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STRUCTURE AND PROPERTIES OF LUCIFERASE

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FROM PHOTOBACTERIUM PHOSPHOREUM

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SUMMARY: The nucleotide sequences of the Lux and Lux and Lux and Lux and Lux genes coding for the alpha and beta subunits, respectively, of luciferase from Photobacterium phosphoreum have been determined. The predicted amino acid sequences of the alpha and beta subunits were shown to be significantly different from other bacterial luciferases with 62 to 88% identity with the alpha subunits and 47 to 71% identity with the beta subunits of other species. Expression of the different luciferases appear to correlate with the number of modulator codons. Kinetic properties of P. phosphoreum luciferase were shown to reflect the bacterium's natural cold temperature habitat.

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In luminescent bacteria, the light emitting reaction is catalyzed by luciferase, a heterodimer made up of an alpha and beta subunit (1,2). The enzyme catalyzes the oxidation of long chain aldehydes and FMNH2 in the presence of molecular oxygen to produce the corresponding fatty acid. The aldehyde substrate for the luciferase reaction is supplied by fatty acid reductase, a luxspecific multienzyme complex made up of acyl-protein synthetase, acyl-CoA reductase and acyltransferase subunits. Long chain fatty acids are activated by the synthetase in an ATP-dependent reaction and are then reduced by the reductase in an NADPH-dependent reaction to produce the aldehyde substrate. The transferase is responsible for diverting the fatty acid from the fatty acid biosynthesis pathway to the luminescent system. The P. phosphoreum lux system has been cloned and shown to have a lux gene organization of luxCDABFE (3), luxCDE coding for the fatty acid luciferase and <u>lux</u>F coding for a flavoprotein. Only the sequences of luxF (4) and luxE (5) have been reported.

At present, the only enzyme purified from any biological system and capable of reducing fatty acids to aldehydes is the

fatty acid reductase complex from Photobacterium phosphoreum (2). As the aldehyde product may be channelled directly from the complex to luciferase and the fatty acid may be recycled from luciferase back to the fatty acid reductase complex, determination of the structure and properties of luciferase from this species is of particular importance since these enzymes may interact. Moreover, the primary structure of the P. phosphoreum luciferase may have diverged significantly from that of other bacterial luciferases since this bacterial species is naturally found in a deep water marine habitat (6).

MATERIALS AND METHODS

The nucleotide sequence of P. phosphoreum DNA containing the <u>luxA</u> and <u>luxB</u> genes was determined in both directions by the dideoxy chain termination method (7) using the modified T7 DNA polymerase provided in the Sequenase DNA sequencing kit (United States Biochemical Corp.). Restriction enzymes, obtained from Bethesda Research Laboratories, were used to create subfragments which were inserted into the M13 sequencing vectors mpl8 and mpl9 Remaining segments were sequenced by using specific oligonuclectides synthesized at McGill University. Analysis of the sequencing data was performed using the DNASIS and PROSIS programs provided by Hitachi Software Engeneering Co., Ltd.

P. phosphoreum luciferase was purified as previously described In vitro luciferase activity was measured by injecting 1.0 ml of 50 μ M FMNH₂ into 1.0 ml 50 mM Na/K phosphate (pH 7.0), 0.2% bovine serum albumin, 0.01% dodecanal containing luciferase (10). The enzyme activity was measured as maximum initial light intensity (I_o), where one light unit (LU) = 5.5 x 10⁹ quanta/s and the first order rate constant for luminescence decay, kL, was determined.

RESULTS AND DISCUSSION

Nucleotide and Amino Acid Sequences. The nucleotide sequence of a DNA fragment containing the P. phosphoreum luxA and luxB genes was determined and is given in figure 1 along with the predicted amino acid sequences of the alpha and beta luciferase The nucleotide sequence in figure 1 extends from the stop codon of luxD, proceeds through luxA and luxB and terminates 12 codons into luxF. The luxD, luxA, luxB and luxF genes are separated by 38, 49 and 25 nucleotides, respectively. sequence consists of an open reading frame of 1071 nucleotides in length coding for the 40.5 kDa luciferase alpha subunit (357 amino acids) with the <u>lux</u>B sequence containing 984 nucleotides in an open reading frame coding for the 37.5 kDa luciferase beta subunit (328 amino acids).

Comparison of the amino acid sequence of the alpha subunit from P. phosphoreum with those from the other luminescent bacteria,

TAA TTACAACTGATACATAAACCAACAAAAGGAATATT ATG AAG TTT GGA AAT ATT TTC TCA TAT CAG CCT CCA GGT GAG TCA CAT AAA GAA GTC ATG GAT CGC TTT 110 GTT CGT TTA GGC GTT GCA TCA GAA GAA CTA AAT TTT GAT ACT TAC TGG CTA GAG CAT CAT TTT ACT GAA TTT GGA CTA ACA GGT AAC CTG TTT GTT GCT 212 V R L G V A 8 E E L N F D T Y W L E H H F T E F G L T G N L F V A TOT GOT AAC TTA CTT GOT CGA ACC AAA CTG AAT GTT GGT ACT ATG ATT GTT CTT CCA ACA GCT CAC CCT GCA CGT CAG ATG GAA GAT TTA TTA CTT 314 TTM GAT CAA ATG TCA AAA GGC CGT TTT AAT TTT GGT GTT GTG CGT GGC TAC CAC AAA GAT TTC CGC GTT TTT GGT GTA ACG ATG GAA GAT TCT CGT GCT 416 ATC ACT GAA GAT TTT CAC ACC ATG ATT ATG GAT GGT ACA AAA ACA GGT CTT CAT ACT GAT GGT AAA AAC ATC GAA TTC CCA GAT GTA AAC GTT TAC CCA 518

I T E D F H T M I M D G T K T G L H T D G K N I E P P D V N V V P GAG GCG TAT TTA GAG AAA ATT CCA ACA TGC ATG ACT GCT GAA TCA GCA ACA ACG ACT TGG CTT GCT GAG CGT GGC TTA CCC ATG GTT CTP AGT TGG ATT 620 E A Y L E K I P T C M T A E S A T T T W L A E R G L P M V L S W I ATT ACA ACG AGT GAA AAG AAA GCT CAA ATG GAA CTC TAT AAT GCT GTT CGA GAT AGC GGT TAC AGT GAA GAG TAC ATT AAA AAC GTT GAT CAC AGT ATG 722 ACC CTC ATC TGT TCT GTA GAT GAA GAT GGC AAA AAA GCT GAA GAT GTG CGT GAG TTT TTA GGT AAT TGG TAT GAT TCA TAC GTA AAT GCA ACC AAT ATC B24
T L I C S V D E D G K K A E D V R E F L G N W Y D S Y V N A T N I TIT AGT GAA AGT AAC CAA ACT CGT GGT TAT GAT TAT CAT AAA GGT CAA AAA GAT TIT GIT GIT CAI GGA CAT ACT AAT ACT AAA CGT CGT GIT GAT TAT 926
F S E S N O T R G Y D Y H K G O K D F V L Q G H T N T K R R V D Y AGC AAC GAT CTA AAC CCT GTA GGT ACA CCT GAA AAA TGT ATT GAA ATT CAG CGT GAT ATT GAT GCA ACA GGT ATT ACT AAT ATT ACC CTT GGT TTC GAA 1028 S N D L N P V G T P E K C I E 1 Q R D I D A T G I T N I T L G F E GCA AAT GGC TCT GAG GAA ATC ATT GCC TCT ATG AAA CGC TTC ATG CAA GTT GCA CCA TTC TTA AAA GAT CCA AAA TAA ATAAATCACTCAGATTAACTTTA 1135 A N G S E E E I I A S M K R F M Q V A P F L K D P K * ATAMATAMATAMAGGAMTATAMAC ANG AMT TITT GGA THA TITC TTC CTC AAC TITT CCT GAA AMT ACA TCG TCA GAA ACA GIT THA GAT AMT ATG ATC AMT ACT GTC 1242 TCT THA GIT GAT AAA GAT TAT AAA AAC TIT ACA ACT GCT THA GIC AAC CAC CAT TIT TCT AAA AAT GGT ATT GTC GGT GCT CCG ATG ACA GCT 1344 S L V D K D Y K N F T T A L V N H H F S K N G I V G A P M T A A S TTC CTA TTA GGA CTA ACT GAA CGT TTA CAT ATT GGT TCT TTA AAT CAA ATT ACA ACG CAT CCG GTT CGT ATT GCA GAA GAA GCA AGT TTG CTT GAT 1446
F L L G L T E R L H I G S L N Q I T T H H P V R I A E E A S L L D CAA ATG TCA GAC AGC CGC TTT ATT CTA GGT CTA AGT GAT TGT GTT AAT TTT GAG ATG GAT TTC TTT AAA CGC CAA CGT GAC TCA CAG CAG CTA CAA TTT 1548
Q M S D S R F I L G L S D C V N F E M D F F K R O R D S O O L O F GAA GCT TGC TAT GAC ATC ATT AAT GAA GCT ATC ACA ACT AAT TAC TGC GCT AAT AAT GAT TTT TAT AAC TTC CCT CGT ATC TCA ATT AAT CCT CAT TGC 1650 E A C Y D I I N E A I T T N Y C A N N D F Y N F P R I S I N P H C TTA AGT AAA GAG AAT ATG AAG CAA TAT ATT TTG GCT TCT AGT GTG AGT GTT GAG TGG GCT GCT AAA AAA GCG CTT CCA CTA ACA TAT CGT TGG AGC GAT 1752 L S K E N M K Q Y I L A S S V S V E W A A K K A L P L T Y R W S D ACC CIT GAA GAT AAA GAG ATT CIT TAT AAG CGT TAT TIA GAA GIT GCA AAG CAT AAT ATT GAC GIT TCT AAT GTC GAG CAT CAG TIC CCG CTG CTT GTA 1854
T L E D K E I L Y K R Y L E V A K H N I D V S N V E H Q F P L L V AAT TTA AAT CAT GAT CGT GAT GTT GCT CAT CAA GAA GCA ACG GCC TAT GTA AGT TAT ATT GCT GAA GTA TAC CCA CAT CTA AAT CAG CAA CAA AAA ATT 1956 N L N H D R D V A H Q E A T A Y V S Y I A E V Y P H L N O Q O K I GCT GAA CTT ATT AGC CAA CAT GCG ATT GGT ACT GAT AAT GAT TAC TAT TCA ACA TTA AAT GCG TTA GAG CGT ACA GGT TCA AAA AAT GTA TTA CTT TCT 2058
A E L I S Q H A I G T D N D Y Y S T L N A L E R T G S K N V L L S ATT ATG AAT AAA TGG AAT TAC GGA GTC TTC TTC GTT AAC 2203

Fig. 1. Nucleotide sequence and corresponding translated amino acid sequences of the <u>luxA</u> and <u>luxB</u> genes of <u>P. phosphoreum</u>. The nucleotides are numbered on the right while the amino acids are numbered below the residue.

Photobacterium leiognathi, Vibrio fischeri, Vibrio harveyi and Xenorhabdus luminescens shows that the sequence from P. phosphoreum is significantly different with 88%, 78%, 62% and 62% identity, respectively. Only 54% of the sequence is conserved in all 5 luciferases (Fig. 2). A comparison of the amino acid sequences of the beta subunits showed even greater differences with only 71%, 61%, 48% and 47% identity, respectively, with only 33% of the amino acids being conserved in the beta subunits of the 5 luciferases (Fig. 3). The most closely related luciferase is that of P. leiognathi; the only other species known to have the same lux gene organization as P. phosphoreum, with luxF located between luxB and luxE.

Only three gaps were introduced in the amino acid sequences to give the maximum number of identities between the luciferase

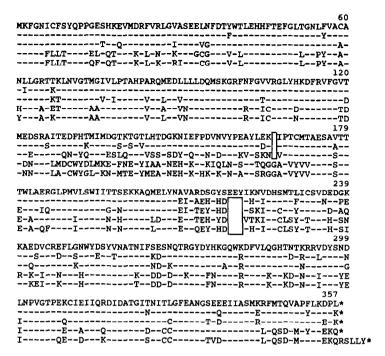


Fig. 2. Amino acid sequence comparison of the luciferase alpha subunits. From top to bottom, the sequences are given in the following order: P. phosphoreum, P. leiognathi (11), Y. fischeri (12), Y. harveyi (13) and X. luminescens (14). Amino acids identical to the corresponding residue in P. phosphoreum are indicated by bars. Gaps are indicated by the boxes.

subunits. The most striking gap occurs at position 210 in the alpha subunits of all species except P. phosphoreum. Since the luxA and luxB (16) genes arose by gene duplication, the alignment of the sequence of these proteins (Fig. 4) indicates that an insertion of nine bases (3 codons) occurred in the luxA gene of P. phosphoreum instead of a deletion in the other species as these 3 codons are missing in all luxB genes. An insertion of six nucleotides (two codons) appears to have occurred near the 5' end of the luxB gene in the Photobacterium species as well as V. fischeri as luxA is missing these two codons. In contrast, the gap located at position 167 of the P. phosphoreum, P. leiognathi and V. fischeri luxA gene appears to have resulted from a deletion as this gap is not present in luxB.

Codon Usage. Certain codons, which are found in very low levels in highly expressed genes in \underline{E} . \underline{coli} have been proposed to limit the rate of weakly expressed genes (17). Strong candidates for these modulator codons include the arginine codons, CGG, AGA and AGG and the isoleucine codon, AUA. The \underline{lux} system of \underline{v} .

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MNFGLFFLNFQPENTSSETVLDNMINTVSLVDKDYKNFTTALVNEHHFSKNGIVGAPMTA
-K------KDGIT--ET----VK--T-I-ST--H-N--F------I
-K-----INSTR--DQ-IEE-LD-HY--QI K-D-LA-Y-N--N--V---L-V
-K-----INST-VQ-QSIVR-QEITEY---I --EQI--Y-N--D-V---L-V
ASFLLGLTERLHIGSLNQVITTHHPVRIAEEASLLDQMSDSRFIIGLSDCVNDFEMDFFK
-G----M-KNAKVA---H------V----C----EG--AF-F--EKSAD-R--N
SG-----KIK----HI------A----C---L-EG---F--EKKD--H--N
RQRDSQQLQFEACYDIINEAITTNYCQANNDFYNFPRISINPHCLSKENMKQYILASSVS
-PVEY--QL--E--E--D-L--G--NPD----S--K--V---AYTPGGPRK-VT-T-HH
VVEWAAKKALPLTYRWSDTLEDKEILYKRYLEVAAKHNIDVSNVEHQFPLLVNLNHDRDV
-----G-----AE--NY-Q--T--EN-V-ITH-D-----I-P--I
--M-----FK-E-N-T--RYAIL-NKT-QQYGV-I-D-D--LTVIA--S-ST
----LG---VF--D-SNAQRKEYAGL-H---QA-GV---Q-R-KLT----Q-V-GEA
I-----GI--IFK-D-SNDVRYEYAE--KA--D-YDV-L-EID--LMI---Y-E-SNK
-K--TR-FISD-VL-MH-NE-FEN-LE-I-AEN-V-NYTECITAAKL-I-KC-A-S----
                      328
FESMKNHDDVVKVINMVNEKTOKNLPSS*
-----KAA-IDL------*
----ADFKG-KEI-D-L-Q--E----*
----EDKAQQRA--DV--AN-V-YHS*
--P-NDLMSQKN---I-DDN-K-YHMEYT*
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Fig. 3. Amino acid sequence comparison of the beta luciferase subunits. From top to bottom, the sequences are given in the following order: P. phosphoreum, P. leiognathi (11), V. fischeri (12), V. harveyi (15) and X. luminescens (14). Amino acids identical to the corresponding residue in P. phosphoreum are indicated by bars. A gap is indicated by the box.

```
MKFGNICFSYQPPGESHKEVMDRFVRLGVASEEL NFDTYWTLEHHFTEFGLTGNLFVA
-N--LFFLNF--ENT-SET-L-NMINTVSLVDKDYK--T-ALVN----SKN-IV-APMT-
CANLLGRTTKLNVGTMGIVLPTAHPARQMEDLLLLLQMSKGRFNFGVVRGLYHKDFRVFG
ASF---L-ER-HI-SLNQ-IT-H--V-IA-EAS--D---DS--IL-LSDCVNDFEMDF-K

VTMEDSRAITEDFHTMIMDGTKTGTLHTDGKNIEFPDVNVYPEAYLEK IPTCMTAESAV
RQRDSQQLQF-ACYDI-NEAIT-NYCQANNDFYN--RISIN-HCLSKENMKQYIL-S-VS

TTTWLAERGLPMVLSWIITTSEKKAQMELYNAVARDSGYSEEYIKNVDHSMTLICSVDED
VVE-A-KKA--LTYR-SD-LED-EILYKR-LE--AKHNID VS--E-QFP-LVNLNH-
GKKAEDVCREFLGNWYDSYVNATNIFSESNQTRGYDYHKGQWKDFVLQGHTNTKRVDYSN
RDV-HQEATAY-VSYIAEVYP HLNQQQKIAELI

DLNPVGTPEKCIEIIQRDIDATGITNITLGFEANGSEEEIIASMKRFMTQVAPFLKDPL*
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Fig. 4. Amino acid sequence comparison of the proteins encoded by $\underline{lux}A$ (line 1) and $\underline{lux}B$ (line 2) of \underline{P} . phosphoreum. Blank spaces have been introduced to maximize identities. Horizontal bars represent residues identical to the corresponding amino acid in $\underline{lux}A$ gene product. Three gaps, corresponding to those in figures 2 and 3 are indicated by boxes.

SOHAI--DNDYY-STLNALER--SK-VL-S--SMKNHDDVVKVINMVNEKIQKN-PSS*

Table I

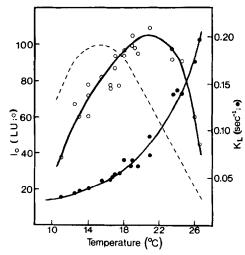
Modulator codon usage and nucleotide ratio in luxA (A) and luxB (B) of P. phosphoreum (PP), P. leiognathi (PL), V. harveyi (VH), Y. fischeri (VF) and X. luminescens (XL)

| CODON | PP | | \mathtt{PL} | | VH | | VF | | XL | |
|-------|-----|-----|---------------|-----|-----|-----|-----|-----|-----|-----|
| | A | В | A | В | A | В | A | В | A | В |
| AUA | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 2 | 6 | 8 |
| CGG | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 1 |
| AGA | 0 | 0 | 0 | 0 | 1 | 3 | 1 | 2 | 2 | 3 |
| AGG | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 |
| AT/GC | 1.6 | 1.9 | 1.3 | 1.7 | 1.3 | 1.2 | 1.7 | 2.0 | 1.7 | 1.7 |

harveyi was proposed to be regulated using a similar modulating system, since the highly expressed lux and lux genes do not have any AUA codons while the lux cand lux genes expressed to a much lower extent, have several AUA codons (18).

In P. phosphoreum, luxA contains none of the above modulator codons, while the <u>lux</u>B gene only contains one CGG codon (Table I). These codons are also very rare in P. leiognathi luxA and luxB, with only one CGG codon located in luxA. The V. harveyi, V. fischeri and X. luminescens luxA and luxB genes contain 5, 15 and 22 of these codons, respectively. The number of modulator codons appears to be independent of the nucleotide composition. of modulator codons correlates with the relative luminescence of the different species. P. phosphoreum has been shown to have luciferase levels that can compose up to 20% of the soluble protein (19), whereas Y. harveyi luciferase can constitute up to 5% of soluble protein (20,21) with <u>V. fischeri</u> containing less luciferase than <u>V. harveyi</u> (22). P. leiognathi has been found to contain levels of luciferase close to that of P. phosphoreum. In contrast, the level of luciferase in X. <u>luminescens</u> is very low (23). results suggest that the content of modulating codons may partially regulate translation in these bacterium.

Kinetic Properties. As the natural habitat of P. phosphoreum is the lower depths of the ocean at cold temperatures, the dependence of the kinetic properties of the P. phosphoreum luciferase on temperature was investigated. Figure 5 shows that although the turnover rate of the enzyme (k_L) , measured from the decay of luminescence, increases with temperature, the maximum light intensity (I_{\circ}) for the reaction occurs at 20 °C. Moreover,



 $\underline{\text{Fig. 5}}.$ Dependence of initial light intensity (Io) and decay rate of light intensity ($k_{\rm L}$) of <u>P</u>. <u>phosphoreum</u> luciferase on temperature. The dashed line (- - -) represents the ratio of the two curves (Io/kL) reflecting the quantum efficiency of the luminescence reaction.

the quantum efficiency of light emission (Io/kL), reflecting the total light emission for a single turnover of the enzyme reaches a maximum at 15 °C. The kinetic properties of P. phosphoreum luciferase appear to be reflective of the natural habitat of these marine species.

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REFERENCES

- (1) Hastings, J., Potrikas, C., Gupta, S., Kurfurst, M., and Makemson, J. (1985) Adv. Microbiol. Physiol. 26, 235- 291
- Meighen, E. A. (1988) Annu. Rev. Microbiol. 42, 151-176 (2)
- Mancini, J., Boylan, M., Soly, R., Graham, A., and Meighen, E. (1988) J. Biol. Chem. 263, 14308-14314 (3)
- Soly, R., Mancini, J., Ferri, S., Boylan, M., and Meighen E. (4)(1988) <u>Biochem. Biophys. Res. Commun.</u> <u>155</u>, 351-358 Soly, R., and Meighen, E. (1991) <u>J. Mol. Biol.</u>, in press
- (5)
- Nealson, K., and Hastings, J. (1979) Microbiol. Rev. 43, (6) 496-518
- Sanger F., Nicklen, S., and Coulson, A. (1977) Proc. Natl. (7) Acad. Sci. USA 74, 5463-5467
- Messing, J. (1983) Methods Enzymol. 101, 20-78 (8)
- Hastings, J., Baldwin, T., and Nicoli, M. (1978) Methods (9) Enzymol. <u>57</u>, 135-152
- Meighen, E., and Bartlet, I. (1980) J. Biol. Chem. 255, (10) 11181-11187
- (11) Illarionov, B., Protopopova, M., Karginov, V., Mertvetsov, N., and Gitelson, I. (1988) Bioorg. Khim. 14, 412-415

- (12) Baldwin, T., Devine, J., Heckel, R., Lin, J., and Shadel, G. (1989) J. Biolumin. Chemilumin 4, 326-341
- (13) Cohn, D., Mileham, A., Simon, M., Nealson, K., Rausch, S., Bonam, D., and Baldwin T. (1985) J. Biol. Chem. 260, 6139-6146
- Szittner, R., and Meighen, E. (1990) J. Biol. Chem. 265, 16581-16587
- (15) Johnston, T., Thompson, R., and Baldwin, T. (1986) J. Biol. Chem. 261, 4805-4811
- (16) Ziegler, M., and Baldwin, T. (1981) Curr. Top. Bioenerg. 12, 65-113
- (17) Grosjean, H., and Fiers, W. (1982) Gene 18, 199-209
- (18) Miyamoto, C., Graham, A., and Meighen, E. (1988) Nucl. Acids Res. 16, 1551-1562
- (19) Wall, L., Rodriguez, A., and Meighen, E. (1984) J. Biol. Chem. 259, 1409-1414
- (20) Hastings, J., Riley, W., and Massa, J. (1965) J. Biol. Chem. **240**, 1473- 1483
- (21) Evans, J., McCracken, S., Miyamoto, C., Meighen, E., and Graham, A. (1983) <u>J. Bacteriol. 153</u>, 543-545
 (22) Gunsalus-Miguel, A., Meighen, E., Nicoli, M., Nealson, K., and Hastings, J. (1972) <u>J. Biol. Chem. 247</u>, 398-404
 (23) Frackman, S., Anhalt, M., and Nealson, K. (1990) <u>J.</u>
- Bacteriol. 172, 5767-5773