World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

GENE EXPRESSION PROFILING AND BIOPHYSICAL CHARACTERIZATION OF LUCIFERIN PROTEIN IN FIREFLY (*LAMPYRIDAE*)

Revathi A., Nithya G. and Shoba K.*

Department of Biochemistry, DKM College for Women, Vellore.

***Corresponding Author: Shoba K.**
Department of Biochemistry, DKM College for Women, Vellore.

Article Received on 22/01/2018 **Article Revised on 12/02/2018** **Article Accepted on 06/03/2018**

ABSTRACT

The Lampyridae are a family of insects in the beetle order Coleoptera. They are winged beetles, commonly called fireflies or lightning bugs. Luciferin (from the Latin Lucifer, "light-bringer") is the light-emitting compound found in organisms that generate bioluminescenceFirefly luciferin is the luciferin, or light-emitting compound, found in many firefly (Lampyridae) species. It is the substrate of luciferase which is responsible for the characteristic yellow light emission from many firefly species. Luciferase can be used as markers to detect blood clots, to tag tuberculosis virus cells, and to monitor hydrogen peroxide levels in living organisms (hydrogen peroxide is believed to play a role in the progression of some diseases, like cancer and diabetes).scientists can now use a synthetic form of luciferase for most research, so the commercial harvest of fireflies has decreased. The nucleotide sequence of luciferin protein is retrieved from NCBI. Through Ace view gene structure analysis of luciferin were carried out. The chromosomal mapping of luciferin gene is done by Gene card. With the help of J cat and Amigo gene expression analysis were done. The phylogenetic analysis of luciferin gene is carry out by clustal W. The 3D structure of firefly luciferin is modeled using Swiss model Server. This work would definitely be useful in the field of Clinical Pathology, Computational Entomology and Cheminformatics.

KEYWORDS:

INTRODUCTION

They are about 2000 species of firefly have been identified. They produce light in its lower abdomen may be yellow, green or pale red, withWavelenght from 510 to 670 nanometers. By the use bioluminescence it attract mates or prey. Light production in fireflies is due to a type of chemical reaction called bioluminescence. The enzyme luciferase acts on the luciferin, inthe presence of magnesium ions, ATP, and oxygen to produce light.

The majority of luciferases have been found in firefly, marine animals like copepods, jellyfish and the sea pans. It also present in fungi (Jack-O-Lantern mushroom) and some of the bacteria. The firefly regulates the flow of oxygen into its abdomen to turn its tail light on or off. Even though a firefly's light is triggered by oxygen, fireflies do not have lungs. Instead, they inhale oxygen through tubes called "tracheoles." Luciferase can be used in blood banks to determine if red blood cells are starting to break down. Genes for luciferase can be genetically engineered into organisms so that they glow when exposed to luciferin. This allows visualization of certain biological processes, stages of infection, and provides other valuable sources of information. Luciferases can be produced in the lab through genetic engineering for a number of purposes. Luciferase genes can be synthesized and inserted into organisms or transfected into cells. Mice, silkworms, and potatoes are just a few of the organisms that have already been engineered to produce the protein.

MATERIALS AND METHOD

The protein sequence of luciferin was retrieved from NCBI.The structural analyses of luciferin gene were done using Ace view.The chromosomal mapping of luciferin gene was done by Gene card.Jcat is used to determine the expression of luciferin gene. AmiGO is the official web based set tools for searching and browsing the Gene Ontology database, which consists of a controlled vocabulary of terms covering biological concepts, and a large number of genes or gene products whose attributeshave been annotated using Gene Ontology terms.The evolutionary relationship of luciferin protein was carried out by ClustalW.The structure of luciferin protein was detected by Dipole movement server.3D structure of luciferin protein was predicted by Swiss model server.

RESULTS AND DISCUSSION

1. Sequence Retrieval-NCBI

Luciferin-Protein

>ADK55065.1 luciferin regenerating enzyme [Lampyristurkestanicus]
MSPTIEIVTERVILGEGPHWDVPSQSLYYVDILGQTLHKYVPSTNTHTKVKIEGGPIGFAIPVEGKPNTF
AIGLGRKIVEVVWDGVSDSVSSLKTLVEVDSEAGFTNNRFNDGKADPTGRLWAGTMGPEPEVGKLEPEKG
TLYTFDRNHRIKAHLKTISISNGLAWNLKLKKMYYIDSPLKTVDQYDYDMVKGEICNRKVIFDFDKHSIP
GIPDGMTIDSEGNLWVAVFDGARILKINPNTSELLTTINFPTQQITCPTFGGPNLEDLYVTSGQLVIEGK
TQPAPAGAVFKVTGVGSKGLPCVNVHL

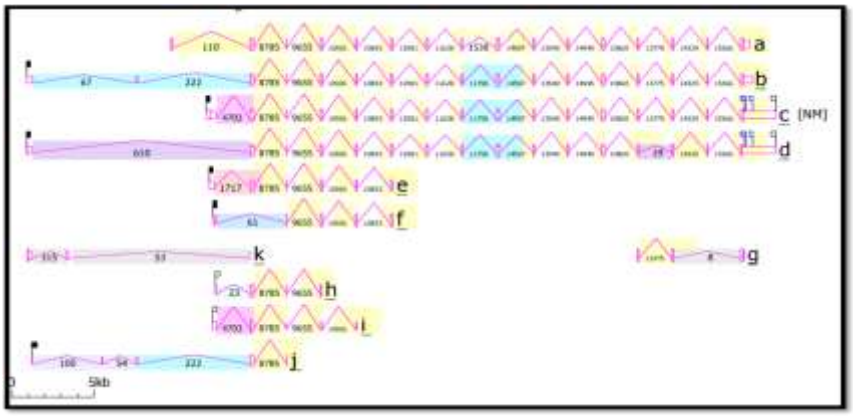
Luciferin-Nucleotide

>GU013474.1Lampyristurkestanicusluciferin regenerating enzyme mRNA, complete cds
ATGTCACCAACCATCGAGATTGTTACAGAACGGGTTATATTAGGAGAAGGTCCACATTGGGATGTTCCCT
CCCAAAGTCTCTACTATGTTGATATATTGGGACAAACTCTTCATAAATACGTACCTTCAACCAATACTCA
CACAAAAGTCAAAATAGAAGGAGGACCGATAGGTTTCGCCATACCTGTTGAAGGTAAACCGAACACATT
T
GCGATTGGACTTGGTCGAAAAATAGTTGAAGTGGTTTGGGATGGCGTCAGCGATTCAGTTTCGAGCTTAA
AGACACTTGTTGAAGTAGATAGCGAAGCGGGATTTACTAATAACAGATTTAATGACGGCAAAGCAGATC
C
AACAGGAAGATTGTGGGCAGGAACGATGGGACCTGAACCAGAAGTTGGAAAGTTAGAGCCAGAAAAAG
GT
The above sequence shows the FASTA format of luciferin protein and nucleotide

2. Gene Profiling

A. Ace View

The above picture shows the gene expression of 15 different primates tissues. The gene expression is coded in 8 equal sized bin (from light green to dark green).Light gray is for weak not-accurately measured expression (2 to 8 reads above intergenic background); dark gray for no expression or no sequence conservation (0 read in gene). The plot to the right shows the distribution of measured expression values in all tissues for all genes (**blue**) and for this gene (**green**)



The above results show the chromosomal mapping of luciferin protein. Here **PINK** colour indicates **good proteins**, **YELLOW** colour indicates **partial** or **not goodproteins**, **and GREEN** colour indicates **uORFs.**

B. Gene Card

The above results shows the gene profiling of luciferin protein, it also shows the expression value.

3. Sequence anatation

Amigo

Gene/product	Gene/product name	Organism	PANTHER family	Type	Source	Synonyms
Q9GV45	Oplophorus-luciferin 2-monooxygenase catalytic subunit	Oplophorus gracilirostris		protein	UniProtKB	LUCI_OPLGR
Luc	Secreted luciferase	Metridia longa		protein	UniProtKB	Q6UQE3_9MAXI
O77206	Dinoflagellate luciferase	Lingulodinium polyedrum		protein	UniProtKB	LUCIF_LINPO
Q26304	Luciferin 4-monooxygenase	Lucicla mingrelica		protein	UniProtKB	LUCI_LUCMI
P08659	Luciferin 4-monooxygenase	Photinus pyralis		protein	UniProtKB	LUCI_PHOPY
luxE	Long-chain-fatty-acid--luciferin-component ligase	Aliivibrio fischeri		protein	UniProtKB	LUXE_ALIFS

The above result shows the evolutionary relationship of luciferin protein.

4. Expression Analysis

J CAT

Table:1 Expression analysis of luciferin gene in homosapiens.

S. NO.	Gene Name	Protein Name	CAI Value
1	LUXR	Lampyridae (luciferin)	0.9553213131312752
2	LUXR	Lingulodinium polyedrum (luciferin)	0.9559359353086416
3	LUXR	Photinus pyralis (luciferin)	0.954629664249828
4	LUXR	Aliivibrio fischeri (luciferin)	0.9507377515922812

The above table shows the expression value of luciferin gene in different Organism.

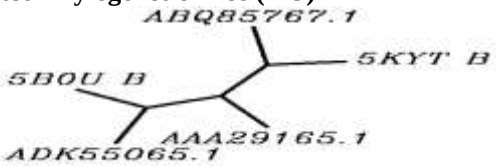
5. Phylogenetic Analysis

Clustal W

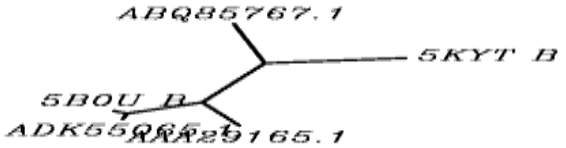
Rooted Phylogenetic Tree (UPGMA)

Rooted Phylogenetic Tree with Branch Length (UPGMA)

Unrooted Phylogenetic Tree (N-J)



Unrooted Phylogenetic Tree with Branch Length (N-J)



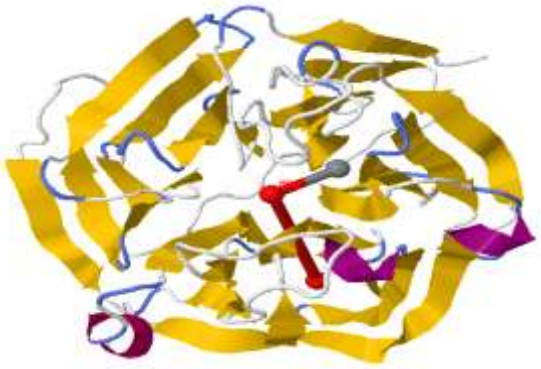
The above pictures shows the different phylogenetic tree format of luciferin gene with evolutionary related gene

6. Biophysical Characterisation
Electrostatic Interaction
Dipole Movement Server

Dipole moment for

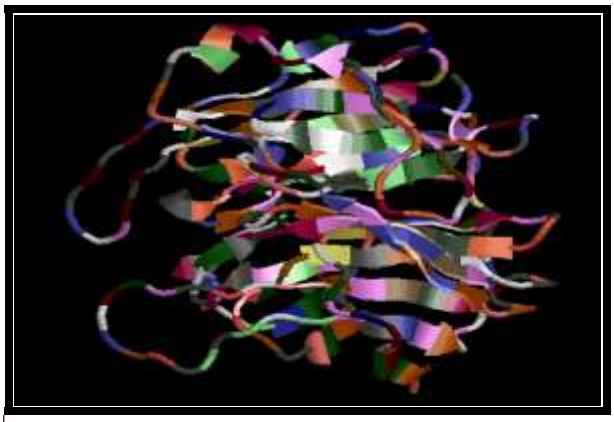
	No. of Chains=1		Spherical							
	No.Atoms	No.Res.	R_M	Pos.Res.	Neg.Res.	Charge	Dipole	Quadrapole	Crg./Nat.	Dip./Nat.
Value	2359.	306.	292.14	30.	35.	-5.	425.	1502.	-0.0021	0.1800
No.Dev.Units	0.50	0.54	0.20	0.32	0.36	-0.23	-0.18	-0.29	-0.13	-0.67

Dipole vector (in atomic units): 38.02 -55.52 57.35
Mass Moments vector: 463.91 532.22 519.27



The above structure shows the Dipole movement of luciferin. Here **Pink** colour indicates-**Helix**, **Blue** colour indicates-**Turns**, **Yellow** colour shows-**Sheets**, White colour shows-Coil region.

7. Structural Analysis
Three Dimensional Structural Analyses
Swiss Model



```
RasMol> SHOW INFORMATION
Secondary Structure ... Calculated
Experiment Technique .. THEORETICAL MODEL (SWISS-MODEL SERVER)
Number of Groups ..... 306
Number of Atoms ..... 2359
Number of Bonds ..... 2416
Number of H-Bonds ..... 200
Number of Helices ..... 3
Number of Strands ..... 35
Number of Turns ..... 33
RasMol>
```

The above 3D structure of luciferin protein was viewed by Rasmol server. Here light grey colour indicates-GLY, Bright red colour indicates-ASP, GLU, Yellow colour indicates-CYS, MET, Pale blue indicates-HIS, Orange colour indicates-SER, THR, Green colour indicates–LEU, VAL, ILE, Dark grey colour indicates-ALA, Deep pink colour indicates-TRP and Blue colour indicates-LYS, AEG

CONCLUSION

The firefly bioluminescence system is universally used as a method of measuring ATP, Ca2+ .Firefly luciferase has been unique marking tool used in various bioimaging

techniques used to detect viral infection and other biological process. Particularly Firefly (*Photinus pyralis*) luciferase is most commonly used for invivo imaging. Both fluorescent proteins and luciferase are referred to as "reporters" because they "report" the location and expression of the target gene. Fluorescent proteins can also mark the location and extent of a tumor. The firefly luciferase gene has been used successfully for the analysis of promoters, transcription, terminator signals, and translational enhancer elements inplants. The protein and nucleotide sequence of luciferin is retrieved from NCBI in FASTA format. Structural analysis of luciferin gene is done by Ace View. The chromosomal mapping is carried out by Gene Card. Gene expression analysis of luciferin gene is done by Amigo and J Cat. Highly expressed genes are selected based on the CAI value. The 3Dstructure of Luciferin protein is modeled using Swiss model server. Insilico approaches have gained immense popularity and have become an integral part of the research that is directed towards drug design and discovery.

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