Polypeptide Folding and Dimerization in Bacterial Luciferase Occur by a Concerted Mechanism in Vivo†

Jenny J. Waddle, Timothy C. Johnston,‡ and Thomas O. Baldwin*

Department of Biochemistry and Biophysics, Texas A&M University, and The Texas Agricultural Experiment Station, College Station, Texas 77843

Received April 27, 1987; Revised Manuscript Received June 5, 1987

ABSTRACT: Bacterial luciferase is a heterodimeric enzyme comprising two nonidentical but homologous subunits, α and β, encoded by adjacent genes, *luxA* and *luxB*. The two genes from *Vibrio harveyi* were separated and expressed from separate plasmids in *Escherichia coli*. If both plasmids were present within the same *E. coli* cell, the level of accumulation of active dimeric luciferase was not dramatically less than within cells containing the intact *luxAB* sequences. Cells carrying the individual plasmids accumulated large amounts of individual subunits, as evidenced by two-dimensional polyacrylamide gel electrophoresis. Mixing of a lysate of cells carrying the *luxA* gene with a lysate of cells carrying the *luxB* gene resulted in formation of very low levels of active heterodimeric luciferase. However, denaturation of the mixed lysates with urea followed by renaturation resulted in formation of large amounts of active luciferase. These observations demonstrate that the two subunits, α and β, if allowed to fold independently in vivo, fold into structures that do not interact to form active heterodimeric luciferase. The encounter complex formed between the two subunits must be an intermediate structure on the pathway to formation of active heterodimeric luciferase.

Proteins composed of multiple folding domains generally fold in a fashion indicating that the individual domains fold independently and are interdependent only through the constraints imposed by the covalent continuity of the polypeptide chain [see Wetlaufer (1981) for a review]. To test this hypothesis, several investigators have studied the folding of proteolytic fragments comprising individual domains to determine the extent to which interdomain interactions alter the pathway and structure of the final product of the folding reaction. For example, the small S-peptide of ribonuclease A clearly contains sufficient information to properly fold and interact with the S-protein to form catalytically active ribonuclease S (Richards & Vithayathil, 1959; Kato & Anfinsen, 1969). However, to carry out comparable experiments with multisubunit enzymes is significantly more complicated, since it is not possible to study directly the folding of individual subunits in the absence of oligomerization of an oligomeric protein.

Bacterial luciferase is not an oligomeric enzyme, but rather a heterodimeric enzyme (Hastings et al., 1969). The active form of the enzyme is an $\alpha\beta$ dimer with a single active center confined primarily if not exclusively to the α subunit (Meighen et al., 1971; Cline & Hastings, 1972); the individual subunits show no detectable activity [Hastings et al., 1969; see Ziegler and Baldwin (1981) for a review]. The two subunits, α and β, are encoded by adjacent genes, *luxA* and *luxB*, respectively. The *luxAB* genes from *Vibrio harveyi* have been cloned (Baldwin et al., 1984) and completely sequenced (Cohn et al., 1985; Johnston et al., 1986) and are translated from a common mRNA (Miyamoto et al., 1985). The genes encoding the two subunits have been separated and expressed from separate compatible replicons under the control of unknown promoters (Gupta et al., 1985); however, the levels of accumulation of active luciferase in those studies were so low as to raise questions regarding the ability of the subunits produced from separate mRNA molecules to associate within the cell. We have therefore separated the two genes, *luxA* and *luxB*, such that they are both expressed from the *lac* promoter on separate pUC vectors.

The immediate goal of the studies reported here was to determine whether the individual subunits of bacterial luciferase can fold independently in vivo into forms approximating the structure achieved by the subunits in the active heterodimeric enzyme. The long-range objective is an understanding of the pathway in vivo of polypeptide folding and assembly of multisubunit enzymes.

Experimental Procedures

Materials. Restriction enzymes were purchased from New England Biolabs and Bethesda Research Laboratories. Phage T4 DNA ligase was obtained from New England Biolabs. Urea was of Ultra-Pure grade from Schwarz/Mann, and ampholines were purchased from LKB. Bacterial growth media were from Difco and Gibco. All other reagents and solvents were of the best commercial grade commonly available.

Bacterial Strains and Plasmids. The MC1009 strain of *Escherichia coli* is *recA*, *recB*, *recC*, *sbcA*, *gal*, *thi*, Δ*trp*, *tonB*, *rpsL*. MC1009 was the gift of Masayasu Nomura. The TB1 strain of *E. coli* is *hsdR*$^-$, *hsdM*$^+$, Δ(*lacproA,B*) (Baldwin et al., 1984). Circular restriction maps of all plasmids used are presented in Figure 1. Construction of plasmids pTB7, pTB718, and pTB104 was described previously (Baldwin et al., 1984).

† Supported by grants from the National Science Foundation (NSF DMB 85-10784), the National Institutes of Health (AG-03697), and the Robert A. Welch Foundation (A865) and by the State of Texas through the Texas Advanced Technology Research Program. The fermentation facilities used in these studies were supported by NIH Biomedical Research Support Instrumentation Grant SO1 RR01712 and DOD Instrumentation Grant P-20862-LS-RI.

* Address correspondence to this author at the Department of Biochemistry and Biophysics.

‡ Present address: Department of Biological Sciences, Murray State University, Murray, KY 42071.

0006-2960/87/0426-4917$01.50/0

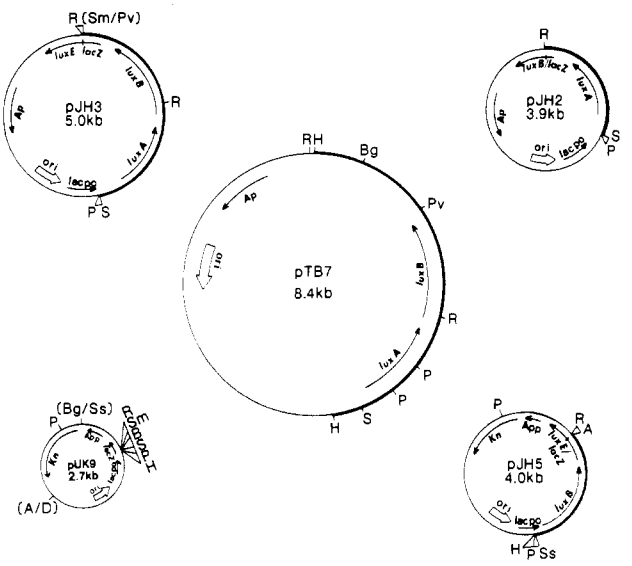

FIGURE 1: Plasmid constructions drawn as circular restriction maps. Gene fusions are shown as a line through the arrow, and the genes are named in the order of transcription. Constructions are described under Experimental Procedures. Abbreviations: A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; D, *Dra*I; H, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II; R, *Eco*RI; S, *Sal*I; Sm, *Sma*I; Ss, *Ssp*I.

Plasmid pJH1 was constructed by ligating the *Sal*I to *Bgl*II fragment containing *luxAB* from pTB718 into pUC9 cut with *Sal*I and *Bam*HI. Plasmid pJH2 was derived from pJH1 by deletion of the *Eco*RI fragment containing the *luxB* gene. Cells carrying pJH2 are Lac^+, Amp^r and produce the α subunit of luciferase.

Plasmid pJH3 was constructed by inserting the *Sal*I to *Pvu*II fragment of pTB7 encoding *luxA* and *luxB* into pUC9 cut with *Sal*I and *Sma*I. Cells carrying pJH3 are bioluminescent with addition of 1-decanal and are Lac^+.

Plasmid pUK9 was constructed by replacing the *Dra*I to *Ssp*I fragment of pUC9 with the 1-kbp[1] *Bgl*II to *Ava*I fragment of pSV2neo (Southern & Berg, 1982), which carries the Kan^r gene from Tn5 (Beck et al., 1982). Cells carrying pUK9 are Amp^s, Kan^r.

Plasmid pJH4 was constructed by cutting pUC9 with *Hin*cII and *Bam*HI and inserting the *Ssp*I to *Bgl*II fragment of pTB7, which contained only *luxB*. Plasmid pJH5 was constructed by digestion of both pJH4 and pUK9 with *Hin*dIII and *Ava*I, ligation, and selection of transformed cells on kanamycin. Cells carrying pJH5 are Lac^+, Kan^r and produce the β subunit of luciferase.

Methods employed in restriction and ligation were the commonly used approaches outlined by Maniatis et al. (1982). The media used were LB supplemented with carbenicillin (100 μg/mL) and/or kanamycin (30 μg/mL) to maintain selective pressure on the cells carrying recombinant plasmids. Cells harboring recombinant plasmids were placed in long-term storage at −70 °C.

Growth Conditions. Cells taken from permanent stock were used to start overnight cultures in LB medium with antibiotics. After being shaken overnight at 37 °C, these cultures were used to inoculate flasks (125 mL) containing sterile media (40 mL) to an initial OD_{600} of 0.025; the cultures were aerated by shaking (100 rpm) in a water bath at 37 °C. Cell density was monitored at 600 nm with 1-cm cuvettes. Samples were pelleted and frozen at −20 °C for assays in vitro of luciferase activity.

Cell Lysis and Luciferase Assays. Cell pellets were resuspended in 500 μL of 0.5 M NaH_2PO_4/K_2HPO_4, 1 mM DTT, and 1 mM EDTA, pH 7.0, incubated with 10 μL of 10 mg/mL lysozyme for 10 min, and sonicated with three 5-s pulses. The luciferase activity in the lysates was determined by the standard $FMNH_2$ injection assay (Hastings et al., 1978). Two photomultiplier photometers were used in these studies: the Turner Designs Model TD-20e luminometer and an instrument comparable to that described by Mitchell and Hastings (1971). The light standard of Hastings and Weber (1963) was used to calibrate the instruments. The TD-20e luminometer had a sensitivity of 3.66×10^5 quanta s^{-1} (light unit)$^{-1}$ (LU), and the Mitchell–Hastings photometer had a sensitivity of 7.58×10^9 quanta s^{-1} (LU)$^{-1}$.

Two-Dimensional Polyacrylamide Gel Electrophoresis. Proteins in cell lysates were resolved by two-dimensional polyacrylamide gel electrophoresis as described by O'Farrell (1975) and modified by Parker and Friesen (1980). Cells were lysed as described, giving a total lysate volume of 400 μL for each sample. An aliquot of 75 μL of each lysate was used for analysis. The first dimension was isoelectric focusing in urea with a pH range of 5–7; the second dimension was 10% polyacrylamide in sodium dodecyl sulfate containing buffers. Coomassie blue stained gels were photographed with an orange filter.

Subunit Complementation in Vivo and in Vitro. Subunit complementation in vivo was analyzed by growing cells containing both pJH2 (α subunit, Amp^r) and pJH5 (β subunit, Kan^r). Subunit complementation in vitro was studied by mixing lysates of cells carrying the individual plasmids. Cells of *E. coli* strain MC1009, carrying both pJH3 and pUK9, both pJH2 and pJH5, pJH2 alone, and pJH5 alone, were grown to an OD_{600} of 1. The cells contained in 10 mL of each culture were pelleted by centrifugation, resuspended in 1 mL of 0.5 M NaH_2PO_4/K_2HPO_4, 1 mM DTT, and 1 mM EDTA, pH 7.0, and lysed in a French pressure cell (SLM Aminco). Supernatants were obtained by centrifugation of lysates in an Eppendorf microfuge at 5 °C for 5 min. Aliquots (200 μL) of lysates or supernatants from cells containing both α and β subunits (pJH3/pUK9 or pJH2/pJH5) were incubated at 5 °C for 24 h; aliquots (200 μL) of lysates or supernatants from cells containing the α subunit alone (pJH2) and the β subunit alone (pJH5) were mixed (final volume 400 μL) prior to incubation. After incubation, aliquots were assayed for luciferase activity.

Renaturation of Prefolded Subunits. *E. coli* cells (MC1009) carrying plasmids pJH3 and pUK9, pJH2 and pJH5, pJH2 alone, or pJH5 alone were allowed to grow overnight and were diluted to an OD_{600} of 0.1. Cells were allowed to grow to an OD_{600} of 1, and 1-mL aliquots were pelleted and frozen at −20 °C. The pellets of cells carrying two plasmids were resuspended in 350 μL of lysis buffer (0.1 M NaH_2PO_4/K_2HPO_4, 1 mM EDTA, 1 mM DTT, pH 7.0); cells carrying pJH2 alone and pJH5 alone were resuspended together in a total of 350 μL of lysis buffer. Cells were disrupted on ice by five 1-s pulses from a Branson probe sonicator. Aliquots (100 μL) of the resulting lysates were diluted 1:5 with 10 M urea in lysis buffer, giving a final concentration of 8 M urea, and incubated at room temperature for 20 min. Native lysates were diluted 1:250 into recovery buffer (0.5 M NaH_2PO_4/K_2HPO_4, 1 mM EDTA, 1 mM DTT, 0.2% bovine serum albumin, pH 7.0), and the denatured lysates were diluted 1:50 into recovery buffer, giving an overall dilution of

[1] Abbreviations: kbp, kilobase pair; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; $FMNH_2$, reduced flavin mononucleotide; LU, light unit; EF-Tu, elongation factor Tu.

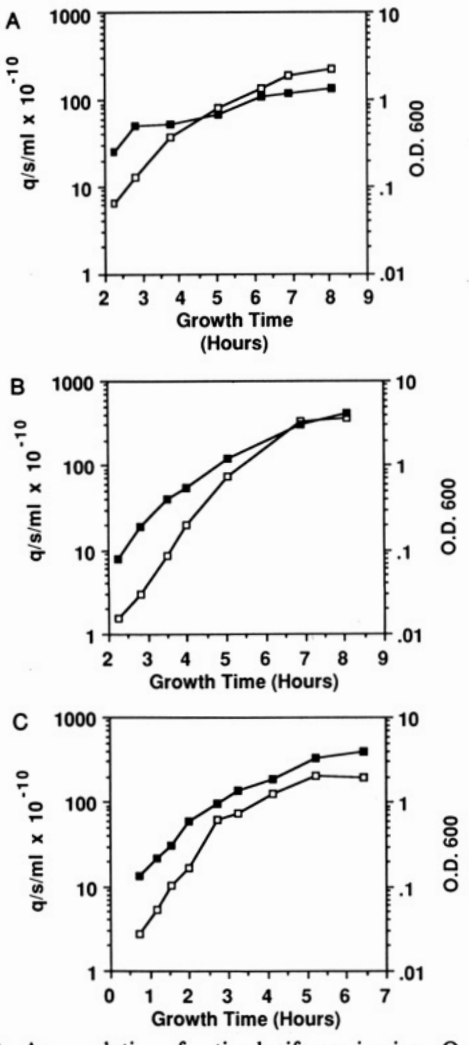

FIGURE 2: Accumulation of active luciferase in vivo. Quanta per second per milliliter was measured by luciferase assays in vitro (open squares). Cell density was measured by absorbance at 600 nm (closed squares). Growth conditions are described under Experimental Procedures for the following cultures: (A) MC1009/pJH3/pUK9, (B) MC1009/pJH2/pJH5, and (C) TB1/pTB7.

protein of 1:250 and a final urea concentration of 0.16 M urea. After 22-h incubation at 5 °C, the solutions were assayed for luciferase activity according to the flavin injection method.

RESULTS

Plasmid Constructions (*Figure 1*). Plasmids pJH2 (α subunit, *Amp*^{r}), pJH5 (β subunit, *Kan*^{r}), and pJH3 ($\alpha\beta$, *Amp*^{r}) were all *Lac*$^+$. The construction of pJH2 allowed an in-frame fusion of the N-terminal 11 residues of the β subunit of luciferase to the α fragment of β-galactosidase at the *Eco*RI site of pUC9 [see Johnston et al. (1986)]. In pJH3 the fusion of the *Pvu*II site downstream from *luxB* in pTB7 with the *Sma*I site of pUC9 resulted in a fusion of the N-terminal 37 residues of the *luxE* gene product to the α fragment of β-galactosidase, resulting in the *Lac*$^+$ phenotype in the background of TB1. In pJH5, the *Bgl*II/*Bam*HI fusion resulted in fusion of the N-terminal 71 residues of the *luxE* gene product [see Johnston et al. (1986)] to the α fragment of β-galactosidase. All three plasmids expressed excellent α-complementing β-galactosidase activity.

Accumulation of Active Luciferase in Vivo. Accumulation of active heterodimeric luciferase transcribed and translated from adjacent genes in cells was compared with luciferase produced in trans from separate plasmids. In all constructions, expression was from the *lac* promoter of the parent pUC plasmids. Plasmid copy number was uncontrolled (Twigg & Sherratt, 1980; Stewart et al., 1986) and not determined. Maintenance of both pJH2 and pJH5 was assured by growth on media containing both kanamycin and carbenicillin. Cells containing pJH3, which encodes both subunits on tandem genes, also contained pUK9, the parent kanamycin resistance conferring plasmid of pJH5, thereby allowing comparisons between cells carrying the separated *luxA* and *luxB* genes and the *luxAB* construction.

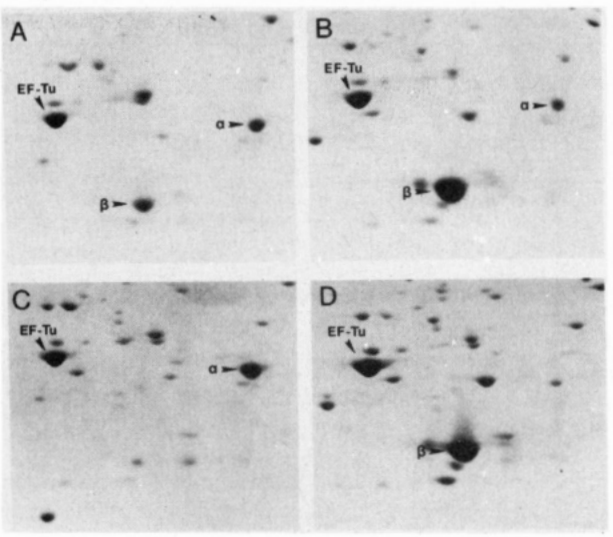

FIGURE 3: Analysis of subunit accumulation by two-dimensional polyacrylamide gel electrophoresis. Regions of interest from Coomassie blue stained gels are shown. Positions of α and β are indicated with arrows, while host protein EF-Tu is marked as a point of reference. Samples are from (A) MC1009/pJH3/pUK9 harvested at an OD_{600} of 1.3, (B) MC1009/pJH2/pJH5 harvested at an OD_{600} of 1.9, (C) MC1009/pJH2 harvested at an OD_{600} of 1.1, and (D) MC1009/pJH5 harvested at an OD_{600} of 2.1.

The results of measurements of luciferase activity in lysates of cells harvested at various times during growth are presented in Figure 2. Figure 2A shows that cells carrying both pJH3 (*luxAB*) and pUK9 grow slowly after reaching an OD_{600} of about 0.5. The cultures did not reach densities greater than an OD_{600} of about 2.0. By comparison, cells carrying both pJH2 (*luxA*) and pJH5 (*luxB*) (Figure 2B) reached optical densities greater than 4.0. Maximal accumulation of active luciferase per milliliter of culture was similar in the two cultures. The differences in growth characteristics were highly reproducible. The growth and luciferase activity in cells carrying the parent plasmid pTB7 are shown in Figure 2C.

Analysis of Subunit Accumulation by Two-Dimensional Polyacrylamide Gel Electrophoresis. Four cultures of cells carrying recombinant plasmids, (A) pJH3 and pUK9, (B) pJH2 and pJH5, (C) pJH2, and (D) pJH5, were grown in Luria broth, and cells contained in 1 mL were harvested by centrifugation and frozen at −20 °C. Cells were lysed, and proteins were resolved by two-dimensional polyacrylamide gel electrophoresis as described under Experimental Procedures. Identical volumes (75 μL) of each lysate were loaded on the first-dimension gels. The results are shown in Figure 3. The locations of the luciferase α and β subunits were determined in two ways: by Western blot analysis (Towbin et al., 1979) with antiluciferase antibody and by spiking samples with purified luciferase.

The polypeptides from *E. coli* carrying both pJH3 (*luxAB*) and pUK9 are shown in Figure 3A. The α and β subunits (arrows) were present in roughly comparable amounts. The polypeptides contained in *E. coli* carrying both pJH2 (*luxA*)

Table I: Subunit Complementation in Vivo and in Vitro

lysates[a]	activity (quanta s^{-1} mL^{-1})	% pJH3/pUK9 lysate activity
pJH3/pUK9[b]	1.54×10^{12}	100
pJH2/pJH5[b]	2.89×10^{11}	18.7
pJH2 + pJH5[c]	2.00×10^{10}	1.3

[a] Cells carrying the plasmids indicated were grown to an OD_{600} of 1, harvested, and lysed as described under Experimental Procedures. [b] Aliquots (200 μL) of the lysates of cells carrying both pJH3 and pUK9, or both pJH2 and pJH5, were incubated for 24 h at 5 °C. [c] Aliquots (200 μL) of the lysates from cells carrying pJH2 alone and from cells carrying pJH5 alone were mixed (total volume 400 μL) and incubated for 24 h at 5 °C.

and pJH5 (*luxB*) are presented in Figure 3B. It is clear in the photograph that the amount of β subunit was much greater than the amount of α subunit; comparison with Figure 3A indicates that the levels of α subunit in the two lysates were similar. The polypeptides contained in *E. coli* carrying pJH2 (*luxA*) are shown in Figure 3C. The α subunit was clearly one of the major proteins in the cell, comparable to the level of EF-Tu. The polypeptides within *E. coli* carrying pJH5 (*luxB*) are shown in Figure 3D. The quantity of β subunit contained in the lysate was actually greater than the level of EF-Tu. No effort was made to precisely quantitate subunits present on the gels.

Subunit Complementation in Vivo and in Vitro. The ability of subunits encoded on separate plasmids within a single cell to interact and fold to form active luciferase was presented in Figure 2B. To determine whether the subunit synthesized and folded in the absence of its normal partner retained the ability to complex with its normal partner, lysates were prepared of cells carrying pJH2 only, pJH5 only, and both pJH2 and pJH5, as described under Experimental Procedures. The lysates of cells containing the individual subunits (pJH2 for α, pJH5 for β) were mixed and incubated at 5 °C for 24 h for comparison with similarly incubated lysates of cells containing both subunits, transcribed either from the same plasmid (pJH3) or from separate plasmids (pJH2/pJH5). The results, which are presented in Table I, were the same for lysates and for supernatants of centrifuged lysates (data not shown).

Subunit association to form active enzyme occurred in the cells containing both subunits, and active luciferase formed was stable during a 24-h incubation at 5 °C. Cell lysates after incubation had essentially the same activity as assays in vitro of cells taken at the same OD_{600} during growth experiments. The highest expression of activity, 1.54×10^{12} quanta s^{-1} mL^{-1}, was from cells carrying pJH3 (α, β) and pUK9 (Table I). This activity is comparable to the activity in fully induced *V. harveyi* (Miyamoto et al., 1987) or in *E. coli* carrying pTB7 (Figure 2C). The level of luciferase activity in cells carrying *luxA* and *luxB* in trans (pJH2 and pJH5) ranged in different experiments from about 20% to 40% of the activity in cells carrying both genes on a single plasmid (pJH3). The lower activity may be accounted for by differences in plasmid copy number, since the copy number of the two plasmids was uncontrolled and not determined. Since luciferase is a heterodimer, the activity would be limited by the limiting subunit, which appears to be α (Figure 3). Furthermore, the luciferase expression seems to be maximal at different stages of cell growth in the two cell types (Figure 2).

As shown in Table I, the activity obtained upon mixing separate lysates containing the α (pJH2) and β (pJH5) subunits was only 1.3% of that in the lysate of cells containing pJH3 (*luxAB*), or 7% of that in the lysate from cells containing *luxA* (pJH2) and *luxB* (pJH5) in trans, in spite of the fact that the lysates containing the individual subunits appeared to have higher subunit concentrations (Figure 3). The results shown in Table I suggest that luciferase subunits encoded in trans can fold and associate productively in vivo, presumably before folding is complete, but that, in vitro, the presumably completely folded individual subunits cannot associate efficiently into active dimers.

Table II: Renaturation of Prefolded Subunits

lysates[a]	denaturant	activity (quanta s^{-1} mL^{-1})	% activity recovered
pJH3/pUK9[b]	none	4.80×10^{11}	
	urea	1.94×10^{11}	40
pJH2/pJH5[b]	none	1.63×10^{11}	
	urea	5.45×10^{10}	33
pJH2 + pJH5[c]	none	$<3.14 \times 10^{8}$	
	urea	3.80×10^{10}	70[d]

[a] Cells carrying the plasmids indicated were grown to an OD_{600} of 1, harvested, and lysed as described under Experimental Procedures. [b] Aliquots of the lysates of cells carrying both pJH3 and pUK9, or both pJH2 and pJH5, were denatured in 8 M urea, diluted into recovery buffer, and assayed following incubation for 22 h at 5 °C. Control samples were not treated with urea. [c] Aliquots of cells carrying pJH2 alone and of cells carrying pJH5 alone were mixed, lysed, and treated as in footnote *b* above. [d] Activity recovered is relative to renatured pJH2/pJH5.

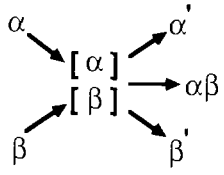

FIGURE 4: Model for concerted folding and dimerization of luciferase. α and β are new partially or completely synthesized subunits. [α] or [β] represents a partially folded intermediate. With complementation, active luciferase (αβ) is formed. Without complementation, noninteractive folded conformation(s) result(s) (α' and/or β').

Renaturation of Prefolded Subunits. To determine if the luciferase subunits seen in the two-dimensional gels of cells carrying pJH2 alone or pJH5 alone could participate in the formation of active luciferase, cells carrying the individual plasmids were mixed and disrupted by sonication, and the proteins were denatured by addition of 8 M urea. Upon renaturation, the mixed lysate attained 70% of the luciferase activity relative to a renatured lysate of cells carrying both plasmids (Table II) and 20% of the luciferase activity of a renatured lysate of cells carrying pJH3 (*luxAB*). This experiment suggests that the failure of undenatured prefolded subunits to interact is not due to covalent modification, such as proteolysis, but that one or both of the individual subunits appear to be conformationally incompetent for dimer formation. It should be stressed that the concentrations of individual subunits were not determined. These results should therefore not be viewed as a quantitative determination of the levels of synthesis of individual luciferase subunits but rather as a qualitative determination that the luciferase subunits are capable of refolding into active heterodimeric luciferase following denaturation in urea.

Discussion

The primary conclusion to be drawn from the experiments reported here, described diagrammatically in Figure 4, is that in vivo the luciferase subunits must interact as either unfolded or partially folded structures, and the final steps in folding must occur by a concerted pathway involving a dimeric structure. If the subunits are allowed (or obliged) to fold independently in vivo, one or the other or both fold into a structure that appears to be experimentally irreversible. That is, mixing of

lysates containing folded subunits results in only a very slow recovery of very small amounts of active luciferase. Cells carrying both plasmids accumulate large amounts of active luciferase, while cells carrying the individual plasmids accumulate large amounts of the individual subunits. Mixing of lysates of cells containing the separate subunits results in formation of very low levels of active heterodimeric luciferase. However, denaturation of proteins within the mixed lysates with urea, followed by renaturation, resulted in formation of large amounts of active luciferase. These studies indicate that the two subunits, α and β, interact in vivo as partially folded structures and that the final steps of folding occur within the heterodimeric structure. If the two subunits are allowed to fold in vivo in the absence of their usual partners, the structure(s) attained is (are) incapable of interacting to form active heterodimeric enzyme even following prolonged incubation times.

Nearly 15 years ago, it was demonstrated that the α and β subunits from *V. harveyi* luciferase (then called "MAV"), purified by column chromatography in 5 M urea, could be renatured separately by diluting out the urea and incubating for 48 h; subsequent mixing of the two presumably completely refolded subunits permitted recovery of 41% of the activity of the untreated native enzyme (Gunsalus-Miguel et al., 1972). The apparent inability, demonstrated in the current studies, of the individual subunits prefolded in vivo to subsequently complement (associate to form active dimer) efficiently in vitro suggests that the conformations of one or both of the individual subunits prefolded in vivo must be different from the conformation of the subunit(s) following unfolding in urea and refolding in buffer.

The second conclusion from this study is that the individual subunits of luciferase appear to be sufficiently stable in *E. coli* to accumulate to high levels. In our original publication of the cloning and expression of the *luxAB* genes in *E. coli*, we reported that we could not detect α subunit in lysates of cells carrying the *luxA*-containing plasmid pTB104 (Baldwin et al., 1984). We have since been able to detect a very low level of α subunit in cells carrying pTB104. In the *luxA*-containing plasmid described in this paper (pJH2), transcription of the *luxA* gene occurred from a *lac* promoter with an in-frame protein fusion between the luciferase β subunit fragment and the α fragment of β-galactosidase. Cells carrying the plasmid pJH2 were Lac^+, giving us confidence that transcription and translation were occurring from the construction. Large amounts of the luciferase α subunit were found in lysates of cells carrying pJH2. Likewise, the *luxB*-containing plasmid (pJH5) and the *luxAB*-containing plasmid (pJH3) were both constructed to give in-frame fusions of the *luxE* gene with the α fragment of β-galactosidase. We have no explanation for the low level of accumulation of α subunit in cells carrying pTB104, but clearly cells carrying pJH2 accumulate large amounts of α subunit.

By forcing both pJH2 and pJH5, carrying Amp^r and Kan^r, respectively, to replicate and express within the same cell, we have confirmed the earlier observations of Gupta et al. (1985). In their construction, the levels of expression of luciferase were disturbingly low, ca. 1000-fold lower than the levels reported here. However, their conclusion that luciferase subunits produced from separate transcripts within the cell could form active luciferase appears to be accurate.

ACKNOWLEDGMENTS

We are deeply indebted to Dr. Miriam M. Ziegler for her advice, suggestions, and criticisms and for her assistance in reading of the manuscript.

REFERENCES

Baldwin, T. O., Berends, T., Bunch, T. A., Holzman, T. F., Rausch, S. K., Shamansky, L., Treat, M. L., & Ziegler, M. M. (1984) *Biochemistry 23*, 3663–3667.

Beck, E., Ludwig, G., Auerswald, E. A., Reiss, B., & Schaller, H. (1982) *Gene 19*, 327–336.

Cline, T. W., & Hastings, J. W. (1972) *Biochemistry 11*, 3359–3370.

Cohn, D. H., Mileham, A. J., Simon, M. I., Nealson, K. H., Rausch, S. K., Bonam, D., & Baldwin, T. O. (1985) *J. Biol. Chem. 260*, 6139–6146.

Gunsalus-Miguel, A., Meighen, E. A., Nicoli, M. Z., Nealson, K. H., & Hastings, J. W. (1972) *J. Biol. Chem. 247*, 398–404.

Gupta, S. C., O'Brien, D., & Hastings, J. W. (1985) *Biochem. Biophys. Res. Commun. 127*, 1007–1011.

Hastings, J. W., & Weber, G. (1963) *J. Opt. Soc. Am. 53*, 1410–1415.

Hastings, J. W., Weber, K., Friedland, J., Eberhard, A., Mitchell, G. W., & Gunsalus, A. (1969) *Biochemistry 8*, 4681–4689.

Hastings, J. W., Baldwin, T. O., & Nicoli, M. Z. (1978) *Methods Enzymol. 57*, 135–152.

Johnston, T. C., Thompson, R. B., & Baldwin, T. O. (1986) *J. Biol. Chem. 261*, 4805–4811.

Kato, I., & Anfinsen, C. B. (1969) *J. Biol. Chem. 244*, 1004–1007.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Meighen, E. A., Nicoli, M. Z., & Hastings, J. W. (1971) *Biochemistry 10*, 4062–4068.

Mitchell, G. W., & Hastings, J. W. (1971) *Anal. Biochem. 39*, 243–250.

Miyamoto, C. M., Graham, A. D., Boylan, M., Evans, J. F., Hasel, K. W., Meighen, E. A., & Graham, A. F. (1985) *J. Bacteriol. 161*, 995–1001.

Miyamoto, C., Byers, D., Graham, A. F., & Meighen, E. A. (1987) *J. Bacteriol. 169*, 247–253.

O'Farrell, P. H. (1975) *J. Biol. Chem. 250*, 4007–4021.

Parker, J., & Friesen, J. D. (1980) *Mol. Gen. Genet. 177*, 439–445.

Richards, F. M., & Vithayathil, P. J. (1959) *J. Biol. Chem. 234*, 1459–1465.

Southern, P. J., & Berg, P. (1982) *J. Mol. Appl. Gen. 1*, 327–341.

Stewart, G. S. A. B., Lubinsky-Mink, S., Jackson, C. G., Cassel, A., & Kuhn, J. (1986) *Plasmid 15*, 172–181.

Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.

Twigg, A. J., & Sherratt, D. (1980) *Nature (London) 283*, 216–218.

Wetlaufer, D. B. (1981) *Adv. Protein Chem. 34*, 61–92.

Ziegler, M. M., & Baldwin, T. O. (1981) *Curr. Top. Bioenerg. 12*, 65–113.