STRUCTURE, FOLDING AND MECHANISM OF BACTERIAL LUCIFERASE

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Introduction

The utility of bacterial luciferase, as well as that of other luciferases and luminescence-associated proteins and enzymes, as a diagnostic tool has been long appreciated. Its value as a means to explore basic principles of biological structure and function has also been widely appreciated and exploited. Recent advances in our understanding of this intriguing enzyme should enhance its utility both as a diagnostic tool and as an instrument of discovery of biochemical and biophysical principles. The structure of the enzyme, an αβ heterodimer, has been determined at high resolution (1), as has the structure of the β_2 homodimer (2, 3), a kinetically stable structure that is most intriguing from a biophysical perspective but has no known biological function (4). The kinetic mechanism of the refolding and assembly reactions has been elucidated, allowing detailed investigations of the processes of chaperone-mediated folding and cotranslational protein folding (5-7). Finally, recent advances in our understanding of the mechanism of the light-producing reaction catalyzed by the enzyme should be of value in refining current diagnostic procedures and in development of the next generation of applications of bacterial luciferase.

Materials and Methods

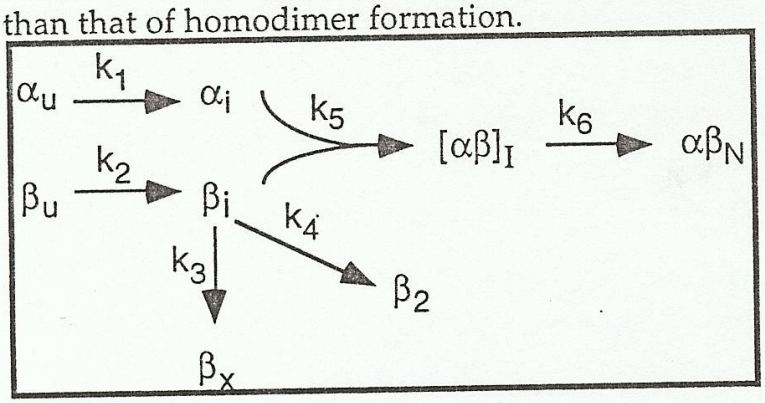
The luciferase used in these experiments was purified from *E. coli* carrying a recombinant plasmid that encodes the *lux*A and *lux*B genes from *Vibrio harveyi* (8). The β_2 homodimer was purified from *E. coli* carrying a similar plasmid that encoded only the *lux*B gene (4). The methods of purification and assay have been reported elsewhere (9).

Results and Discussion

The functional form of bacterial luciferase is an αβ heterodimer (10). The two subunits are homologous (11), and not surprisingly, they assume the same three dimensional fold, the well-known $(\beta/\alpha)_8$ or TIM barrel fold (1). The $(\beta/\alpha)_8$ barrel fold begins with a β strand that is followed by a loop, an α-helix that returns to the N-terminal end of the β-strand, and a second loop to bring the polypeptide back adjacent to the beginning of the first β-strand. The second β-strand is then parallel to the first, and this process continues through 8 β-strands, which form a barrel in the middle of the structure, and 8 α-helices that are on the exterior surface of the protein. In all known $(\beta/\alpha)_8$ barrel enzymes, the active center is comprised of residues within the loops that connect the C-terminal ends of the β-strands to the α-helices (2). Bacterial luciferase is known to have a single active center on the α subunit (10). Even though the structure that has been determined does not have the flavin substrate bound, we can be confident of the location of the active center by analysis of the locations of the sites of mutations and of chemical modification reactions that modify the activity of the enzyme (13). The reactive thiol of the α subunit, α106, as well as a series of residues shown to reside in or near the active center, surround a central cavity in the α subunit at the C-terminal ends of the β-strands, as would be expected based on other $(\beta/\alpha)_8$ barrel enzymes.

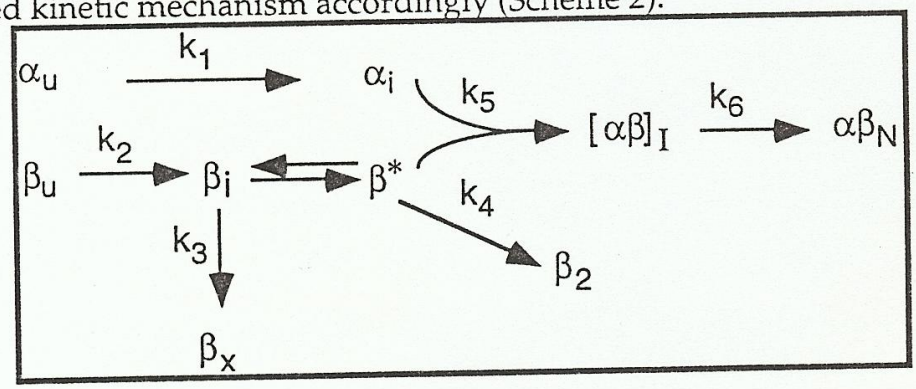
The function of the β subunit in the bioluminescence reaction remains a mystery. It appears that no residues in the β subunit contribute directly to the active center, but the association between the two subunits appears to stabilize a conformation of the α subunit that is not well-represented in the population of conformers that exist for the free α subunit. This hypothesis is supported by recent advances in our study of the folding and assembly of the subunits of the enzyme.

Detailed investigations of the folding and assembly of the active, heterodimeric form of bacterial luciferase have resulted in a proposed kinetic mechanism (Scheme 1). The individual subunits fold upon dilution from urea-containing buffers through multiple kinetic steps to yield dimerization-competent species, α_i and β_i. These species assemble to yield a dimeric but enzymatically inactive form, $[\alpha\beta]_I$, which isomerizes to yield the active heterodimer, αβ. The second order rate constant for the dimerization reaction (k_5) appears to be rather slow, 2400 $M^{-1} \cdot s^{-1}$ (5), and the homodimerization reaction (k_4) yielding β_2 is even slower, ca. 150 $M^{-1} \cdot s^{-1}$ (14). Thus, formation of the native enzyme appears to be under kinetic control. That is, the native enzyme is formed rather than β_2 because the rate of the heterodimerization reaction is faster than that of homodimer formation.



Scheme 1.

A plausible explanation for the slow rate of heterodimer assembly came from studies of the chaperone-mediated folding of the luciferase subunits (6). The chaperone GroEL can bind both α_i and β_i, but it does not bind β_2, $[\alpha\beta]_I$ or αβ (6). In a refolding reaction containing GroEL, GroES, α_i and β_i, and ATP, the reduction in the concentrations of α_i and β_i (due to binding by GroEL) should have slowed the rate of formation of the active enzyme accordingly. However, the chaperone did not slow the rate of formation of the active luciferase, suggesting that the structure of one or both of the subunits after release from the chaperone was somehow different from the α_i and β_i forms. Other experiments showed that the rate enhancement was due to the β subunit, not the α subunit (6). These observations have caused us to modify the proposed kinetic mechanism accordingly (Scheme 2).



Scheme 2

This modified kinetic mechanism incorporates an equilibrium between two forms of the β subunit, β_i and β^*. At equilibrium, the predominant form is β_i, while the form that associates with the α subunit is β^*. Due to the low concentration of the β^* species, the rate of the association reaction would be expected to be very slow. Likewise, the rate of the homodimerization reaction would be expected to be exceedingly slow. The role of the chaperone then is to redistribute the β subunit equilibrium, supplying the β^* form for association with the α subunit. The bimolecular reaction of α with the β_i form appears to occur near the diffusion limit (6). In the absence of the α subunit, the β^* form isomerizes back to the β_i form.

These studies on the folding and assembly of the luciferase heterodimer have been greatly facilitated by the heterodimeric structure and by the exquisite sensitivity, ease and enormous dynamic range of the bioluminescence reaction. Bacterial luciferase is an excellent model system for investigating the mechanisms by which flavoproteins can activate triplet state oxygen for reaction with a variety of biological molecules. The bacterial luciferase-catalyzed reaction is one of the more thoroughly studied flavoprotein monooxygenase reactions, but the mechanism of light emission has remained unclear.

Our understanding of the kinetic mechanism of the bacterial luciferase-catalyzed reaction is now well developed (15, 16). Of general interest in the study of bioluminescent reactions in general, many of which show single turnover reactions, is the fact that the kinetic fate of an apparent long-lived intermediate in the bacterial bioluminescence reaction has been misinterpreted for many years. When reduced flavin mononucleotide is injected into a solution of bacterial luciferase, O_2 and a long chain aldehyde, the intensity of emitted light rises rapidly to a peak and decays, apparently exponentially. It has been *assumed* that the decay of bioluminescence parallels the decay of a specific intermediate (17), specifically the C4a peroxydihydroflavin intermediate (18). The detailed investigation of the kinetic mechanism showed that the decay of the light is in fact a very complicated function of numerous rate constants. Studies with bacterial luciferase, and probably with other luciferases, that ascribe changes in the rate of decay of bioluminescence to a specific chemical step should be regarded as tentative unless a thorough kinetic analysis has been undertaken.

Recently, investigation of the effects of isotopic substitution in the aldehyde substrate on the kinetics of the reaction has allowed elimination of certain proposed mechanisms as inconsistent with the data (16). The dioxirane mechanism remains viable but not proven (19). It is noteworthy that model chemical studies have shown that decay of dioxiranes can elicit luminescence from fluorescent sensitizers (20).

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