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Covalent structure of subunits of bacterial luciferase: NH_2-Terminal sequence demonstrates subunit homology*

(evolution/bioluminescence/gene duplication)

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ABSTRACT The heterodimeric subunit structure of bacterial luciferase was demonstrated more than 10 years ago. The enzymes from both *Beneckea harveyi* and *Photobacterium fischeri* have since been studied in detail; they each consist of two nonidentical subunits, designated α and β. Both are required for bioluminescence activity, with the active center apparently confined to the α subunit. Amino acid sequence analysis of the NH_2 termini of the α and β subunits of the *B. harveyi* and *P. fischeri* luciferases not only confirms the earlier observation that the α subunits are homologous but also demonstrates that the NH_2-terminal sequences of the β subunits of the luciferases from the two genera are homologous. Furthermore, within each luciferase, the NH_2-terminal sequences of the α and β subunits are similar, suggesting the possibility that the genes coding for α and β may have arisen by gene duplication, presumably prior to divergence of the lines leading to present-day luminous bacteria.

Bacterial luciferase catalyzes the mixed function oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long-chain saturated aldehyde to yield the corresponding carboxylic acid, oxidized flavin, and blue-green light ($\lambda_{max} \approx 490$ nm) (2). The enzyme is a heterodimer (αβ) with subunit molecular weights obtained from electrophoresis in sodium dodecyl sulfate of 42,000 and 37,000 for *Beneckea harveyi* α and β, respectively, and 41,000 and 38,000 for the corresponding *Photobacterium fischeri* subunits (3).[¶]

The structural nonidentity of the luciferase α and β subunits was first demonstrated clearly by separation of the subunits on columns of DEAE-cellulose in urea-containing buffers (5) and by electrophoretic resolution in polyacrylamide gels containing urea or sodium dodecyl sulfate (3). The α and β subunits have been shown to have different amino acid compositions (3) and tryptic peptide maps (4). However, the discovery of three identical tryptic peptides between the higher molecular weight subunits from the *P. fischeri* and *B. harveyi* luciferases suggested that the α subunits of the luciferases from these two genera are homologous (4).

The functional nonidentity of the luciferase α and β subunits was first demonstrated by chemical modification of the enzyme (6, 7) and has been confirmed by mutant enzyme analysis (8), additional chemical modification studies (9, 10), differences in susceptibility to proteases (11), and numerous ligand binding studies (12–17). The enzyme has a single flavin-binding site. The binding of reduced flavin has been demonstrated by using a kinetic technique (12), fluorescence quenching (13), and circular dichroism (14). The binding of oxidized flavin has been demonstrated by equilibrium dialysis (15) and fluorescence quenching and circular dichroism (16, 17). Luciferases that have mutant α subunits and show alterations in reduced flavin binding or bioluminescence emission spectrum also show alterations in oxidized flavin binding (8, 17). Furthermore, by careful analysis of the bioluminescence quantum yield under conditions of limiting $FMNH_2$ or limiting enzyme, it has been demonstrated that the stoichiometry of the bioluminescence reaction is one $FMNH_2$ per luciferase αβ dimer (14).

The rather compelling conclusion drawn from all of these observations is that the luciferase α and β subunits are quite distinct, both structurally and functionally. We have now found that the α and β subunits of the luciferases from two different species are in fact strikingly similar in NH_2-terminal amino acid sequence.

EXPERIMENTAL PROCEDURE

Cells of *B. harveyi* and *P. fischeri* (18) were grown in a 250-liter New Brunswick fermenter (19). The luciferases were purified and the subunits were separated by published methods (19, 20).

The sequencer utilized in these studies was a Beckman model 890 C and was loaned to the Physiology Course at the Marine Biological Laboratory by Beckman Instruments, Inc. The program utilized was the Fast Quadrol program (072172C) developed by Beckman. The solvents and reagents were all purchased from Beckman.

The 3-phenyl-2-thiohydantoin derivatives of the amino acids were identified by thin-layer chromatography on 20 × 20 cm Brinkmann F254 thin-layer plates. Two methods were used. The two-solvent system of Jeppson and Sjoquist (21) was utilized as the primary method. Also, a solvent system developed specifically for use in identifying the phenylthiohydantoin derivatives of lysine, aspartate, asparagine, glutamate, glutamine, threonine, and serine was used to confirm the identification of these occasionally recalcitrant residues. This solvent system consists of chloroform/isopropanol/xylene/propionic acid, 30:5:2:1 (vol/vol).

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Abbreviation: MMD, minimal mutation distance.

* A preliminary report of this work has appeared in abstract form (1).

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¶ Note that the designations of the *B. harveyi* ("MAV") α and β subunits were originally reversed (3); the subsequent change to the presently accepted α and β designations was made on the basis of the tryptic peptide homologies found by Meighen *et al.* (4) between the higher molecular weight subunits from *P. fischeri* (α) and *B. harveyi* ("β" in ref. 3; α in ref. 4 and all subsequent publications).

Table 1. Comparison of NH_2-terminal sequences of luciferase subunits

Sequence pair*	No. of compared positions	No. of identities	% identities	MMD†	Significance,‡ %
Bh α/Pf α	14	8	57	7	<0.01
Bh β/Pf β	14	10	71	7	<0.01
Bh α/Bh β	22	9	41	17	<0.01
Pf α/Pf β	15	6	40	9	<0.01
Bh α/Pf β	14	6	43	7	<0.01
Pf α/Bh β	14	5	36	9	<0.01

* The NH_2-terminal sequences of the *B. harveyi* (Bh) α and β subunits and the *P. fischeri* (Pf) α and β subunits are shown in Fig. 1.
† The sum of the MMDs (24) for all positions compared.
‡ The significance level at which the hypothesis of no common ancestry can be rejected for the observed MMD, according to the alignment statistic of Moore and Goodman (25).

RESULTS AND DISCUSSION

Each subunit was degraded twice; the observed NH_2-terminal sequences are presented in Fig. 1. The subunits utilized in these experiments were estimated to be >95% pure by sodium dodecyl sulfate gel electrophoresis (22), so the observed similarity in amino acid sequence cannot be due to contamination of one subunit with the other. The criterion for each assignment was an unambiguous chromatographic identification in each of the three solvent systems used in both degradations.

The yield decreased dramatically after step 10 on both α subunits and on the β subunit of *P. fischeri*. A comparable decrease occurred after step 18 on the *B. harveyi* β subunit. These decreases in yield apparently resulted from the well-known cyclization of glutamine (23), the residue observed at step 11 of both α subunits and the *P. fischeri* β subunit and at step 19 of the *B. harveyi* β subunit. Consistent with this suggestion is the observation that the holdover (amount of residue n-1 observed at step n of the degradation) did not increase after the decrease in yield. Of 82 identifications attempted, the chromatographic system allowed unambiguous identification of 78 residues; the remaining 4 positions (designated by parentheses in Fig. 1) could not be identified with the required level of confidence and therefore were deleted from any comparisons.

Each subunit's NH_2-terminal sequence was compared, position by position, with each of the other three subunits' sequences, with respect both to degree of identity and to minimal mutation distance (MMD) (24). The results are presented in Table 1. The substantial number of identities (57%) observed between the sequences of the α subunits of the two luciferases confirms the earlier suggestion of α subunit homology from tryptic peptide comparisons (4). The degree of identity between the β subunits from the two species (71%) suggests a similar homologous relationship between the β subunits of the two species which had not been detected in the tryptic peptide maps.‖ The substantial degree of α/α and β/β homology suggested by the NH_2-terminal sequences is not surprising in view of the phylogenetic relationship between the *Beneckea* and *Photobacterium* genera (18).

The surprising result which is apparent in Table 1 is the degree of identity between the α and β subunits of the luciferases, which ranged between 36 and 43%. Although the number of positions compared represents only a small part of the whole polypeptide chains (about 5%), the relatedness of this section of the α and β chains can be evaluated by the alignment statistic of Moore and Goodman (25). This treatment, which is based on the assumption of an equal and independent probability of each nucleotide pair at each alignment position, permits a statistical distinction between the ***null hypothesis*** (absence of common ancestry for two sequences) and the most plausible ***alternative hypothesis*** (presence of common ancestry) by comparison of the observed MMD (24) with a randomly generated collection of alignments. Using a table provided by Moore and Goodman (25), we find that, for every sequence pair compared in Table 1, the similarity is so great that the null hypothesis can be rejected at a level of significance of <0.01%. In other words, the probability that the NH_2-terminal sequences of the α and β subunits did *not* share a common ancestral sequence is <0.01%, a very strong indication indeed that these sequences *did* arise from a common ancestor.

The striking degree of homology of the NH_2-terminal sequences of the α and β subunits from two different species suggests the possibility that the luciferase α and β genes may have arisen by gene duplication prior to divergence of the lines leading to present-day luminous bacteria. Definitive proof of this suggestion, however, must await the determination of the entire amino acid sequences of both subunits, and perhaps the three-dimensional structure of the enzyme.

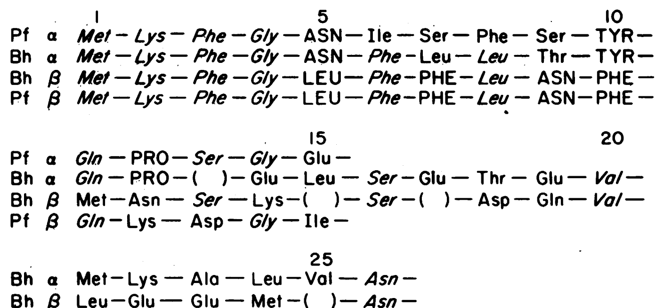

FIG. 1. NH_2-terminal amino acid sequences of bacterial luciferase subunits. Identities in the sequences of *P. fischeri* (Pf) and *B. harveyi* (Bh) subunits are emphasized by italics or capital letters; parentheses indicate positions not determined unambiguously.

‖ Failure to detect identical tryptic peptides in different polypeptide chains is by no means evidence against evolutionary relatedness; the α and β chains of human hemoglobin, which are identical in 40% of their residues (26), have no tryptic products in common except free lysine (27).

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