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Isolation and expression of a cDNA encoding *Renilla reniformis* luciferase

(bioluminescence/*Renilla* luciferase/green fluorescent protein/gene expression)

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ABSTRACT **_Renilla reniformis_ is an anthozoan coelenterate capable of exhibiting bioluminescence. Bioluminescence in _Renilla_ results from the oxidation of coelenterate luciferin (coelenterazine) by luciferase [_Renilla_-luciferin:oxygen 2-oxidoreductase (decarboxylating), EC 1.13.12.5]. _In vivo_, the excited state luciferin–luciferase complex undergoes the process of nonradiative energy transfer to an accessory protein, green fluorescent protein, which results in green bioluminescence. _In vitro_, _Renilla_ luciferase emits blue light in the absence of any green fluorescent protein. A _Renilla_ cDNA library has been constructed in λgt11 and screened by plaque hybridization with two oligonucleotide probes. We report here the isolation and characterization of a luciferase cDNA and its gene product. The recombinant luciferase expressed in _Escherichia coli_ is identical to native luciferase as determined by SDS/PAGE, immunoblot analysis, and bioluminescence emission characteristics.**

Renilla reniformis (class Anthozoa) is a bioluminescent soft coral found in shallow coastal waters of North America, which displays blue-green bioluminescence upon mechanical stimulation (1, 2). The components involved in *Renilla* bioluminescence have been described in detail (3). *Renilla* luciferase [*Renilla*-luciferin:oxygen 2-oxidoreductase (decarboxylating), EC 1.13.12.5] catalyzes the oxidative decarboxylation of coelenterazine in the presence of dissolved oxygen to yield oxyluciferin, CO_2, and blue light (λ_{max} = 480 nm) (4). This reaction has a bioluminescence quantum yield of ≈7%. The stoichiometry of this reaction and the detailed mechanism leading to excited-state formation have been described (4, 5).

The color of *in vitro*-catalyzed bioluminescence changes from blue to green upon addition of submicromolar amounts of an energy-transfer acceptor green fluorescent protein (GFP), which has been purified from *Renilla* and characterized (6). This green fluorescence (λ_{max} = 509 nm) is identical to the *in vivo* emission in *Renilla*. The energy-transfer process is nonradiative; an increase in both the quantum yield (6) and calculated lifetimes has been determined for this process (7). Luciferase and GFP form a specific 1:1 rapid equilibrium complex in solution (8).

The elucidation of mechanisms involved in nonradiative energy transfer processes as well as determination of detailed structural information on both luciferase and GFP have been hindered by a lack of material. To overcome this, we have cloned, sequenced, and expressed in *Escherichia coli* a cDNA encoding *Renilla* luciferase.§

MATERIALS AND METHODS

Amino Acid Sequence Determination of *Renilla* Luciferase. Native *Renilla* luciferase was isolated as described (4). Purified luciferase was digested with *Staphlococcal* protease V-8 (9). The resulting peptides were purified by HPLC and subjected to NH_2-terminal Edman sequencing as described (10). Based on these peptide sequences two 17-base oligonucleotide probes were synthesized with an Applied Biosystems DNA synthesizer at the Molecular Genetics Instrumentation Facility at the University of Georgia.

Construction of a cDNA Library in λgt11. Live *R. reniformis* were collected at the University of Georgia Marine Institute located at Sapelo Island. The animals were frozen immediately in liquid N_2 and stored at −80°C. Frozen tissue was ground to a fine powder in liquid N_2 with mortar and pestle. Total RNA was isolated from the frozen powder by the guanidine thiocyanate method (11), and poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography (12). cDNA was synthesized by the method of Gubler and Hoffman (13). Phosphorylated *Eco*RI linkers (Collaborative Research) were ligated to the cDNAs, which were then digested with *Eco*RI. Separation of cDNA from free linkers after *Eco*RI digestion as well as size selection of cDNAs were accomplished by electrophoresis in low-melting-temperature agarose (NuSieve, FMC) (14). cDNAs were ligated into the *Eco*RI site of λgt11 (15). The library was amplified in Y1088 cells (16) by a plate method (17).

Isolation and DNA Sequence Determination of a Luciferase cDNA. Oligonucleotide probes were 5′ end-labeled with T4 polynucleotide kinase (Bethesda Research Laboratories) and [γ-^{32}P]ATP (3000 Ci/mmol; 1 Ci = 37 GBq; ICN) to specific activities ≥1 × 10^8 cpm/μg (18). A total of 6 × 10^5 recombinant plaque-forming units were screened by plaque hybridization (19). Phage DNA was isolated as described (20). A luciferase cDNA, isolated from the clone λRLuc-6, was subcloned into the M13 sequencing vectors mp18 and mp19, and sequencing templates were prepared (21). The DNA sequence of both strands was determined by the dideoxynucleotide chain-termination technique by using a Sequenase kit (United States Biochemical) and [α-^{35}S]dATP (400 Ci/mmol; Amersham) (22). The M13 universal primer and a λgt11 sequencing primer (Amersham) were used to prime the sequencing reactions.

Expression of Recombinant Luciferase (r-luciferase). Positive clones were converted to lysogens in *E. coli* Y1089 cells (16). Lysogens were grown at permissive temperatures and induced with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). Crude cell extracts were prepared and assayed for luciferase activity as described below. The plasmid pTZR-Luc-1 was constructed by ligation of a 2.2-kilobase-pair (kbp) *Eco*RI/*Sst* I λRLuc-6 restriction fragment into the plasmid pTZ18R (Pharmacia), which contains the *lacZ′* gene. *E. coli* TG-1 cells (23) were transformed with pTZRLuc-1 (24). Single colonies were isolated and grown at 37°C in LB

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Abbreviations: GFP, green fluorescent protein; IPTG, isopropyl β-D-thiogalactopyranoside; r-luciferase, recombinant luciferase; ORF, open reading frame.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M63501).

medium containing ampicillin (100 μg/ml) to an OD_{600} = 0.6–0.8 unit and induced with 1 mM IPTG for 4 hr. The cells were centrifuged at 10,000 × *g* and frozen solid at −20°C. The pellets were thawed and resuspended 1:5 in 10 mM EDTA, pH 8, and lysozyme at 4 mg/ml (Sigma). After 20-min incubation at 25°C, the cells were placed on ice for 1 hr and then sonicated for 30 sec with a Branson cell disrupter. The cell lysate was clarified by centrifugation at 30,000 × *g*. The clarified lysate was used in subsequent bioluminescence assays and emission studies.

Assay for *Renilla* Luciferase Activity and Determination of Emission Spectra. Bioluminescence assays (4) were done with a Turner model TD-20e luminometer, and peak light intensities were determined. Bioluminescence intensity was converted to quanta per second by calibrating the instrument relative to a radioactive ^{63}Ni light standard that emits in the 460- to 480-nm region (25). Corrected emission spectra were collected on an on-line computerized fluorimeter (26). A 100-μl sample of a clarified pTZRLuc-1 cell extract was added to 1 ml of luciferase assay buffer (4) or to 1 ml of "energy-transfer buffer" containing 1×10^{-6} M GFP (8). An excess of coelenterazine (0.47 mM) dissolved in MeOH was added to maintain a strong emission signal.

Genomic Southern Blot Analyses. A 790-bp *Eco*RI/*Bam*HI cDNA restriction fragment was labeled to specific activities $\geq 1 \times 10^9$ cpm/μg with both [α-^{32}P]dATP and dCTP (4000 Ci/mmol, ICN) by the random hexamer-priming method (27). Genomic DNA was isolated from *Renilla* by a guanidine thiocyanate method developed for coelenterate DNA isolation (D. Prasher, personal communication). DNA samples were digested with the appropriate enzymes and resolved in a 0.8% agarose gel, followed by transfer to nitrocellulose filters (Schleicher & Schuell) (28). Aqueous hybridizations and washes were done at high stringencies as described for a homologous probe (17).

Electrophoretic Analysis of Protein. Protein samples were analyzed on 12.5% SDS/PAGE gels that were fixed and stained with Coomassie blue as described (29). Immunoblots were done as described (30). Proteins were transferred to nitrocellulose (Schleicher & Schuell) and incubated in a 1:50 dilution of rabbit anti-native luciferase antibody. Detection of the secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit IgG) signal was determined according to the vendor's instructions (Bio-Rad).

Computer-Facilitated DNA and Amino Acid Sequence Analyses. The DNA sequence was compiled and manipulated using MicroGenie sequence software (Beckman).

RESULTS

Synthesis of Luciferase Oligonucleotides. Seven luciferase peptides (V8-1–V8-7) were purified by HPLC, and their amino acid sequences were determined. Two of the peptides contained regions of relatively low codon degeneracy. Amino acid sequence from these regions was used to synthesize two mixed-sequence 17-base oligonucleotide hybridization probes with the following sequences; RLP-1 {GAR-AAYAAY-TTY-TTY-GT} and RLP-2 {AAR-AAR-TTY-CCN-AAY AT}, which are 32- and 64-fold degenerate, respectively.

Nucleotide and Deduced Amino Acid Sequence Analyses of *Renilla* Luciferase. Six clones were isolated from the *Renilla* cDNA library. One clone, λRLuc-6, hybridized to both oligonucleotide probes. The cDNA insert could not be isolated after *Eco*RI digestion, as one of the linker sites was lost during cloning. A double digest with *Eco*RI and *Sst* I produced a 2.2-kbp fragment that contained a 1.2-kbp cDNA with 1 kbp of λgt11 DNA at the 3′ end. This fragment was subcloned into the M13 vectors mp18 and mp19. DNA sequencing provided the locations of six base restriction sites (Fig. 1), which were used to generate specific sequencing subclones. The entire 1.2-kbp luciferase cDNA was sequenced on both strands.

The cDNA, excluding the *Eco*RI linkers, is 1196 nucleotides long and encodes an open reading frame (ORF) of 314 amino acids (Fig. 2). Although an ATG in-frame codon is found at the 5′ end of the cDNA, the intrinsic mRNA may contain additional 5′ coding nucleotides. If the first ATG codon in the ORF is designated as the initiation codon, the predicted 311 amino acid sequence is essentially identical in size (34 kDa) and composition to native *Renilla* luciferase (4).

Comparison of the deduced amino acid sequence with the native peptides reveals that λRLuc-6 encodes a luciferase cDNA (Fig. 2). One discrepancy lies at amino acid residue 222, which is leucine in the peptide sequence but tryptophan in the deduced sequence. Sequencing autoradiograms from this region of the clone have been examined carefully and found free of any irregularities. The protein sequence also contains a consensus N-linked glycosylation site (Asn-Xaa-Ser) beginning at residue 92.

Genomic Southern Analysis. A *Renilla* genomic Southern blot was probed with a 790-base-pair (bp) *Eco*RI/*Bam*HI luciferase cDNA restriction fragment (Fig. 3). The *Bam*HI digest, lane A, contains two hybridizing bands as does the *Sma* I digest, lane B. The *Bgl* II digest, lane C, contains four bands. If luciferase is encoded by a single gene containing no introns, a single band would be expected in the *Bam*HI and *Sma* I digests, as these two sites are not spanned by the hybridization probe. Similarly, two bands would be expected in the *Bgl* II digest. That the *Bam*HI and *Sma* I digests contain two hybridizing bands shows either that there is more than one luciferase gene or that the luciferase gene(s) has introns containing *Bam*HI and *Sma* I sites. The four bands seen in the *Bgl* II could be explained by two very large introns containing *Bgl* II sites. When genomic DNA was digested with restriction enzymes having no sites within the cDNA sequence, there were always at least two or more hybridizing bands (data not shown). These results suggest that luciferase is encoded for by more than one gene, which may or may not contain introns.

Luciferase Expression in *E. coli*. The λRLuc-6 lysogen is capable of low-level r-luciferase expression as determined by light emission from clarified, crude extracts (5×10^{10} hv·sec^{-1}·ml^{-1}). When these cells are induced with 1 mM IPTG, light emission decreases by 2-fold; this happens because the cDNA is reversely oriented with respect to the λgt11 *lacZ* promoter. Presumably, when IPTG is absent, the luciferase gene is transcribed from a promoter in the right end of λgt11, as reported (31).

The 2.2-kbp *Eco*RI/*Sst* I fragment was subcloned into the plasmid pTZ18R, which uses the *lacZ* promoter. The ORF of

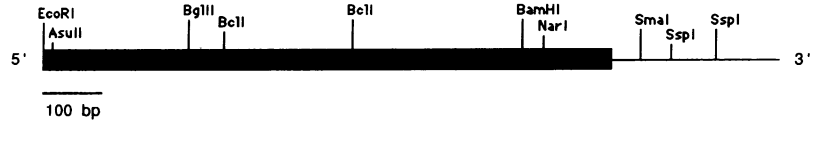

FIG. 1. Location of six base restriction enzyme sites within the luciferase cDNA. The boxed region defines the ORF. The *Eco*RI site at the 5′ end is a synthetic linker site.

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                 *           *           *           *           *           *
  1  AGC TTA AAG ATG ACT TCG AAA GTT TAT GAT CCA GAA CAA AGG AAA CGG ATG ATA ACT GGT   60
  1  Ser Leu Lys Met Thr Ser Lys Val Tyr Asp Pro Glu Gln Arg Lys Arg Met Ile Thr Gly   20

                 *           *           *           *           *           *
 61  CCG CAG TGG TGG GCC AGA TGT AAA CAA ATG AAT GTT CTT GAT TCA TTT ATT AAT TAT TAT  120
 21  Pro Gln Trp Trp Ala Arg Cys Lys Gln Met Asn Val Leu Asp Ser Phe Ile Asn Tyr Tyr   40

                 *           *           *           *           *           *
121  GAT TCA GAA AAA CAT GCA GAA AAT GCT GTT ATT TTT TTA CAT GGT AAC GCG GCC TCT TCT  180
 41  Asp Ser Glu Lys His Ala Glu Asn Ala Val Ile Phe Leu His Gly Asn Ala Ala Ser Ser   60

                 *           *           *           *           *           *
181  TAT TTA TGG CGA CAT GTT GTG CCA CAT ATT GAG CCA GTA GCG CGG TGT ATT ATA CCA GAT  240
 61  Tyr Leu Trp Arg His Val Val Pro His Ile Glu Pro Val Ala Arg Cys Ile Ile Pro Asp   80

                 *           *           *           *           *           *
241  CTT ATT GGT ATG GGC AAA TCA GGC AAA TCT GGT AAT GGT TCT TAT AGG TTA CTT GAT CAT  300
 81  Leu Ile Gly Met Gly Lys Ser Gly Lys Ser Gly Asn Gly Ser Tyr Arg Leu Leu Asp His  100

                 *           *           *           *           *           *
301  TAC AAA TAT CTT ACT GCA TGG TTT GAA CTT CTT AAT TTA CCA AAG AAG ATC ATT TTT GTC  360
101  Tyr Lys Tyr Leu Thr Ala Trp Phe Glu Leu Leu Asn Leu Pro Lys Lys Ile Ile Phe Val  120

                 *           *           *           *           *           *
361  GGC CAT GAT TGG GGT GCT TGT TTG GCA TTT CAT TAT AGC TAT GAG CAT CAA GAT AAG ATC  420
121  Gly His Asp Trp Gly Ala Cys Leu Ala Phe His Tyr Ser Tyr Glu His Gln Asp Lys Ile  140

                 *           *           *           *           *           *
421  AAA GCA ATA GTT CAC GCT GAA AGT GTA GTA GAT GTG ATT GAA TCA TGG GAT GAA TGG CCT  480
141  Lys Ala Ile Val His Ala Glu Ser Val Val Asp Val Ile Glu Ser Trp Asp Glu Trp Pro  160

                 *           *           *           *           *           *
481  GAT ATT GAA GAA GAT ATT GCG TTG ATC AAA TCT GAA GAA GGA GAA AAA ATG GTT TTG GAG  540
161  Asp Ile Glu Glu Asp Ile Ala Leu Ile Lys Ser Glu Glu Gly Glu Lys Met Val Leu Glu  180

                 *           *           *           *           *           *
541  AAT AAC TTC TTC GTG GAA ACC ATG TTG CCA TCA AAA ATC ATG AGA AAG TTA GAA CCA GAA  600
181  Asn Asn Phe Phe Val Glu Thr Met Leu Pro Ser Lys Ile Met Arg Lys Leu Glu Pro Glu  200

                 *           *           *           *           *           *
601  GAA TTT GCA GCA TAT CTT GAA CCA TTC AAA GAG AAA GGT GAA GTT CGT CGT CCA ACA TTA  660
201  Glu Phe Ala Ala Tyr Leu Glu Pro Phe Lys Glu Lys Gly Glu Val Arg Arg Pro Thr Leu  220

       +     *           *           *           *           *           *
661  TCA TGG CCT CGT GAA ATC CCG TTA GTA AAA GGT GGT AAA CCT GAC GTT GTA CAA ATT GTT  720
221  Ser Trp Pro Arg Glu Ile Pro Leu Val Lys Gly Gly Lys Pro Asp Val Val Gln Ile Val  240

                 *           *           *           *           *           *
721  AGG AAT TAT AAT GCT TAT CTA CGT GCA AGT GAT GAT TTA CCA AAA ATG TTT ATT GAA TCG  780
241  Arg Asn Tyr Asn Ala Tyr Leu Arg Ala Ser Asp Asp Leu Pro Lys Met Phe Ile Glu Ser  260

                 *           *           *           *           *           *
781  GAT CCA GGA TTC TTT TCC AAT GCT ATT GTT GAA GGC GCC AAG AAG TTT CCT AAT ACT GAA  840
261  Asp Pro Gly Phe Phe Ser Asn Ala Ile Val Glu Gly Ala Lys Lys Phe Pro Asn Thr Glu  280

                 *           *           *           *           *           *
841  TTT GTC AAA GTA AAA GGT CTT CAT TTT TCG CAA GAA GAT GCA CCT GAT GAA ATG GGA AAA  900
281  Phe Val Lys Val Lys Gly Leu His Phe Ser Gln Glu Asp Ala Pro Asp Glu Met Gly Lys  300

                 *           *           *           *           *           *
901  TAT ATC AAA TCG TTC GTT GAG CGA GTT CTC AAA AAT GAA CAA TAATTACTTT GGTTTTTTAT   960
301  Tyr Ile Lys Ser Phe Val Glu Arg Val Leu Lys Asn Glu Gln                        314

               *           *           *           *           *           *
963   TTACATTTTT CCCGGGTTTA ATAATATAAA TGTCATTTTC AACAATTTTA TTTTAACTGA ATATTTCACA  1032

1033  GGGAACATTC ATATATGTTG ATTAATTTAG CTCGAACTTT ACTCTGTCAT ATCATTTTGG AATATTACCT  1102

1103  CTTTCAATGA AACTTTATAA ACAGTGGTTC AATTAATTAA TATATATTAT AATTACATTT GTTATGTAAT  1172

1173  AAACTCGGTT TTATTATAAA AAAA                                                    1196
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FIG. 2. Nucleotide sequence and translated amino acid sequence of the *Renilla* luciferase cDNA. Putative and known translation control elements, as well as oligonucleotide hybridization sites, are underlined. Positions of native luciferase peptide sequences are boxed and, except at one residue (+), are identical to the deduced amino acid sequence obtained from the luciferase cDNA. Some of the native luciferase peptide sequences overlap at glutamic acid residues.

the cDNA is not in frame with the *lacZ'* gene ORF of pTZ18R. Supernatants were prepared from IPTG-induced pTZRLuc-1 cells, as described, and the level of luciferase expression was measured by the standard luciferase assay. A high level of r-luciferase activity, 2×10^{15} hv·sec^{-1}·ml^{-1}, is detected in clarified crude extracts of pTZRLuc-1 cells. This level of activity is 7-fold greater than in uninduced pTZR-Luc-1 cells.

A prominent protein band (M_r = 34,000) migrating to the position of native luciferase is seen after SDS/PAGE of pTZR-Luc-1 crude extracts (Fig. 4). Crude extracts of IPTG-induced pTZRLuc-1 cells were analyzed by immunoblotting (Fig. 5). The protein band that reacts with antiluciferase antibody, lane B, corresponds to the same band seen in the Coomassie-stained gel (Fig. 4). Native luciferase was used as a positive control, lane A. No signal is detectable in the crude extract of pTZ18R cells, lane C. A duplicate filter incubated with preimmune serum showed no detectable signal.

Bioluminescence Emission Spectra. The r-luciferase-catalyzed bioluminescence emission spectrum (Fig. 6*a*) is

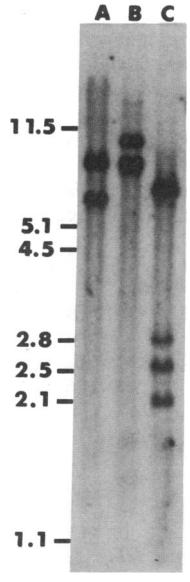

FIG. 3. Genomic Southern blot of *R. reniformis* DNA hybridized to a 790-bp *Eco*RI/*Bam*HI luciferase cDNA fragment. Genomic DNA had been digested with the following restriction enzymes: *Bam*HI (lane A); *Sma* I (lane B); and *Bgl* II (lane C). Samples were resolved in a 0.8% agarose gel and transferred to nitrocellulose. Each lane contains 20 μg of digested genomic DNA. Molecular size markers are in kbp.

very similar to that seen with native luciferase (32). The r-luciferase emission spectrum has a λ_{max} = 480 nm and a slight shoulder at 400 nm, which correspond to emission from the excited-state oxyluciferin monoanion and neutral species, respectively. Disproportionation between these species is sensitive to environmental factors (7); thus, this spectrum indicates the strong similarity of the active-site environment between r-luciferase and the native enzyme. Although an increase in quantum yield has yet to be determined, r-luciferase can clearly transfer energy in the presence of *Renilla* GFP (Fig. 6*b*). The emission spectrum dramatically shifted

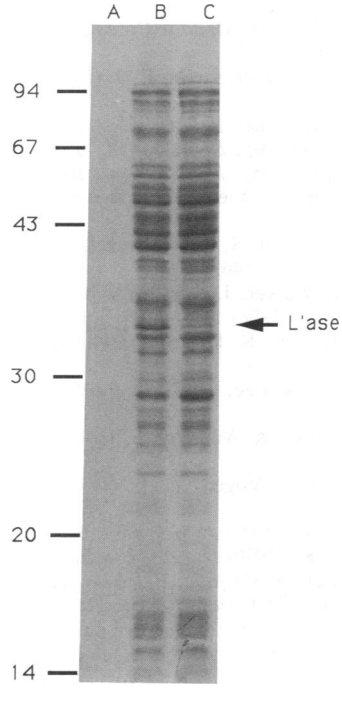

FIG. 4. SDS/PAGE analysis of total protein from IPTG-induced *E. coli* cells transformed with either pTZRLuc-1 or pTZ18R. Ten-milliliter cultures were grown to an OD_{600} = 0.8 and induced with 1 mM IPTG for 4 hr. One-milliliter of cell culture, OD_{600} = 5.0, was pelleted and resuspended in 0.5 ml of SDS sample buffer. Samples were boiled for 5 min, and 20 μl was loaded per lane: native luciferase (10 μg) (lane A); pTZRLuc-1 cells (lane B); and pTZ18R cells (lane C). Molecular weight ($M_r \times 10^{-3}$) standard positions are indicated. Arrow shows position of native luciferase (L'ase).

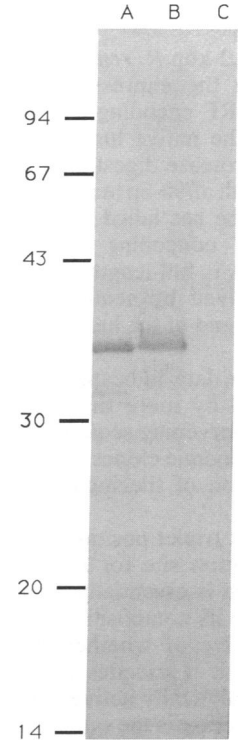

FIG. 5. Immunoblot analysis of total protein. Sample preparation and electrophoresis were the same as in Fig. 4. Native luciferase (2 μg) (lane A); 10 μl of pTZRLuc-1 cell extract (lane B); and 10 μl of pTZ18R cell extract (lane C). Molecular weight ($M_r \times 10^{-3}$) standard positions are indicated.

from the broad emission band generated by r-luciferase to the narrow, structured emission band (λ_{max} = 509) seen when GFP is present. The emission spectrum generated with r-luciferase and GFP is very similar to the same spectrum generated with native luciferase and GFP (33).

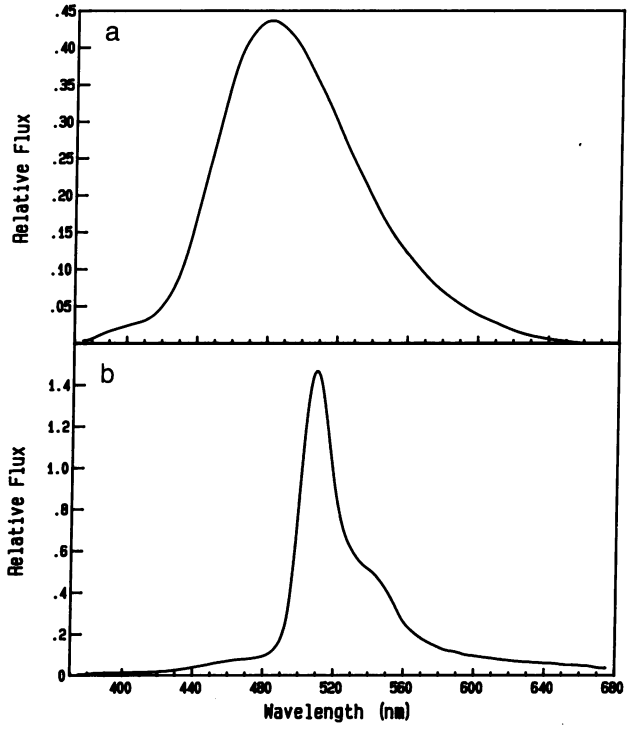

FIG. 6. Bioluminescence emission spectra generated with crude r-luciferase and r-luciferase plus GFP. Crude pTZRLuc-1 cell extracts were prepared, as described, and 100 μl, $\approx 1 \times 10^{-6}$ M r-luciferase, as determined by peak light emission, was used to generate each spectrum. (*a*) Emission spectrum of crude r-luciferase. (*b*) Spectrum that results when 1×10^{-6} M *Renilla* GFP is added to crude r-luciferase in energy-transfer buffer.

DISCUSSION

This work describes the isolation of a 1.2-kbp *R. reniformis* luciferase cDNA capable of directing the expression of r-luciferase. The cDNA contains an ORF encoding a 314-amino acid sequence in which all of the native luciferase peptide sequences obtained from V8-protease digestion are found. Rescreening the cDNA library with a 790-bp luciferase cDNA fragment as a hybridization probe has failed to produce other clones that contain the 5′ noncoding region; therefore, whether the luciferase cDNA is full-length is not known. This uncertainty can be resolved by sequencing genomic clones corresponding to the 5′ end of the luciferase gene.

The genomic Southern hybridization data indicates that *Renilla* luciferase is probably encoded by more than one gene, which may or may not contain intervening sequences. Further characterization of luciferase genomic clones will be required before the genetic organization of the luciferase gene(s) can be defined.

A putative initiation codon located at triplet position 4 of the ORF may be the translation initiation site for *Renilla* luciferase; the 311-amino acid sequence is essentially identical to native luciferase with respect to its composition and predicted molecular weight. Irrespective of whether this cDNA is full length, the luciferase that it encodes is expressed in pTZRLuc-1 cells and is catalytically active. The expression data demonstrate that r-luciferase is the same size as native luciferase on SDS/PAGE gels and is reactive with polyclonal rabbit antibodies raised against native *Renilla* luciferase. Expression of r-luciferase from the plasmid pTZRLuc-1 is "leaky" because activity can be detected from uninduced cell cultures. The luciferase cDNA ORF is not in frame with the short *lacZ′* ORF contained in this construct. Any translation product initiating at the β-galactosidase sequence of pTZRLuc-1 would be terminated at a stop codon immediately adjacent to the putative initiation codon in the luciferase cDNA. Thus, the r-luciferase seen in SDS/PAGE gels does not contain any β-galactosidase sequence. We propose that expression of r-luciferase by pTZRLuc-1 is due to a translation coupling mechanism (34).

r-luciferase displays two very important characteristics of native luciferase: the ability to catalyze coelenterazine oxidation with the concomitant emission of blue light (λ_{max} = 480 nm) and the ability to transfer energy to *Renilla* GFP with the production of green light (λ_{max} = 509 nm). The two emission bands at 400 nm and 480 nm in the r-luciferase spectrum verify the strong similarity between the native and recombinant proteins and suggest that the integrity of the luciferase active site has been maintained. Furthermore, that energy transfer occurs in the presence of GFP shows that the luciferase domain(s) required for the interaction between luciferase and GFP is present in r-luciferase. Once pure r-luciferase is available, energy transfer quantum yield measurements will offer a more quantitative determination of the efficiency of the nonradiative energy-transfer process. Finally, the data demonstrate that N-linked glycosylation is not required for luciferase activity because *E. coli* do not perform this modification (35). r-luciferase in *E. coli* crude extracts behaves like the native enzyme by every criterion examined thus far.

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