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Expression and DNA Sequence of the Gene Coding for the *lux*-Specific Fatty Acyl-CoA Reductase from *Photobacterium phosphoreum*

Chan Yong Lee* and Edward A. Meighen[1]

**Eulji University School of Medicine, Taejon 301-112, Korea*
[1]Department of Biochemistry, McGill University, Montreal, Canada H3G-1Y6

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The nucleotide sequence of the *luxC* gene coding for *lux*-specific fatty acyl-CoA reductase and the upstream DNA (325 bp) of the structural gene from bioluminescent bacterium, *Photobacterium phosphoreum*, has been determined. An open reading frame extending for more than 20 codons in 325 bp DNA upstream of *luxC* was not present in both directions. The *lux* gene can be translated into a polypeptide of 54 kDa and the amino acid sequences of *lux* specific reductases of *P. phosphoreum* shares 80, 65, 58, and 62% identity with those of the *Photobacterium leiognathi*, *Vibrio fischeri*, *Vibrio harveyi*, and *Xehnorhabdus luminescens* reductases, respectively. Analyses of codon usage, showing that a high frequency (2.3%) of the isoleucine codon, AUA, in the *luxC* gene compared to that found in *Escherichia coli* genes (0.2%) and its absence in the *lux*A and B genes, suggested that the AUA codon may play a modulator role in the expression of *lux* gene in *E. coli*. The structural genes (*luxC*, *D*, *A*, *B*, *E*) of the *P. phosphoreum* coding for luciferase (α, β) and fatty acid reductase (r, s, t) polypeptides can be expressed exclusively in *E. coli* under the T7 phage RNA polymerase/promoter system and identification of the [^{35}S]methionine labelled polypeptide products. The degree of expression of *lux* genes in this system, high level of *luxA*, *B* genes whereas low level of *luxC*, *D* genes, were consistent with the analyses of codon usage. High expression of the *luxC* gene could only be accomplished in a mutant *E. coli* 43R. Even in crude extracts, the acylated acyl-CoA reductase intermediate as well as acyl-CoA reductase activities could be readily detected.

Key words: Bioluminescence, *lux*, *Photobacterium*, acyl-CoA reductase

Bioluminescent bacteria are the most abundant and widespread of the luminescent organisms found in marine, freshwater, and terrestrial habitats. Their primary habitat is in the ocean forming symbiotic, saprophytic, and parasitic relationships as well as in a free-living mode (11, 21). The luminescent species that have been extensively investigated are the marine bacteria; *Vibrio harveyi*, *Photobacterium fischeri* (formally known as *Vibrio fischeri* (12)), *Photobacterium phosphoreum* and *Photobacterium leiognathi*, and the terrestrial bacterium, *Photorhabdus luminescens* (formally known as *Xenohabdus luminescens* (3)). Other luminescent bacteria that have been found, but less studied include some strains of freshwater pathogens *Vibrio cholera*, the aerobic species, *Shewanella hanedai*, and the obligate symbionts of flashlights fishes, deep-sea angler fishes, and the pyrosomes (10).

P. phosphoreum is a luminescent marine bacterium which has been found as a symbiont in certain osseous fish including Morid, Macrourid, and Opisthoprocid (9). Although luciferase has been purified from several strains, *P. phosphoreum* (NCMB844) is the only strain from which the enzyme required for synthesis of aldehyde substrate has also been purified (16). The light-emitting reaction in bioluminescent marine bacteria is catalyzed by a mixed function oxidase, luciferase, a heterodimeric protein consisting of α and β subunits with approximate molecular sizes of 43 and 38 kDa, respectively. As substrates the reaction requires a long chain aldehyde (RCHO), reduced FMN, and molecular oxygen that are closely related to the basic metabolites of the cell (16, 17). The products of the luciferase catalyzed reaction are a long chain fatty acid (RCOOH), oxidized flavin, water, and light.

$$RCHO + FMNH_2 + O_2 \rightarrow RCOOH + FMN + H_2O + light$$

The aldehyde substrate for the luminescence reaction is supplied by fatty acid reductase, a multienzyme complex in *P. phosphoreum* consisting of three different

* To whom correspondence should be addressed.
(Tel) 82-42-259-1698; (Fax) 82-42-259-1047
(E-mail) cylee@emc.eulji.ac.kr

polypeptide subunits; acyl-protein synthetase (50 kDa), acyl-CoA reductase (58 kDa), and acyl-transferase (34 kDa) (16, 17).

The luminescent systems in marine bacteria require genes (*luxA* and *B*) coding for luciferase as well as genes (*luxC, D, E*) coding for fatty acid reductase enzymes for light emission (17). Although luciferase and fatty acid reductase genes are common *lux* genes in luminescent bacteria, new *lux* genes are continually being discovered in specific luminescent bacteria, adding to their complexity and diversity. In the marine *Photobacterium* and *Vibrio* genera, the *luxE* gene is immediately followed by the *luxG* gene, which codes for a protein related in sequence to enzymes involved in electron transport and flavin reduction (1). In some *Photobacterium* species, a gene (*luxF*) related in sequence to the luciferase gene is present between *luxB* and *luxE*. The *luxF* gene codes for nonfluorescent flavoprotein of unknown function containing a flavin adduct covalently linked with tetradecanoic acid (20). Interestingly, only *Photobacterium* strains with *luxF* have another gene, *luxL*, located approximately 600 bp upstream from the start of the *lux* operon transcribed in the opposite direction (22). *Lux*L is a lumazine protein that binds lumazine and/or riboflavin and modulates the spectrum and efficiency of light emission in these species (22).

In this study, we report the nucleotide sequence of the *luxC* gene from *P. phosphoreum* as well as the DNA extending upstream of the *lux* structural genes. The polypeptide products coded by the major *lux* genes (*luxC, D, A, B, E*) are identified by specific expression of *P. phosphoreum* DNA fragments in the T7-phage RNA polymerase/promoter system in *E. coli* and high expression of the *luxC* gene is obtained in mutant *E. coli*.

Materials and Methods

Materials

Acrylamide, N,N-methylene bisacrylamide, ATP, NADPH, FMN, β-mercaptoethanol, reagents for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), molecular weight standards, ampicillin, kanamycin, and rifampicin were all obtained from Sigma Co. [^{3}H]Tetradecanoic acid (30 Ci/mmol) was prepared by New England Nuclear (NEN) by reduction of cis-9-tetradecanoic acid with tritium gas in the presence of 5% palladium on a carbon catalyst. [^{3}H]Tetradecanoyl-CoA (1 Ci/mmol) was prepared from the radioactive fatty acid as previously described (25). [^{35}S]methionine (800 Ci/mmol) was also purchased from NEN. [^{35}S]deoxyadenosine 5'-(α-thio) triphosphate (>1,200 Ci/mmol) was purchased from DuPont. All restriction endonucleases, calf intestinal alkaline phosphatase, and T4 DNA ligase were obtained from Boehringer Mannheim.

Bacteria and plasmids

The bacterial strains used in these studies were; *Photobacterium phosphoreum* NCMB 844, *Escherichia coli* RR1 and *E. coli* K38-1 (31). For transformation, *E. coli* cells were grown in LB medium and treated with calcium as previously described (28). The plasmids, pT7-3, pT7-4, and pT7-5, were used as cloning vectors. The pT7-plasmids and helper plasmid pGP1-2 were generous gifts from S. Tabor and C.C. Richardson (31).

Cell growth and lysis

P. phosphoreum cells were grown in 3% complex media (23) at 19°C. *E. coli* RR1 harbouring one of the plasmids, PpEE.pT7-5, PpSB.pT7-4, PpSG.pT7-3, and PpSE.pT7-4, were grown at 19°C in LB broth containing 100 μg/ml ampicillin. The increase in OD_{660} and *in vivo* luminescence (LU/ml) were followed with growth. Light intensity was measured with a photomultiplier tube, where one light unit (LU) equals 1×10^{10} quanta/sec, based on the standard of Hastings and Weber (8). When the pT7-plasmids containing the *lux* DNA were used to produce high levels of the *lux* proteins for analyses of their functions by acylation with fatty acid or fatty acyl-CoA, *E. coli* cells harbouring the helper plasmid pGP1-2 and the desired pT7-plasmid were grown in LB-broth at 30°C until an OD_{660} of 0.3 was reached. The expression system was then induced by incubation at 42°C for 25 min and grown for an additional 3~4 h at 19°C before using the cells for *in vitro* acylation or extraction of proteins.

For *in vitro* analysis, cells were isolated by centrifugation (15,000 × g, 15 min) and frozen -20°C. Cells were thawed and suspended in lysis buffer [1 mM phosphate (pH 7.0), 0.2 M NaCl, 10% glycerol, 0.25 mM dithiothreitol (DTT)] to give a constant concentration of cells (OD_{660} × volume [ml]=20). The cell suspension was sonicated (four times, 20 sec each), centrifuged to remove cellular debris, and the supernatant had a final concentration of 50 mM in phosphate buffer (pH 7.0).

S1 nuclease protection assay and Southern transfer

S1 nuclease protection assay was performed by procedures described previously (30) except that 100 μg of RNA was used for the hybridizations. An *Eco*RI 4.3 kbp double-stranded DNA fragment (PpEEpT7-5 in Fig. 1) was coprecipitated with *P. phosphoreum* mRNA, and the pellet was resuspended in hybridization buffer (80% formamide, 40% PIPES, pH 6.6, 0.4 M NaCl, 1 mM EDTA), heated to 75°C for 10 min, then slowly cooled to 50°C for 12~16 h. The hybrid mixture was then diluted to a final volume of 0.3 ml with 30 μl of 10 × S1 nuclease buffer (0.3 M NaOAc, pH 4.6, 1 M NaCl, 10 mM $Zn(OAc)_2$, 50% glycerol) and H_2O. S1 nuclease reactions were carried out for 30 min at 37°C with the indicated amount of enzymes. Reaction products were phenol extracted, and 2 μl of 6 × alkaline load buffer (300 mM NaOH, 6 mM

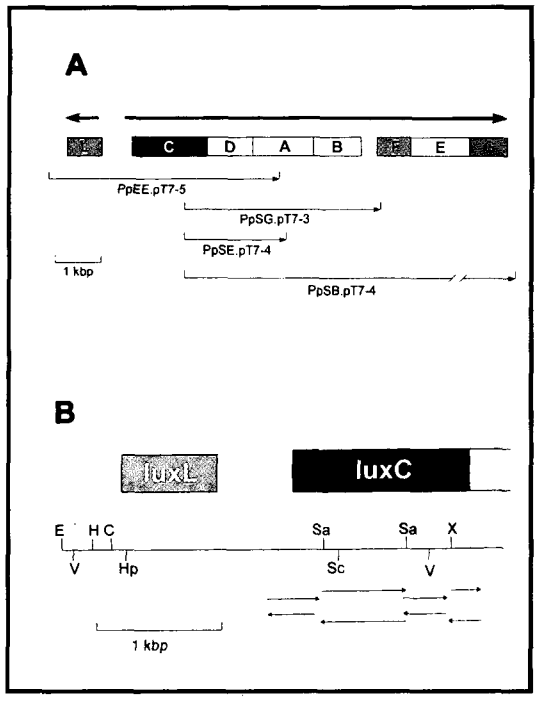

Fig. 1. Physical map of the *lux* operon of *Photobacterium phosphoreum* (A) and cloning strategy for sequencing of *Photobacterium phosphoreum luxC* DNA (B). The locations of restriction fragment of *P. phosphoreum lux* DNA (PpEE, PpSG, PpSE, and PpSB) inserted into pT7 for expression in *Escherichia coli* are indicated in A. Direction of transcription is indicated by dark arrows (A). The arrows below the restriction map (B) represent the direction and extent of DNA sequences read from each clone. B, *Bam*H1; C, *Cla*I; E, *Eco*RI; G, *Bgl*II; H, *Hind*III; Hp, *Hpa*I; Sa, *Sal*I, Sc, *Sac*I; V, *Eco*RV; X, *Xba*I.

EDTA, 18% Ficoll (type 400, Pharmacia), 0.25% bromophenol blue) were added. The samples were applied onto a 1% agarose gel, and run at 25 V for 4 h in running buffer (50 mM NaOH-1 mM EDTA). The gel was soaked to TBE solution containing ethdium bromide after neutralization in a solution of 1 M Tris (pH 7.6)-1.5 M NaCl with stirring, and then transferred in 10 × SSC (0.15 M sodium citrate pH 7.5, 1.5 M NaCl) by blotting to Hybond-Nylon at room temperature for 16 hours. Hybridizations were performed with *P. phosphoreum Sal*I-*Bam*HI DNA fragment labelled [γ-^{32}P]CTP (0.1 pmol, 1.2 × 10^6 cpm/pmol) at 65°C overnight.

Expression of P. phosphoreum proteins using the bacteriophage T7 RNA polymerase/promoter system

Expression of the *P. phosphoreum* proteins was carried out essentially as described for expression of the T7 gene (31). *E. coli* RR1 or K38-1 cells harbouring both the helper plasmid pGP1-2 and the pT7-plasmids containing the DNA of interest located after the T7 promoter were grown at 30°C in LB broth containing 100 μg/ml ampicillin and 50 μg/ml of kanamycin until an OD_{660} of 0.3 was reached. The cells were then harvested by centrifugation and the cell pellet washed twice with M9 minimal media before resuspension at an OD_{660} of 0.25 in M9 minimal media plus all amino acids except methionine. The cells were then incubated at 30°C for 1 h before inducing the expression of the T7 RNA polymerase by incubation at 42°C for 15 min. Rifampicin (200 μg/ml) was then added to inhibit *E. coli* RNA polymerase and incubation continued at 42°C for an additional 10 min. The cells were then placed at 30°C for 20 min before 10 μCi of [^{35}S]methionine was added to 1 ml of cells. After 12 min, the cells were isolated by centrifugation (13,000 × g, 1 min) and 80 μl of SDS sample buffer (62.5 mM Tris pH 6.8, 12.5% glycerol (v/v), 1.25% SDS, 0.18 M β-mercaptoethanol, 1 mM EDTA) was added to the pellet. After boiling the sample for 2 min, the protein was resolved by SDS-PAGE on a 12% gel and [^{35}S]methionine labelled polypeptides identified by fluorography.

In vitro acylation

Cell extracts (50 μl), prepared as described above, were added to a total volume of 100 μl 50 mM phosphate (pH 7.0) containing 22 μM [^{3}H]tetradecanoyl-CoA (1 Ci/mmol), and 0.25 mM DTT. After 2 min, the reaction was stopped by mixing with an equal volume of (1:1) SDS-PAGE sample buffer (lacking β-mercaptoethanol) and 5 mM N-ethylmaleimide, boiled for 5 min and applied on a 12% polyacrylamide gel.

Fatty acyl-CoA reductase activity

Acyl-CoA reductase activity was measured in buffer A (50 mM phosphate pH 7, 0.25 mM dithiothreitol, 10 mM $MgSO_4$, and 10% glycerol) using a luciferase coupled assay. Extracts, mixed with 4 μg of *P. phosphoreum* luciferase, were incubated for 4 min with 5 μM myristoyl-CoA and 0.1 mM NADPH for acyl-CoA reductase activity before measurement of the aldehyde product by the intensity (in LU) of the light on injection of $FMNH_2$.

SDS-PAGE and fluorography

SDS-PAGE was performed using the system of Laemmli (13) with 12% polyacryl-amide resolving gel and 5% stacking gel. Gels were stained with Coomassie Brillant Blue R, destained, treated with Enhance, dried and exposed to Kodak X-Omat AR film.

Sequencing

DNA containing *luxC* and upstream regions of *luxC* was subcloned into the sequencing vector M13mp18 and M13mp19. Sequencing reactions were performed by the method of the dideoxy chain termination method (27) using kits containing Klenow DNA polymerase or T7 DNA polymerase. All sequences were read in both directions using overlapping DNA fragments. Analysis of the data was performed using the program of DNASIS provided by Hitachi Software Engineering Co., Ltd.

Nucleotide sequence accession number

The nucleotide sequence in this paper has been deposited with the EMBL/GenBank Data Libraries under accession number L21989.

Results and Discussion

A 4.8 kb *Eco*RI fragment from *P. phosphoreum* containing the *luxCD* genes as well as an upstream region of the *luxC* gene (PpEE) was cloned (Fig. 1A) (15). The strategy for the analysis of the nucleotide sequence of the *luxC* gene and the upstream DNA is outlined in Fig. 1B. Subfragments of the DNA were cloned into M13 and the sequence of both strands of DNA determined.

The nucleotide sequence of the *luxC* gene is given in Fig. 2. An open reading frame extends 1,434 nucleotides downstream from the first ATG codon and codes for a polypeptide of 478 amino acids with a molecular mass of 54 kDa in excellent agreement with the molecular weight of the reductase polypeptide, predicted as 58 kDa from SDS gel electrophoresis (32), required for synthesis of the aldehyde substrate for the bioluminescent reaction. An analysis of amino acid sequences of *lux* specific reductases demonstrated that the sequence of *P. phosphoreum* reductase shares 80, 65, 58, and 62% amino acid identity with those of the *P. leiognathi*, *V. fischeri*, *V. harveyi*, and *X. luminescens* reductases, respectively (Fig. 3 and Table 1). The ribosome recognition site (Shine-Dalgarno sequence), AGGAGA, can easily be identified 10 bp upstream from the initiation codon of *luxC*.

The open reading frames encoding any protein greater than 3 kDa in molecular mass within 325 base pairs

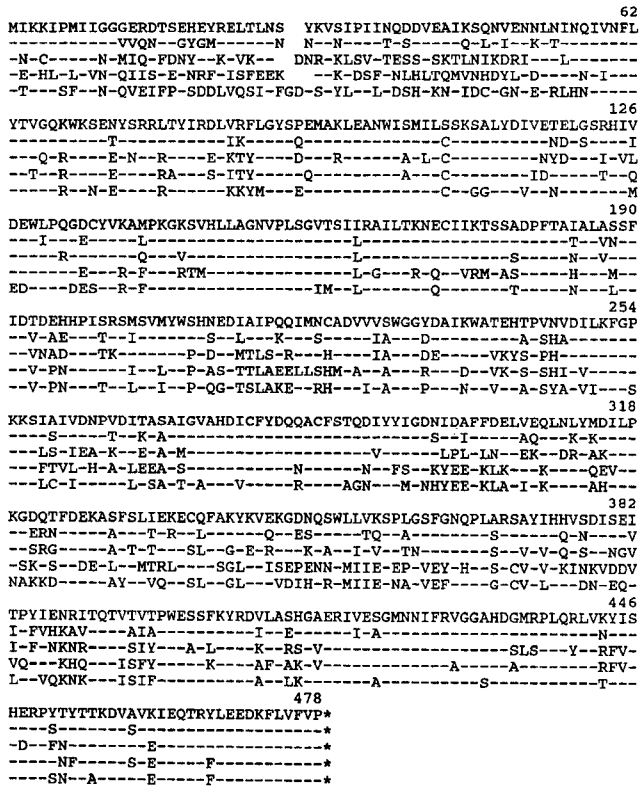

Fig. 3. Alignment of the amino acid sequence of fatty-acyl CoA reductase from various species of bioluminescent bacteria. lane 1, *P. phosphoreum* (this study); lane 2, *P. leiognathi* (14); lane 3, *V. fischeri* (2); lane 4, *V. harveyi* (19); lane 5, *X. luminescens* (18). Amino acids identical with the *P. phosphoreum* protein are indicated as bars (–).

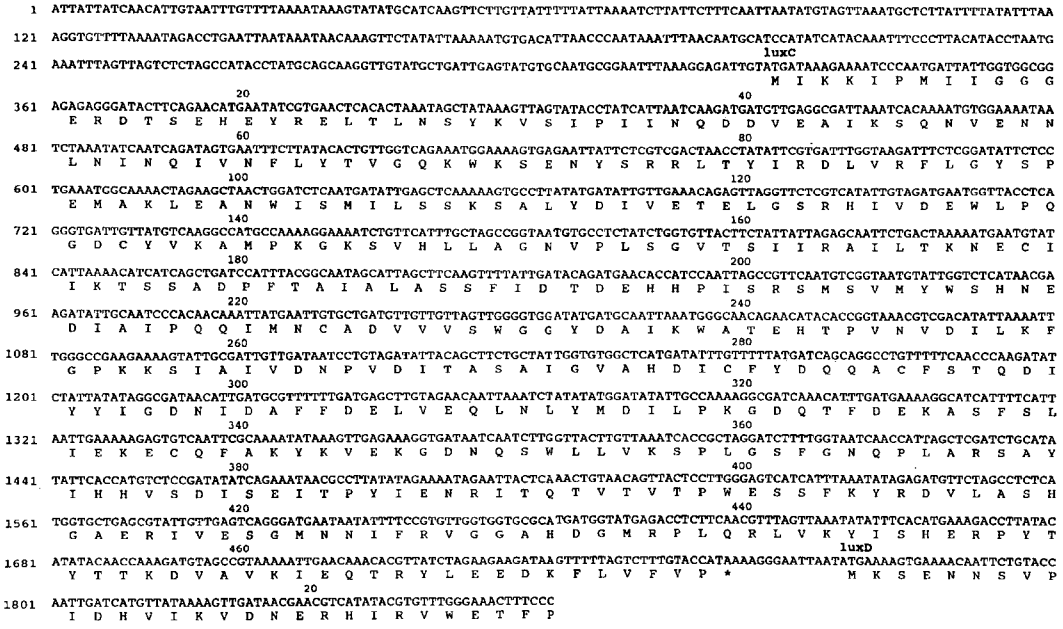

Fig. 2. Nucleotide and deduced amino acid sequences of the *luxC* gene of *Photobacterium phosphoreum*.

Table 1. Amino acid identities (%) between the acyl-CoA reductase from different species of bioluminescent bacteria. Pl, *P. leiognathi*; Pp, *P. phosphoreum*; Vf, *V. fischeri*; Vh, *V. harveyi*; Xl, *X. luminescens*

Pl	Vf	Vh	Xl	
80	65	58	62	Pp
	66	57	63	Pl
		57	58	Vf
			61	Vh

upstream from the start site of *luxC* gene were absent in both directions. In contrast to the *P. leiognathi lux* system (14), which shows that open reading frames greater than 35 codons starting with an ATG initiation codon could not be detected in either orientation extending for 774 bp upstream of *luxC* gene, an open reading frame (*luxL*) coding for the lumazine protein of 204 amino acid polypeptides transcribed in the opposite direction to the *lux* gene is located 657 bp upstream of the *luxC* gene in *P. phosphoreum* (Fig. 1A, C.Y. Lee, unpublished sequence data).

It is likely that this intergenic region contains the regulatory elements for the *lux* operon (17, 19). The GC content of the specific intergenic region decreases to less than half of that for the *lux* coding region (Fig. 4). Especially, a cross region extending 100 to 400 bps upstream of the *luxC* gene itself, the average GC content drops significantly. In this 300 bp span of DNA, the average GC content is only 18% raising the possibility that this DNA may be involved in a specific and/or regulatory role. The low GC content in the DNA surrounding the 5'-terminus of *luxC* may provide a clue in understanding the regulation of *P. phosphoreum*. Immediately downstream, separated only by 12 bp, is the sequence of *luxD*, which has been previously reported (5).

In order to elucidate the 5'-end of the *lux* mRNA, total mRNA was isolated from *P. phosphoreum*, hybridized to the DNA (PpEE) encompassing the 5' region of the *luxC* gene, and treated with S1 nuclease. As shown in Fig. 5 lane 2, the partially labelled DNA fragment of 2.6 kbp was generated by the protection of 5' mRNA for the *lux* operon. The result supported that the mRNA of *P. phosphoreum* for the transcription of the *lux* operon was started in front of the *luxC* region according to the size of the DNA band.

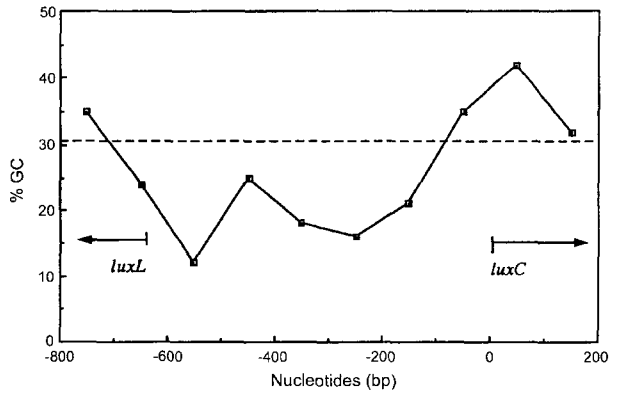

Fig. 4. Plot of the G/C content of the intergenic region between the *lux*C and *lux*L. The % GC content averaged over 100 nucleotides is given. The dotted line indicates the average % GC content for *lux*C and *lux*L (31%).

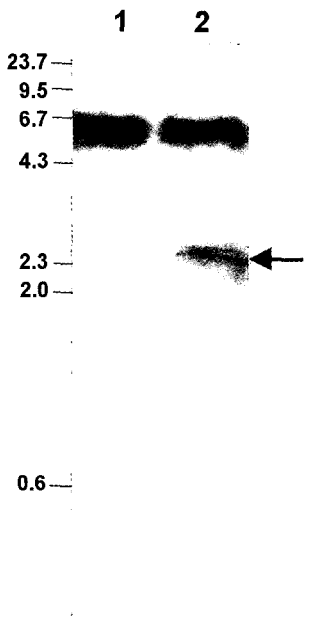

Fig. 5. S1 Nuclease protection assay of *P. phosphoreum*. The single stranded DNA containing the 5' region of the structural gene from the *lux* operon including *lux*CDE (PpEE) were tested for RNA protection by S1 nuclease. lane 1, PpEE hybridizes with tRNA; lane 2, PpEE hybridized with *P. phosphoreum* mRNA. The position of each DNA fragment DNA of λHindIII DNA markers (23.7 kbp, 9.5 kbp, 6.7 kbp, 4.3 kbp, 2.3 kbp, 2.0 kbp, and 0.6 kbp) is shown on the left.

Analysis of the codon usage of *luxC* reveals a major difference observed for the other *lux* genes (*A*, *B*) as well as that found in *E. coli*. Within the *luxC* gene, a high level of codons were found in which the cognate tRNA are minor species in *E. coli* (7). These codons are generally absent or present at very low levels in strongly expressed genes in *E. coli* and have been proposed to limit the rate of translation of weakly expressed genes. Particularly strong coordinates for this putative role as modulator codons are the isoleucine codon, AUA, and the arginine codons, CGG, AGA, and AGG (7). Other minor codons could also limit the rate of translation. In *E. coli*, the AUA codon is utilized at a level of less than 0.3% varying from an average of 0.04% in strongly expressed genes to 0.5% in weakly expressed genes (7). The codon AUA for Ile is used in a total eleven times out of 47 Ile codons accounting for 2.3 % of the total number of codons in *luxC* of *P. phosphoreum*. In contrast, *luxA* and *B* do not contain any AUA codons (6) while this codon only appears at 0.2 %

Table 2. Frequency of use of Ile codon, in the *lux* genes from *P. phosphoreum and E. coli.* The sequence data were obtained from the following: *luxC* (this study), *luxD* (5), *luxA* and *B* (6), *luxE* (29), and *E. coli* (7)

Gene	AUA codons	total Ile codons	% AUA of total Ile	total[a] codons	% AUA of total codons
luxC	11	47	23.4	479	2.3
luxD	8	27	29.6	307	2.6
luxA	0	19	0.0	358	0.0
luxB	0	20	0.0	329	0.0
luxE	5	34	14.7	374	1.3
E. coli			2.8		0.2

[a]Includes stop codon

frequency in *E. coli* (7) proteins (Table 2).

The bacteriophage T7 RNA polymerase/promoter system was used for expression of the *P. phosphoreum lux* genes. The pT7-*P. phosphoreum* DNA constructs utilized are depicted in Fig. 1A. The 10.5 kbp *Sal*I-*Bam*HI DNA fragment (PpSB) was inserted into the polylinker region of pT7-4. Expression of this recombinant plasmid in *E. coli* cells (containing the pGP1-2 plasmid which expresses T7 RNA polymerase) and analysis of ^{35}S-labelled proteins by SDS-PAGE and fluorography are shown in Fig. 6, lane 1. Five major polypeptides with molecular masses of 50, 43, 38, 34,

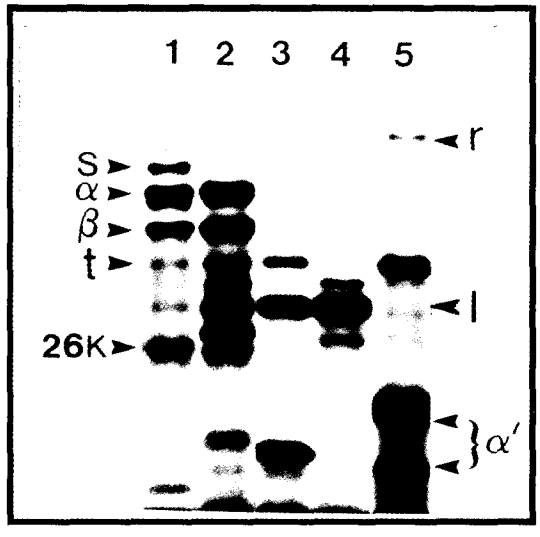

Fig. 6. Expression of pT7-*P. phosphoreum* recombinant plasmids. A fluorograph of [^{35}S]methionine-labelled proteins separated by SDS-PAGE is presented. Expression of the following recombinant plasmids in *E. coli* cells is depicted: lane 1, PpSB. pT7-4; lane 2, PpSG.pT7-3; lane 3, PpSE.pT7-4; lane 4, pT7-3; lane 5 PpEE.pT7-5. The positions of the migration of the purified *lux* structural polypeptides (r, s, t, α, β) from *P. phosphoreum* are indicated. Truncated α polypeptides are indicated as α'. The pT7-3 and pT7-4 plasmids contain the lactamase gene transcribed in the same direction as the T7 promoter, while pT7-5 have the β-lactamase gene in the opposite direction. Therefore, the β-lactamase gene will also be expressed under T7 promoter in plasmids pT7-3 and pT7-4. β-lactamase polypeptide is denoted by l.

and a doublet at 26 kDa were synthesized. The 43- and 38-kDa polypeptides migrated at the same position as α and β subunits of luciferase coded by the *lux*A and *lux*B genes, respectively. The 50- and 34-kDa polypeptides have the same mobility as synthetase(s) and transferase (t) polypeptides of the fatty acid reductase complex which are encoded by the *luxE* and *luxD* genes, respectively. The polypeptides at 26 kDa correspond to the molecular mass of the *luxF* and *luxG* gene products.

Expression of a 4.3-kbp *Sal*I-*Bgl*II fragment combined with the pT7-3 plasmid (PpSG.pT7-3) is presented in Fig. 6, lane 2. The gene products coded by *luxD*, *luxA*, and *luxB* (t, α, β) are still expressed, but the *luxE* gene product (s) is missing. PpSE.pT7-4 expresses the transferase polypeptide (t) coded by *luxD* gene. In addition the lower molecular weight polypeptide of 19 kDa (α') obtained upon expression of PpSE. pT7-4 in lane 3 corresponds to a truncated α subunit. The bands, marked by l, between the 26-kDa doublet and 34-kDa transferase primarily arise from expression of the β-lactamase gene (Fig. 6, lanes 2, 3, and 4). The largest polypeptide expressed is the β-lactamase precursor, the highly labeled middle band is the processed enzyme, and the lower band is due to a product from the pT7 plasmid transcribed in the same direction as the β-lactamase gene (31).

A polypeptide corresponding to the 58-kDa reductase (r) can be detected in the recombinant plasmid PpEE.pT7-5 (Fig. 6, lane 5). As shown in Fig. 6, the *luxA* and *luxB* genes are expressed very well and their products α and β subunits are highly labelled in SDS-PAGE, whereas *luxC* and *luxD* gene are expressed at low levels. These results are consistent with the analyses of codon usage of AUA for Ile in Table 2. The frequency of AUA codon in *luxC* and *luxD* genes are much greater than that found in other *lux* genes particularly the highly expressed luciferase genes. Although the utilization of codons may be very different in the two bacteria, the absence of the AUA codon in the two other *lux* genes with complete nucleotide sequences, *luxA* and *luxB*, might suggest that in the *P. phosphoreum luxC* and *D* genes, the AUA codon may play a modulator role. The *luxA* and *B* genes are known to be strongly expressed in *P. phosphoreum* with the luciferase enzyme accounting for up to 20% of the total soluble protein as well (32).

Synthesis of the fatty aldehyde substrate for the bioluminescence reaction is catalyzed by a *lux* specific fatty acid reductase complex coded by the *luxCDE* genes. The transferase (LuxD) diverts tetradecanoic acid from lipid biosynthesis to the bioluminescent pathway (4). The initial step in conversion of tetradecanoic acid to fatty aldehyde involves activation of fatty acid with ATP by synthetase (LuxE) to produce an enzyme-bound fatty acyl-AMP intermediate (26). A cysteinyl residue on the synthetase subunit then reacts with the activated acyl group to form an acylated synthetase with release of

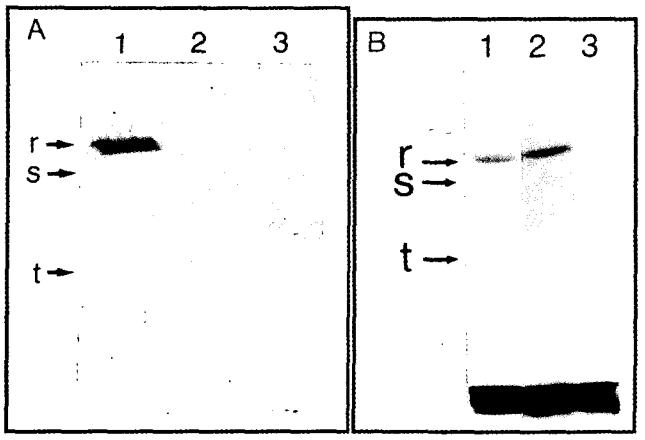

Fig. 7. Labeling of extracts with [^{3}H]tetradecanoyl-CoA of *P. phosphoreum* and *E. coli* containing pT7-*P. phosphoreum* plasmid using the T7 RNA polymerase system (A) and in the mutant *E. coli* 43R system (B). Lane 1, *P. phosphoreum* native strain grown to OD_{660}=3.6 (1,800 LU/ml); lane 2, *E. coli* K38-1[PpEE.pT7-5] (A) and *E. coli* 43R [PpEE.PT7-5] (B) grown to OD_{660}=3.0; lane 3, *E. coli* K38-1 [pT7-5] (A) and *E. coli* 43R[pT7-5] (b) grown to OD_{660}=3.0. All lanes contain 50 μg of protein.

AMP (33). Finally, the fatty acyl group is transferred onto the reductase subunit (LuxC) forming a covalent acylated intermediate before being reduced to aldehyde with NADPH (33). The reductase can accept and receive acyl groups from fatty acyl-CoA as well as the acyl-synthetase intermediate (24). This function originally provided the basis for designating this enzyme as an acyl-CoA reductase.

Identification of the reductase subunit in the T7 system was accomplished by acylation with the radioactive acyl-CoA *in vitro*. Fig. 7A, lanes 1-3 show the acylation of proteins with [^{3}H]tetradecanoyl-CoA of *P. phosphoreum* and *E. coli* K38-1 with recombinant plasmid PpEE. pT7-5. The acylated polypeptides of reductase and transferase with [^{3}H]tetradecanoyl-CoA from the extracts of *E. coli* containing PpEE.pT7-5 were very weakly labelled by the T7 RNA polymerase system in Fig. 7A, lane 2. However, transformation of an *E. coli* mutant 43R gave high level of expression which was strong enough to determine not only the identification of the acyl-CoA reductase peptide easily on the SDS-PAGE (Fig. 7B), but also to detect acyl-CoA reductase activities which could readily be measured in the crude extract even in the absence of a compatible plasmid containing the gene for the T7 RNA polymerase. This result is similar to that observed on expression of the *V. harveyi lux* system (19), indicating that a repressor or inhibitor may have been eliminated in the mutant *E. coli*. The high expression of the *luxC* gene coding for the *lux* specific acyl-CoA reductase from *P. phosphoreum*, provides the basis for future studies requiring expression of mutants of the enzyme.

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