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STRUCTURE AND PROPERTIES OF LUCIFERASE FROM PHOTOBACTERIUM PHOSPHOREUM

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SUMMARY: The nucleotide sequences of the luxA and luxB 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.

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 $FMNH_2$ 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 lux-specific 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 reductase subunits, luxAB coding for the alpha and beta subunits of luciferase and luxF 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

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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 luxA and luxB 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 mp18 and mp19 (8). Remaining segments were sequenced by using specific oligonucleotides 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 (9). In vitro luciferase activity was measured by injecting 1.0 ml of 50 μM $FMNH_2$ 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) = 5.5×10^9 quanta/s and the first order rate constant for luminescence decay, k_L, 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 subunits. 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. The luxA 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 luxB 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,

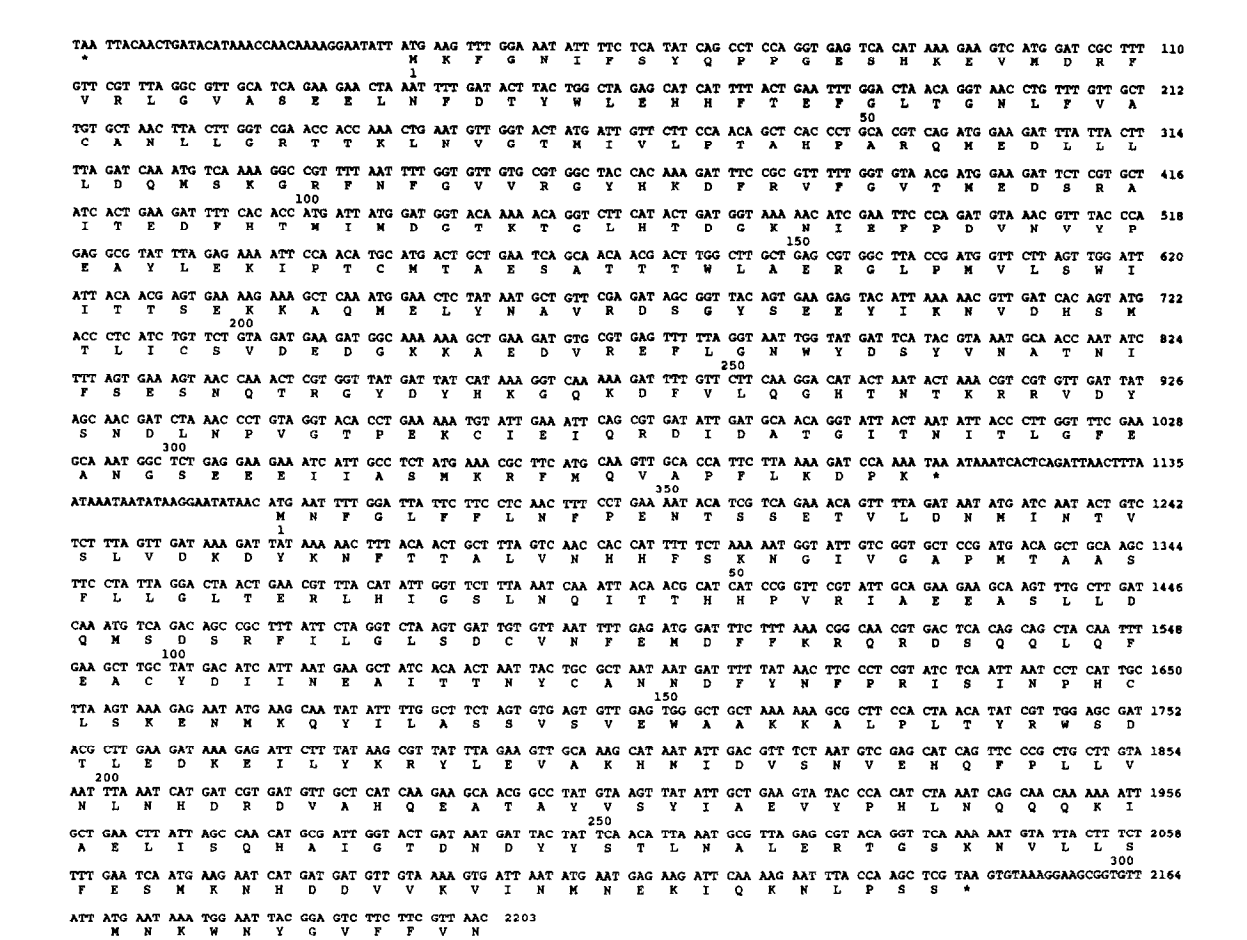

Fig. 1. Nucleotide sequence and corresponding translated amino acid sequences of the luxA and luxB genes of P. phosphoreum. The nucleotides are numbered on the right while the amino acids are numbered below the residue.

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

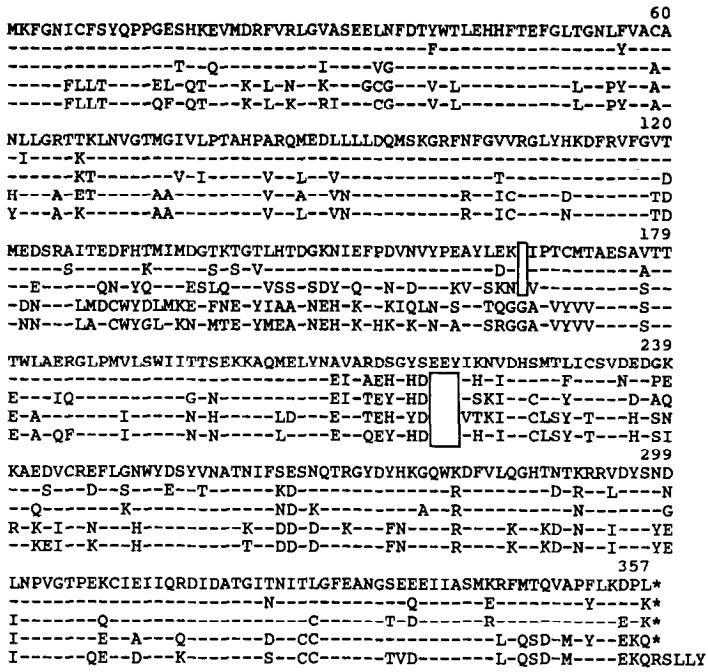

Fig. 2. Amino acid sequence comparison of the luciferase alpha subunits. From top to bottom, the sequences are given in the following order : P. phosphoreum, P. leiognathi (11), V. fischeri (12), V. harveyi (13) and X. luminescens (14). Amino acids identical to the corresponding residue in P. phosphoreum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

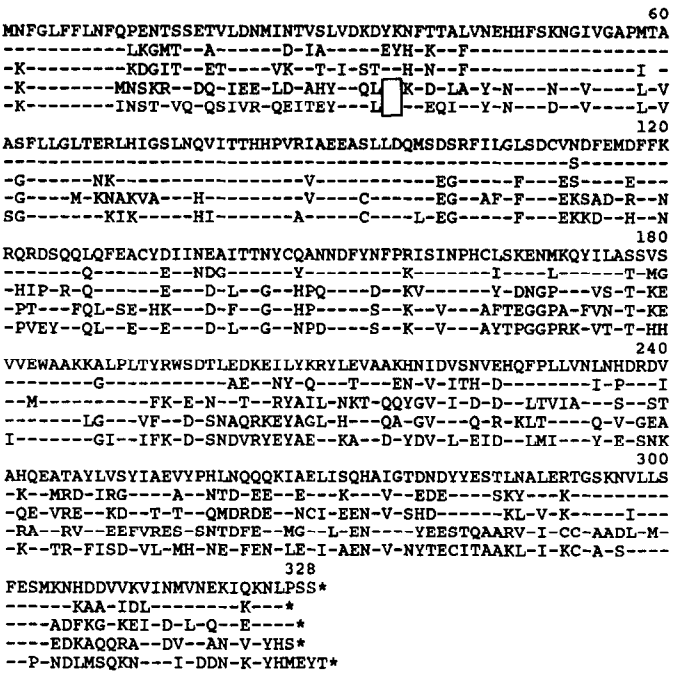

Fig. 3. Amino acid sequence comparison of the beta luciferase subunits. From top to bottom, the sequences are given in the following order : P. phosphoreum, P. leiognathi (11), V. fischeri (12), V. harveyi (15) and X. luminescens (14). Amino acids identical to the corresponding residue in P. phosphoreum are indicated by bars. A gap is indicated by the box.

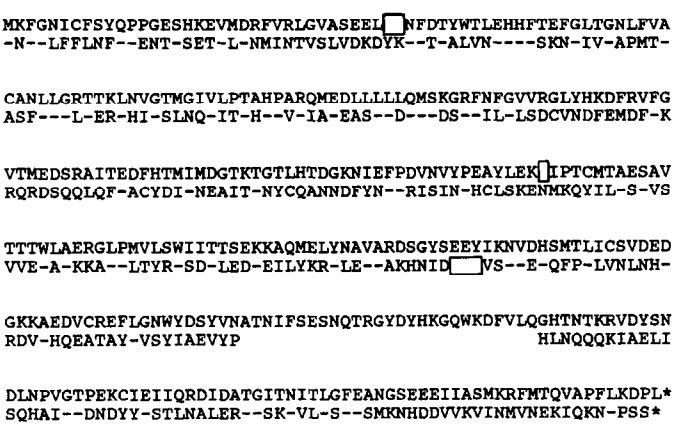

Fig. 4. Amino acid sequence comparison of the proteins encoded by luxA (line 1) and luxB (line 2) of P. phosphoreum. Blank spaces have been introduced to maximize identities. Horizontal bars represent residues identical to the corresponding amino acid in luxA gene product. Three gaps, corresponding to those in figures 2 and 3 are indicated by boxes.

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

	PP		PL		VH		VF		XL	
CODON	A	B	A	B	A	B	A	B	A	B
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 luxA and luxB genes do not have any AUA codons while the luxC and luxE 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 luxB 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. The level 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 V. harveyi luciferase can constitute up to 5% of soluble protein (20,21) with V. fischeri containing less luciferase than V. harveyi (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. luminescens is very low (23). These 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_o) for the reaction occurs at 20 °C. Moreover,

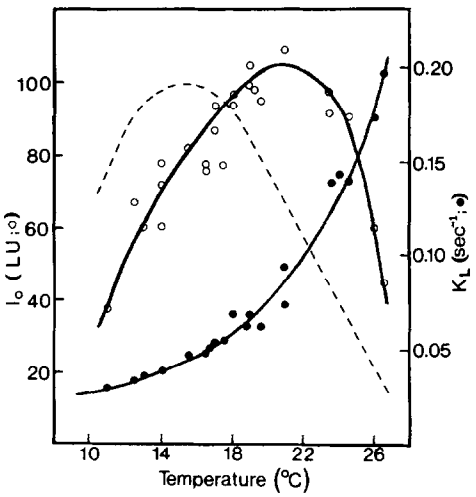

Fig. 5. Dependence of initial light intensity (I_o) and decay rate of light intensity (k_L) of P. phosphoreum luciferase on temperature. The dashed line (- - -) represents the ratio of the two curves (I_o / k_L) reflecting the quantum efficiency of the luminescence reaction.

the quantum efficiency of light emission (I_o/k_L), 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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