Sequence and biochemical similarities between the luciferases of the glow-worm *Lampyris noctiluca* and the firefly *Photinus pyralis*

Graciela B. SALA-NEWBY,* Catherine M. THOMSON and Anthony K. CAMPBELL

Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

A full-length clone encoding *Lampyris noctiluca* (British glow-worm) luciferase was isolated from a complementary DNA (cDNA) expression library constructed with mRNA extracted from light organs. The luciferase was a 547-residue protein, as deduced from the nucleotide sequence. The protein was closely related to those of other lampyrid beetles, the similarity to *Photinus pyralis* luciferase being 84 % and to *Luciola* 67 %. In contrast, *Lampyris* luciferase had less sequence similarity to the luciferases of the click beetle *Pyrophorus*, at 48 %. Engineering *Lampyris* luciferase *in vitro* showed that the C-terminal peptide containing 12 amino acids in *Photinus* and 9 amino acids in *Lampyris* was essential for bioluminescence. The pH optimum and the K_m values for ATP and luciferin were similar for both *Photinus* and *Lampyris* luciferases, although the light emitted by the latter shifted towards the blue and was less stable at 37 °C. It was concluded that the molecular and biochemical properties were not sufficient to explain the glowing or flashing of the two beetles *Lampyris* and *Photinus*.

INTRODUCTION

Luciferases (EC 1.13.12.7) from luminous beetles generate light by catalysing the oxidative decarboxylation of a common luciferin, a benzothiazole, in the presence of ATP, Mg^{2+} and oxygen. The luciferase from the firefly *Photinus pyralis* has been extensively studied and it is used widely to measure ATP and a variety of metabolites [1]. Cloning of the complementary DNA (cDNA) coding for beetle luciferases has extended their use as indicators of the control of gene expression and covalent modification of proteins within live cells [2–4]. We have shown also that the C-terminus appears to provide a solvent cage necessary for high-quantum-yield chemiluminescence [5,6].

Luminous beetles belong to the superfamilies Elateroidea and Cantharoidea. The former comprises a single family, Elateridae, from which four luciferases have been cloned and sequenced from the click beetle *Pyrophorus plagiophthalamus* [7]. The Cantharoidea, in contrast, contains four luminous families: Homalisidae, Teleusidae, Phengodidae and Lampyridae. Four luciferases have been cloned and sequenced from the Lampyridae, showing more than 60 % sequence homology [8–11]. Two features characterize the light emission from the beetles: the colour and the flashing pattern. The colour of the light emitted from luminous beetles ranges from green ($\lambda_{max.} \approx 543$ nm) to red ($\lambda_{max.} \approx 620$ nm), and is determined by the active centre of the luciferase [12,13]. The luciferases exhibit similar spectra to the colour *in situ*. Each luminous beetle emits a distinctive flashing pattern, recognized by the opposite sex of the species [14]. The flash may last a few milliseconds, as with adult *Photinus*, or may be a glow lasting for several hours, as in the glow-worm *Lampyris* [15]. It is now almost 50 years since the requirement for ATP in beetle luciferase bioluminescence was discovered [16], yet there is still no definitive molecular explanation for the kinetics or colour of the light emitted by the beetles [1,17,18].

In Britain there are two luminous beetles, *Lampyris noctiluca* and the rare *Phosphaenus hemiptera* [1]. *Lampyris* can be found glowing throughout Britain, from May to August, although July is the favoured month. Green light ($\lambda_{max} \approx 550$ nm) is emitted by the glow-worm at all stages of the life cycle, including the eggs within a few days of laying. The larvae and male have only two photophores on what is essentially the last abdominal segment, which appear to glow only when the beetle is disturbed. In addition to these photophores, the sessile female has two large light organs in the fourth and fifth segments that respond to the hormone octopamine. These glow green for several hours to attract the male, which, like most other beetles, is able to fly ([15,17] and A. K. Campbell, unpublished work).

The aim of the work described in this paper was to sequence and characterize the luciferase from *Lampyris*, to determine whether the amino acid sequence or enzymic properties could account for any of the characteristics of the glow-worm's glow, in contrast with the flash of the firefly *Photinus*. Although firefly luciferase has been used as an intracellular indicator, it is susceptible to inhibition by several intracellular components as well as by its product. A further objective therefore was to determine whether *Lampyris* luciferase has properties advantageous to its use inside cells, and whether its green emission makes it more sensitive to detection by the blue-sensitive photocathodes normally employed to measure chemiluminescence [1].

EXPERIMENTAL

Materials

Glow-worms were collected at Cosmeston Country Park, Cardiff, during July and August. Oligonucleotide primers were prepared by using an Applied Biosystems 392 DNA synthesizer. The sequences of oligonucleotides 105 and 100 were previously described [5]. Oligonucleotides lox2, GCTTTGAGGTTGTA-

Abbreviations used: cDNA, complementary DNA; p.f.u., plaque-forming units.

* To whom correspondence should be addressed.

The nucleotide sequence shown in Figure 1 will appear in the EMBL, DDBJ and Genbank Nucleotide Sequence Databases under the accession number X89479.

GAAGTTCC (sense), and lox3, GGTGGCAGCAGCCAACTC (antisense), hybridize to the λ EXlox phage upstream and downstream of the inserted cDNA respectively, allowing the amplification of any cloned DNA. Other oligonucleotides were: GW1, TTTCGCGAGGGGAGCTCCAC (antisense), GW3, GAGTAAGCTCAACTCCC (antisense), GW12, CACCTAATACGACTCACTATAGGGAGAATGGAAGATGCA (includes a T7 promoter), and GW11, TTAGATCTCCCTGATTTTTCTTC (antisense; hybridizes to the 3′ end of the glow-worm cDNA to delete 27 bp). Reagents to isolate RNA, Ultraspec, and to construct and screen the cDNA library were from AMS Biotechnology, Witney, Oxon, U.K. Luciferin was from Boehringer. Other molecular biology reagents, Magicλ and the coupled transcription–translation system, TNT, were from Promega U.K. [γ-^{32}P]ATP (6000 Ci/mmol) and stabilized [^{35}S]methionine (1000 Ci/mmol) were purchased from Amersham International. All other A. R. grade reagents were from Sigma and Fisons. DNA purification kits were from Qiagen Ltd, nylon membranes from Pall Europe, Portsmouth, Dorset, U.K., and nitrocellulose was from Millipore.

General molecular biology methods

Plaque lifts, oligonucleotide end labelling and hybridization, plaque purifications and solutions used were performed as described in [19].

Bacterial strains

Escherichia coli strain ER1647 [tet^R, str^R F-, λ-, *trp*-31, *his*-1, *rsp*L104 (StrR), *fhu*A 2Δ(*lacz*)r1, *sup*E44, *xyl*-7, *mtl*-2, *met*B1, *rec*D 1014, *mcr*A 1272::Tn10, Δ(*mrc*B-*hsdRMS*-mrr-)2::Tn10] was used to plate the primary library, for titering and screening with oligonucleotide probes. *E. coli* strain BM25.8 [F′, traD36, lac *I* ^{q}*lacZ* ΔM15 *proAB*/*supE thi* Δ (*lac-proAB*)λ*imm*434(P1), Cm^R, kan^R] was used for automatic subcloning of pure plaques because it is lysogenic for phages 1 and P1 and therefore expresses the P1 *cre* recombinase, which excises the plasmid from phage at specific sites. *E. coli* strain BL21(DE3)pLysE [F$^-$, ompT, rB$^-$mB$^-$, (DE3)pLysE, CmR] is a lysogen that carries the T7 RNA polymerase gene under the control of the *lac*UV5 promoter. Infection with λ EXlox recombinants followed by IPTG induction enables the expression of cDNA-encoded polypeptides fused to the T7 gene 10 protein. *E. coli* strain HB101 was used to generate plasmids.

Generation and screening of the cDNA library

Total RNA was isolated from 16 tails and poly(A)$^+$ mRNA was eluted from an oligo(dT)–Sepharose column. A unidirectional cDNA library was constructed from mRNA (4 μg) in the λ EXlox phage [20], which includes, upstream of the cDNA insertion site, T7 transcription and translation signals and DNA encoding 260 amino acids of the gene 10 protein. To induce the expression of the fusion proteins, BL21[DE3]pLysE were infected with recombinant phage followed by induction and capture of the coded proteins on nitrocellulose according to the AMS Biotechnology protocol. Rabbit polyclonal antibodies raised against *Photinus* luciferase and goat anti-rabbit IgG alkaline phosphatase conjugate were used for immunodetection. λ phage was collected from purified plaques in SM buffer [19], DNA was extracted by boiling 10 μl of virus suspension with 40 μl of 20 mM Tris/HCl, pH 8.0, containing 2 mM EDTA and 1% (w/v) NP40. The size of the insert was determined by PCR performed on extracted phage DNA with primers lox2 and lox3. Plasmid was generated from plaque-purified phage by infection of *E. coli* BM258. Plasmids were used to transform *E. coli* HB101 and pure plasmid 16.1 was prepared by using Qiagen and sequenced allowing the design of the oligonucleotide GW1. λ DNA was prepared from *E. coli* ER1647 infected with 10^5 plaque-forming units (p.f.u.) using Magicλ according to the manufacturer's instructions. Purified λ phage DNA (1 μg) was amplified by using oligonucleotide lox2 and GW1 sense and antisense respectively (Figure 1). The 500 bp fragment obtained was partly sequenced by using GW1, and an oligonucleotide that hybridized to its 5′ end was designed (GW3; Figure 1). End-labelled GW3 was used to screen plaque lifts obtained by infection of *E. coli* ER1647 with 2×10^5 p.f.u.

Determination and analysis of the DNA sequences

Three plasmids, 62.10.1 (containing the complete sequence of the luciferase), 61.18.1 and 16.1 were sequenced. λ DNA amplified by using oligonucletides lox2, and GW1 was also sequenced. Double-stranded DNA sequencing was performed by the dye-dideoxy chain terminator method with the Applied Biosytems Model 373A DNA sequencer. The cDNAs were sequenced in both orientations by using a total of 13 oligonucleotides. DNA and protein analysis were performed with Genepro5. Protein alignment was carried out with the Clustal program.

Generation of luciferases *in vitro*

The cDNAs coding for glow-worm and firefly luciferase and variants were generated by PCR from plasmids 62.10.1 and A10.6.1 respectively, as described [5]. They were transcribed–translated in one step with the TNT system according to the manufacturer's instructions. The amount of protein produced was calculated by including [^{35}S]methionine in the transcription–translation mix [5]. The specific activities were determined in three independent experiments and the results are expressed as mean (range).

Assay of luciferase activity

Buffer (100 μl) containing 20 mM Tris/acetate, pH 7.75, 0.3 mM dithiothreitol, 0.2 mM EDTA, 1 mg/ml BSA, 12 mM magnesium acetate, 1 mM ATP and 0.2 mM luciferin was added to 4 μl of translation mixture and luciferase activity was measured in a home-built chemiluminometer [1] as chemiluminescent counts integrated for 10 s.

Characterization of the glow-worm luciferase

Luciferases were synthesized by using TNT, precipitated with 64% saturated ammonium sulphate and resuspended in 0.4 translation volumes of 20 mM Tris/acetate, pH 7.75, 0.3 mM dithiothreitol and 0.2 mM EDTA. The pH optimum curves were performed in quadruplicate in Tris/Mes buffers as previously described [21]. The discontinuous spectra of both luciferases were measured in the presence of 4 μM sodium pyrophosphate to stabilize light emission, at pH 6.5 and 8.0. The light-emitting solutions were dispensed in 96-well microtitre plates that were successively covered with narrow-band interference filters with maximum transmittance at 496, 519, 524, 555, 570, 582, 595, 612 and 629 nm. The light emitted was measured with a Photek bioluminescence imaging CCD camera (Photek Ltd., St. Leonards-on-Sea, Sussex, U.K.). Corrections for the decay in the light emitted by the enzymes were performed with uncovered wells. The spectra were corrected for the transmittance of the filters but not for the quantum efficiency of the camera. The K_m values, time courses of light emission and the temperature stabili-

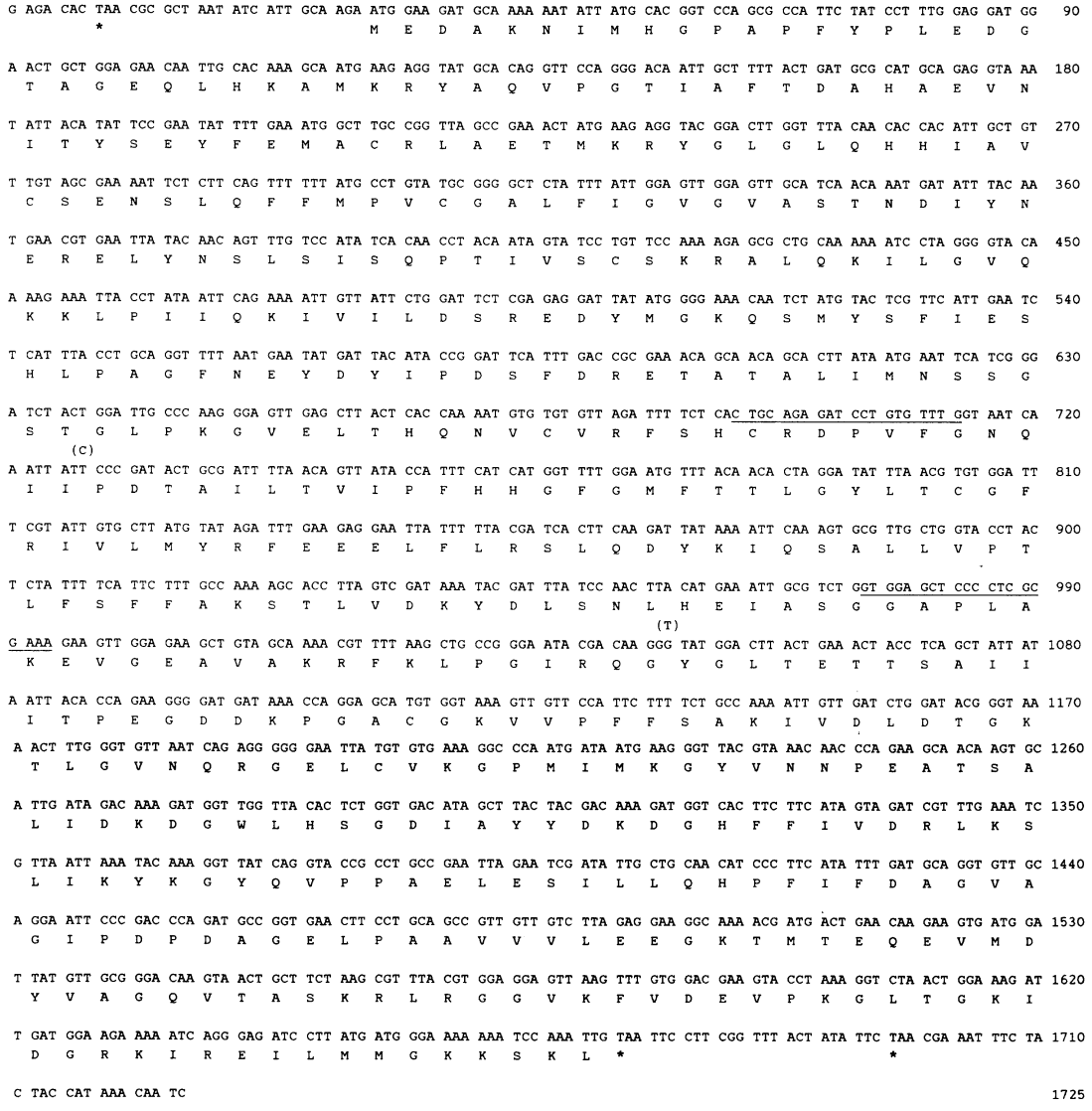

Figure 1 Glow-worm luciferase cDNA

Nucleotide and deduced amino acid sequences of the cDNA insert of clone 62.10.1. An asterisk indicates the presence of stop codons. Underlined nucleotides correspond to the sites where the oligonucleotides GW3 and GW1 hybridized. Bases between parentheses correspond to clone 61.18.1.

ties of the enzymes were measured in medium approaching the intracellular milieu, containing 100 mM potassium glutamate, 10 mM NaCl, 10 mM magnesium acetate, 1 mM potassium phosphate, 0.2 mM EDTA, 1 mg/ml BSA and 20 mM Tris/Mes buffer, pH 7.2. The counts corresponding to the chemiluminescent peak from quadruplicate measurements were plotted by using the Hanes–Woolf equation to calculate K_m values.

RESULTS

Isolation of a full-length clone of *Lampyris noctiluca* luciferase cDNA

A library of 1.1×10^6 p.f.u. was generated. Immunoscreening showed that 1% of the plaques reacted with the anti-firefly antibody. Among 91 positive plaques screened for size by PCR the one with the longest insert (800 bp) was converted to a plasmid. The insert in plasmid 16.1 was sequenced. The open reading frame coded for a protein with 80% sequence similarity to firefly luciferase. As the frequency of full-length clones was less than 1%, we obtained a probe closer to the 5′ end of the sequence by amplification of λ phage DNA followed by sequencing of the fragment. The oligonucleotide GW3 (Figure 1) was designed and used to screen plaque lifts obtained by infection of ER1647 with 2×10^5 p.f.u. Positive plaques were selected and screened for size of inserts by PCR; from 83, only 3 (61, 62 and 66) contained inserts longer than 1300 bp. Pure plaques were converted to plasmids, and clones 62.10.1 and 61.18.1 contained inserts of 1800 and 1400 bp respectively. Plaques from clones 62 and 61 produced immunodetectable protein, but the stain from clone 62 was very weak. BL21(DE3) transformed with clone 62.10.1 generated light in the presence of luciferin.

Nucleotide and protein sequence

The cDNA sequence from clone 62.10.1 showed that the only open reading frame coded for a protein of 547 residues (Figure

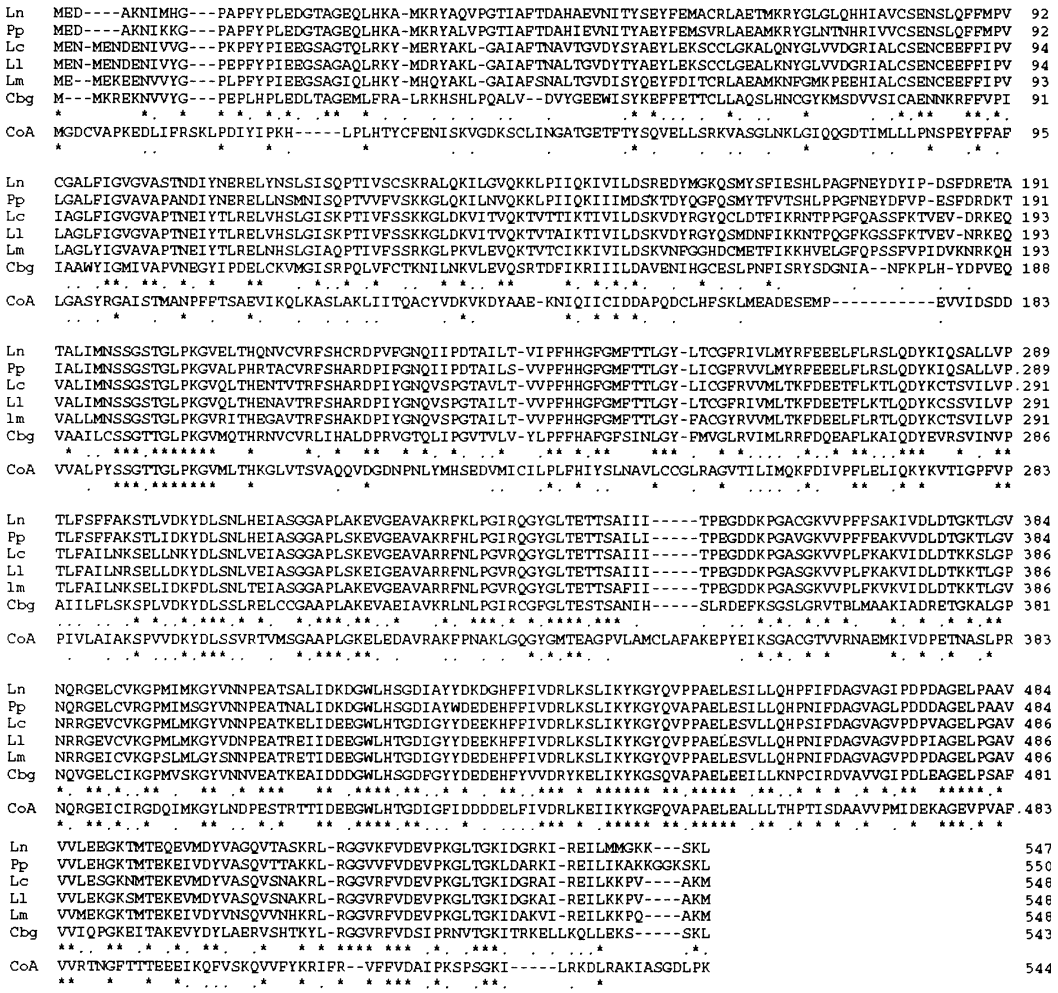

Figure 2 Alignment of the amino acid sequences of luciferases and an acyl-CoA ligase

The amino acid sequences of the luciferase from *Lampyris noctiluca* (Ln), *Photinus pyralis* (Pp), *Luciola cruciata* (Lc), *Luciola lateralis* (Ll), *Luciola mingrelica* (Lm) and *Pyrophorus plagiophthalamus*, green-emitting variety (Cbg), are compared. Also the sequence of the luciferases is compared with that of 4-coumarate-CoA ligase from parsley (CoA). Dashes indicate gaps introduced to aid alignment. An asterisk below a position indicates a fully conserved residue, whereas a dot signifies a strongly conserved position.

1), between one and three amino acids shorter than the other lampyrid luciferases that have been sequenced previously. The open reading frame contained a stop codon upstream of the start codon that might stop the generation of a fusion protein and hence hamper the selection of full-length clones based on immuno-screening or light screening. Clone 61.18.1 had two base pair differences from the full-length clone (Figure 1), but the base pair changes did not cause a change in the amino acid sequence.

The amino acid sequences of the beetle luciferases are highly homologous (Figure 2). The sequence of the glow-worm luciferase showed 84% similarity to the luciferase from *Photinus*, 65–68% to those of the three species of *Luciola* but was less similar to the luciferases from *Pyrophorus*, with only 48% homology. However, the amino acid compositions of all the luciferases when grouped by class are extremely similar. They all contain 31–32% of external amino acid (RNDQEHK), 35–38% of internal amino acid (ILMFV) and 35–38% ambivalent (ACGPSTWY).

Like beetle luciferases, several prokaryotic and eukaryotic enzymes convert MgATP to AMP by covalent binding to their carboxylate-containing substrate. Some of these enzymes are also acyl-CoA ligases. All these enzymes share a highly conserved region rich in G, S and T followed by a conserved K [22]. This region in the glow-worm luciferase falls between residues 195 and 206 (Figures 1 and 2). However, sequence similarity between the luciferases and the above category of enzymes is not limited to this motif, as shown by the alignment of the luciferases with the 4-coumarate-CoA ligase from parsley, *Petroselinum crispum* (Figure 2). Up to residue 194 overall sequence similarity between the luciferases is 22%, and that between the luciferases and CoA ligase 8%, whereas, from residue 195 to the C-terminus, the overall sequence similarities between the luciferases themselves and the CoA ligase are much higher, being 45% and 30% respectively.

Requirement of the C-terminus in glow-worm luciferase

Amplification of *Lampyris* and *Photinus* cDNA with oligonucleotides containing the T7 promoter produced cDNAs of the correct length (Figure 3A),, which when transcribed and translated *in vitro* generated proteins of approx. 61 kDa (Figure 3B). The specific activities of the two luciferases were 2.5×10^{19}

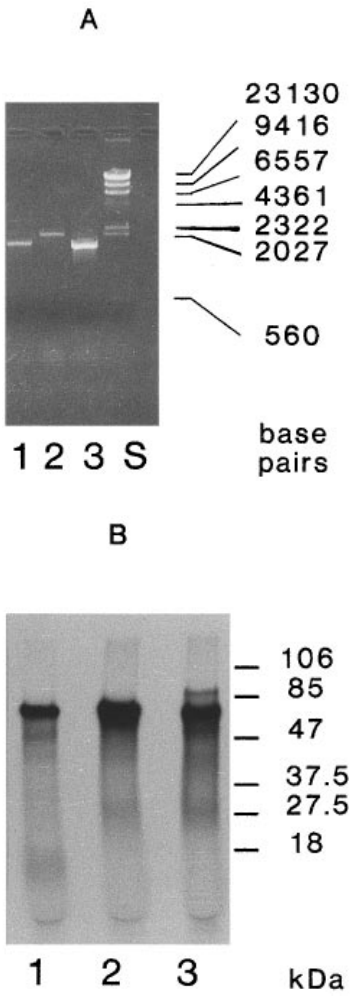

Figure 3 cDNAs and recombinant glow-worm and firefly proteins

(**A**) Agarose gel electrophoresis of the cDNAs coding for full-length *Photinus* (lane 1) and *Lampyris* (lane 2) luciferases and *Lampyris* luciferase lacking nine residues (lane 3). Size markers are shown in lane S. (**B**) The corresponding [^{35}S]methionine-labelled proteins synthesized by transcription–translation *in vitro* were separated by SDS/PAGE under reducing conditions.

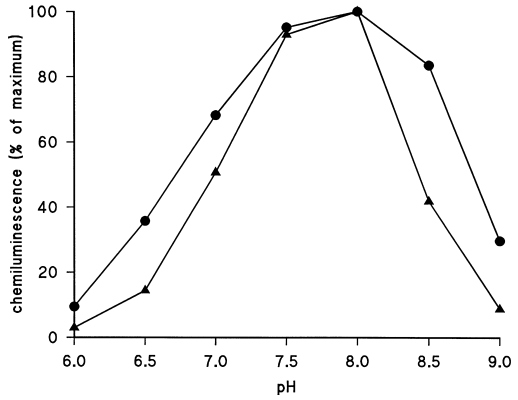

Figure 4 Effect of pH on glow-worm (▲) and firefly (■) luciferase activities

Chemiluminescent counts per 10 s were recorded and activity was expressed as a percentage of maximum. A representative experiment performed in quadruplicate is shown.

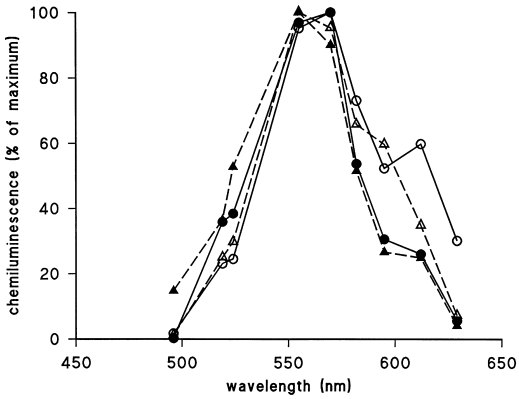

Figure 5 Discontinuous luminescence spectra

The light emitted by the glow-worm (△,▲) and firefly (○,●) luciferases at nine wavelengths was recorded at pH 6.5 (open symbols) and pH 8.0 (filled symbols) in quadruplicate. Results are expressed as a percentage of maximum.

$[(1.2–5) \times 10^{19}]$ and 4.5×10^{19} $[(1.7–10) \times 10^{19}]$ chemiluminescent counts per 10 s per mol of protein respectively. To investigate whether the C-terminus of *Lampyris* luciferase was required in a similar manner to that demonstrated for *Photinus* [5,6], a variant of the glow-worm luciferase lacking the last nine residues at the C-terminus was constructed (Figures 3A and 3B). The loss of catalytic activity of this 538-residue glow-worm variant to $1.2 \pm 0.4\%$ of the respective wild-type mimicked that of firefly luciferase lacking 12 residues, which was also 538 residues long [5].

Biochemical characterization of the glow-worm luciferase

To compare the glow-worm and firefly luciferases the following parameters were measured: pH optima, spectra, K_m values for ATP and luciferin, thermal stabilities and decay characteristics of the light emission.

Both luciferases had a pH optimum at approx. 8, with the glow-worm luciferase showing a somehow narrower pH optimum curve (Figure 4). The colour of the light emitted by the glow-worm was greener than that of the firefly. Although the spectra, measured discontinuously, looked similar, there was a reproducible shift of the glow-worm luciferase towards the green ($\lambda_{max.} \approx 555$ nm), compared with that of the firefly ($\lambda_{max.} \approx 570$ nm). A decrease in pH from 8 to 6.5 resulted in a broadening of the spectrum towards the red without changing the $\lambda_{max.}$ (Figure 5).

The apparent K_m values for ATP and luciferin for the glow-worm and firefly luciferases were very similar, being 62.4 and 51.6 μM for ATP, and 3.9 and 5.3 μM for luciferin respectively. The time course of the light emitted by both luciferases was also measured in the presence or absence of pyrophosphate (Figure 6). Pyrophosphate, a product of the reaction, reverses product inhibition via the phosphorolysis reaction with luciferyl and oxyluciferyl AMP, liberating luciferase that can then continue to produce light [23]. The decay in the light emission was similar for both enzymes under the two conditions. However, firefly luciferase did produce a reproducibly faster decay, suggesting that it was slightly more sensitive to product inhibition.

The thermal stabilities of the luciferases were measured in 'intracellular medium'. The glow-worm luciferase appeared to

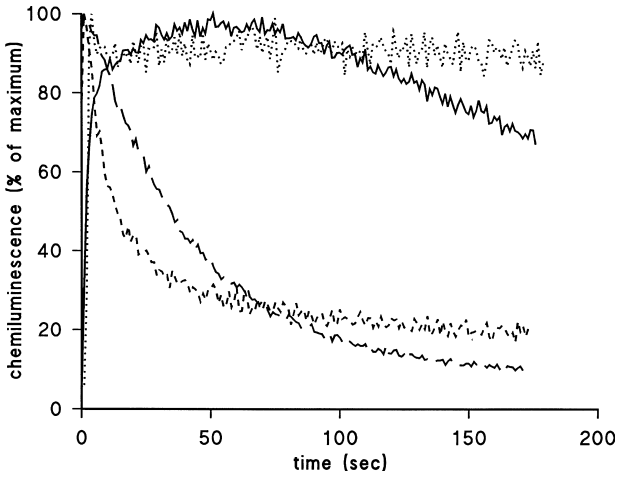

Figure 6 Time course of the light emitted by the luciferases

Luciferin at a final concentration of 0.2 mM was injected at time 0 to start the reaction and counts per second were recorded. A representative experiment is shown. The activity of the luciferases was the same in each case. The order of addition of the reagents did not seem to affect the kinetics of light emission [35]. Glow-worm luciferase in the absence (--- ---) and presence (——) of 4 μM pyrophosphate; firefly luciferase in the absence (– – –) and presence (· · ·) of 4 μM pyrophosphate.

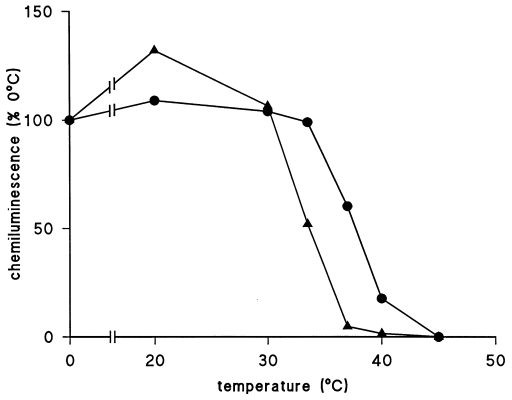

Figure 7 The thermal stability of the luciferases

The glow-worm (▲) and firefly (●) luciferases were incubated for 30 min at the indicated temperatures. The remaining activity was measured and expressed as a percentage of the activity of the enzyme stored at 0 °C. The means from three separate experiments conducted in triplicate are shown.

be less stable than that of the firefly, having lost 50% of its activity after incubation at 33.5 °C for 30 min whereas, under the same conditions, the firefly luciferase lost none (Figure 7). A small but reproducible increase in the activity of the luciferases was detected after incubation at 20 °C but the cause is unknown.

DISCUSSION

The results reported here show that the luciferase responsible for catalysing the light-emitting reaction in the glow-worm *Lampyris noctiluca*, although three residues shorter, has 84% sequence similarity with that of the firefly *Photinus pyralis*. *Lampyris* luciferase also has a C-terminus that showed the same key characteristics as that of *Photinus*. The terminal peptide, containing 12 residues in *Photinus* and 9 residues in *Lampyris* luciferase, was essential for bioluminescence. This result highlighted the importance of residue Leu^{539}, because the other amino acids removed are not conserved except for the peroxisomal targeting signal (Figure 2), which does not seem to be required for bioluminescence [6,24]. This is consistent with our hypothesis that the C-terminus forms the solvent cage necessary for high-quantum-yield chemiluminescence. There was also a Val at position 217, in a region that cannot be mutated without having a major deleterious effect on luciferase activity [21, 24a].

There seemed to be no significance in the sequence itself, the biochemical characteristics of specific activity, pH optimum and K_m, or the luminescence *in vitro*, in determining the kinetics of light emission of the beetles themselves. It is therefore most likely that the patterns of light emitted *in vivo* are controlled by the environment within the cell. Morphological evidence has suggested that photocytes are under neural control [25]. Addition of the neurotransmitter octopamine, present in firefly light organs, caused the glow-worm to glow for at least 6 h ([26] and A. K. Campbell, unpublished work). Octopamine stimulated adenylate cyclase in firefly-lantern homogenates, and cholera toxin, a G_s stimulator injected into live fireflies to activate lantern adenylate cyclase, caused the light organs to develop a sustained glow [26,27]. The key question is therefore how the chemiluminescent reaction is triggered and maintained *in vivo*. Oxygen availability can produce a flash with isolated luciferase. But although it was originally proposed as the trigger *in vivo*, the anatomy of the light organ and other experiments appear to have refuted this mechanism as a means of controlling flashing. It is also known that, *in vitro*, the presence of pyrophosphatase allowed the isolation of luciferase–oxyluciferin complexes containing AMP [28]. Because pyrophosphate can reverse product inhibition *in vitro*, it is possible that the concentration of this metabolite plays a role in neural control [23,25,29].

The glow-worm luciferase emitted light of $\lambda_{max.} \approx 555$ nm and the spectrum of the light emitted was broadened towards the red at pH 6.5. The activity and the colour of the light produced by several luciferases are sensitive to factors known to denature the enzymes partly. Low pH, increase in temperature, 0.2 M urea and divalent cations at millimolar concentrations produce a shift of the light-emission peak towards the red [1]. Two hypotheses have been proposed to explain the difference in the colour of the light emitted by the luciferases and the changes that different agents can cause. The first proposed that the dianion of oxyluciferin was responsible for the yellow–green emission and the monoanion emitted red light, with the active centre of the enzymes providing the different environments to account for the spectra seen [1]. A similar cause of changes in spectra has been proposed for coelenterazine bioluminescence [30]. Recently a second explanation proposed that the key determinant of colour is the topology of the two rings of luciferin, in particular the two extreme conformations where the rings are coplanar or at an angle of up to 90° to each other. Each different enzyme determines this angle by multiple interactions [18]. The amino acids responsible for the different colours of the light emitted by the various luciferases have been difficult to pinpoint. Sets of amino acids capable of producing a change in the spectrum have been found for *Pyrophorus* luciferases [31]. Mutant luciferases of *Luciola cruciata* ($\lambda_{max.}$ 562 nm) emitting light of $\lambda_{max.}$ between 558 and 612 nm were generated by single amino acid substitutions that did not cause a change in the predicted secondary structure [32]. We generated the firefly luciferase mutant V217R that emitted greener light than the wild-type [21]. Amino acids responsible for spectral changes are present between positions corresponding to residues 217–431 of the glow-worm luciferase. However, five of those amino acids mapped to positions 217–250,

making this region of low sequence similarity (Figure 2) a good candidate for luciferin binding.

Our results also show that no biochemical characteristics were identified that might make the *Lampyris* luciferase more suitable as an intracellular indicator for ATP than that of *Photinus*. In fact the specific activity determined for the recombinant glow-worm protein was $64 \pm 12\%$ of that of the firefly luciferase, in spite of the fact that its green emission might have been expected to make it more sensitively detectable by blue-sensitive photon-counting photomultipliers. However, the increased temperature sensitivity of the glow-worm luciferase might make it a slightly better reporter for the study of gene control elements for high-turnover mRNAs and unstable proteins in live cells [33].

The present report supports the hypothesis that the evolutionary precursor protein of beetle luciferases was an acyl-CoA ligase [34]. However, an intriguing finding was the high degree of homology between luciferases from two different genera. Despite this sequence similarity their bioluminescence signalling systems are the most different [14]. In their natural environment luminous beetles emit light only at dusk, although addition of octopamine will cause them to luminesce during the day ([26] and A. K. Campbell, unpublished work]. The intracellular signal responsible for mixing the components of the bioluminescent reaction seems to be cyclic AMP. Continued elevation of cyclic AMP results in a glow [27]. This is unlikely to be caused by an effect on luciferase itself because neither firefly nor glow-worm luciferase contains a protein kinase A recognition site [21]. The challenge now is to explain how cyclic AMP initiates the light reaction and how the light is switched off. A further interesting evolutionary question is how two beetles with very similar luciferases could have evolved such different communication signals.

We thank A. Trimby for technical assistance and J. Hoy for running the automatic sequencer, and Dr. J. Kendall and Dr. M. Badminton for helpful discussions. This work was funded by the Arthritis and Rheumatism Council (programme grant No. C0091).

REFERENCES

1 Campbell, A. K. (1988) Chemiluminescence: Principles and Applications in Biology and Medicine, Ellis Horwood, Chichester
2 Campbell, A. K. and Sala-Newby, G. (1993) in Fluorescent and Luminescent Probes for Biological Activity (Mason, W. T., ed.), pp. 58–82, Academic Press, London
3 Sala-Newby, G. B. and Campbell, A. K. (1992) FEBS Lett. **307**, 241–244
4 White, M. R. H., Maduko, M., Amet, L., Elliot, G., Braddock, M., Kingsman, A. J. and Kingsman, S. M. (1995) J. Cell Sci. **108**, 441–455
5 Sala-Newby, G., Kalsheker, N. and Campbell, A. K. (1990) Biochem. Biophys. Res. Commun. **172**, 477–482
6 Sala-Newby, G. B. and Campbell, A. K. (1994) Biochim. Biophys. Acta **1206**, 155–160
7 Wood, K. V., Lam, Y. A., Seliger, H. H. and McElroy, W. D. (1989) Science **244**, 700–702
8 De Wet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R. and Subramani, S. (1987) Mol. Cell. Biol. **7**, 725–737
9 Tatsumi, H., Masuda, T., Kajiyama, N. and Nakano, E. (1989) J. Biolumin. Chemilumin. **3**, 75–78
10 Tatsumi, H., Kajiyama, N. and Nakano, E. (1992) Biochim. Biophys. Acta **1131**, 161–165
11 Devine, J. H., Kutuzova, G. D., Green, V. A., Ugarova, N. N. and Baldwin, T. O. (1993) Biochim. Biophys. Acta **1173**, 121–132
12 Selinger, H. H. and McElroy, W. D. (1964) Proc. Natl. Acad. Sci. U.S.A. **52**, 73–81
13 Viviani, V. R. and Bechara, E. J. H. (1993) Photochem. Photobiol. **58**, 615–622
14 Lloyd, J. E. in Bioluminescence in Action (Herring, P. J., ed.), pp. 241–272, Academic Press, London
15 Newport, G. (1857) J. Proc. Linn. Soc. **1**, 40–71
16 McElroy, W. D. (1947) Proc. Natl. Acad. Sci. U.S.A. **33**, 342–345
17 Ramdas, L. A. and Venkiteshwaran, L. P. (1931) Nature (London) **128**, 726–727
18 McCapra, F., Gilfoyle, D. J., Young, D. W., Church, N. J. and Spencer, P. (1994) in Bioluminescence and Chemiluminescence (Campbell, A. K., Kricka, L. J. and Stanley, P. E., eds.), pp. 387–391, John Wiley, Chichester
19 Sambrook, J., Fritsch, E. F. and Maniatis, T. (eds.) (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
20 Palazzolo, M. J., Hamilton, B. A., Ding, D., Martin, C. H., Mead, D. A., Mierendorf, R. C., Raghavan, K. V., Meyerowitz, E. M. and Lipshitz, H. D. (1990) Gene **88**, 25–36
21 Sala-Newby, G. B. and Campbell, A. K. (1991) Biochem. J. **279**, 727–732
22 Babbit, P. C., Kenyon, G. L., Martin, B. R., Charest, H., Slyvestre, M., Scholten, J. D., Chang, K.-H., Liang, P.-H. and Dunaway-Mariano, D. (1992) Biochemistry **31**, 5594–5604
23 Rhodes, W. C. and McElroy, W. D. (1958) J. Biol.Chem. **233**, 1528–1537
24 Gould, S. J., Keller, G. A., Hosken, N., Wilkinson, J. and Subramani, S. A. (1989) J. Cell Biol. **108**, 1657–1664
24a Waud, J. P., Sala-Newby, G. B., Matthews, S. B. and Campbell, A. C. (1996) Biochim. Biophys. Acta, in the press
25 Buck, J. (1978) in Bioluminescence in Action (Herring, P. J., ed.), pp. 419–460, Academic Press, London
26 Nathanson, J. A. (1979) Science **203**, 65–67
27 Nathanson, J. A. (1985) J. Cycl. Nucleot. Prot. Phosphoryl. Res. **10**, 157–166
28 Gates, B. and DeLuca, M. (1975) Arch. Biochem. Biophys. **169**, 616–621
29 Hastings, J. W. and Buck, J. (1956) Biol. Bull. **111**, 101–113
30 Shimomura, O. (1995) Biochem. J. **306**, 537–543
31 Wood, K. V., Lam, Y. A., McElroy, W. D. and Selinger, H. H. (1989) J. Biolumin. Chemilumin. **4**, 31–39
32 Kajiyama, N. and Nakano, E. (1991) Protein Eng. **4**, 691–693
33 Thompson, J. F., Hayes, L. S. and Lloyd, D. B. (1991) Gene **103**, 171–177
34 Wood, K. V. (1991) in Bioluminescence and Chemiluminescence: Current Status (Kricka, L. J. and Stanley, P. E., eds.), pp. 11–14, John Wiley and Sons, Chichester
35 DeLuca, M. and McElroy, W. D. (1974) Biochemistry **13**, 921–925

Received 10 July 1995/7 September 1995; accepted 13 September 1995