. P. J. M. *J. Am. chem. Soc.* **99**, 3870–3871 (1977).
5. McCapra, F. *Acct. chem. Res.* **9**, 201–208 (1976).
6. Badcock, J. R. & Merrett, N. R. *Nat. envir. Res. Coun. New. Jers.* **5**, 4 (1972).
7. Gafni, A. & Steinberg, I. Z. *Photochem. Photobiol.* **15**, 93–96 (1972).
8. Luk, C. L. & Richardson, F. S. *J. Am. chem. Soc.* **96**, 2006–2009 (1974).
9. Steinberg, I. Z. in *Biochemical Fluorescence* Vol. I (eds Chen, R. F. & Edelhoch, H.) 79–113 (Dekker, New York, 1976).
10. Koo, J.-Y., Smidt, S. P. & Schuster, G. B. *Proc. natn. Acad. Sci. U.S.A.* **75**, 30–33 (1978).
11. White, E. H., Midno, J. A. & Umbreit, M. *J. Am. chem. Soc.* **97**, 198–200 (1975).
12. McElroy, W. D. & Deluca, M. in *Bioluminescence in Action* (ed. Herring, P.) 109–127 (Academic, London, 1978).
13. McCapra, F. in *Bioluminescence in Action* (ed. Herring, P.) 49–73 (Academic, London, 1978).
14. Wampler, J. E. in *Bioluminescence in Action* (ed. Herring, P.) 9–48 (Academic, London, 1978).