(S6-D) Reaction Mechanism of Bacterial Bioluminescence

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Bioluminescent organisms are widely distributed and comprise a diverse set of species, such as bacteria, dinoflagellates, fungi, fish, insects and squid. The light emitting reaction in bacteria involves the concomittant oxidation of reduced Flavin Mononucleotide and long chain fatty aldehyde by molecular oxygen into oxidized Flavin Mononucleotide and coresponding fatty acid with emission of blue-green light peaked at around 490 nm catalyzed by bacterial luciferase (1).

Substrate Supply

The flavin mononucleotide is reduced again by NAD(P)H:FMN oxidoreductase using NADH or NADPH from electron transport pathway, and fatty acid is reduced to corresponding fatty aldehyde (usually tetradecanal) by fatty acid reductase complex composed of three different proteins, a reductase (γ), a transferase (δ), and a synthetase (ε) which are encoded in lux operon as Lux C, D, and E, respectively, using 1 NADPH and ATP as electron and energy source (2). Although NAD(P)H or flavin are very well known electron carrier in biological oxidoreduction reaction, this bacterial luminescence system use very unusual electron carrier, fatty acid/fatty aldehyde pair. The conversion of oxidation energy into photoenergy occur at the active site of bacterial luciferase.

Bacterial luciferase

Luciferases of all luminous bacterial species so far studied are

homologous heterodimeric protein consisting of two subunits, α and β, with molecular weight of ca. 40,000 and 35,000, respectively. These two polypeptides and fatty acid reductase complex with 3 polypeptides are encoded in lux operon in the order of luxC, luxD, luxA, luxB, and luxE. For light emission, bacterial luciferase, reduced FMN, fatty aldehyde and molecular oxygen are the minimum necessity. Redued FMN binds to luciferase to form intermediate I which in turn reacts with molecular oxygen generating intermediate II (L'ase:FMN-4a-OOH) which shows a remarkable stability permitting its isolation and characterization even at room temperature (3). The key step in bacterial bioluminescence reaction is the reaction of this intermediate II and fatty aldehyde at the active site of luciferase producing fatty acid and electronically excited L'ase:FMNH-4a-OH (intermediate IV) which converts into free luciferase, FMN and water after light emission. In the absence of fatty aldehyde, II decays to form FMN and hydrogen peoxide with little or no light emission. Hastings and collegues have proposed the formation of FMNH-4a-hydroperoxyhemiacetal (III) of aldehyde by reaction of II and fatty aldehyde and consequent breakdown of III via Baeyer-Villiger type degradation leading to the direct formation of the siglet electronically excited IV as the first (primary) excited species. However, some new findings now call for a reevaluation of this key aspect.

Color Tuning Protein in luminescence System

In the presence of a lumazine protein (LP) which is also found to be encoded in lux operon as luxL, the bioluminescence kineics are changed, quantum yield is increased and, most importantly, the emission blue shifted (475 nm emission maximum). Since this is an endogonic shift, it cannot be attributed to a Forster energy transfer from electronically excited IV (490

nm emission maximum) to LP (4). An FMN-containing yellow fluorescence protein (YFP) has also been found to affect the luciferase bioluminescence kinetics, intensity, and color. Although YFP has a lower electronically excited enegy level than IV, energy transfer from IV* to YFP cannot account for the observed spectral shift to 540 nm (5). Instead of normal FMN, 2-thio FMN was used as a substrate which shows about 5 % light intensity and 560 nm emission maximum, and still YFP can shift the emission spectrum to 540 nm (endogonic shift like lumazine protein case).

Baeyer-Villiger .vs. CIEEL

A series of 8-substituted FMN were used as a substrate, and contrary to what expected in Baeyer-Villiger type cleavage the general trend was the more electron donating group was substituted at 8-position, the faster the reaction velocity is. Chemically Initiated Electron Exchange Luminescence (CIEEL) mechnism which was accepted as one of major chemiluminescence reaction mechanism was suggested as a new model for bacterial bioluminescence raction supported by all previous results.

Fatty acid as a Primary excited species ?

CIEEL mechanism support the possibility that fatty acid can be generated as a electronically excited product as well as IV*, and this excited fatty acid was proposed as a primary excited product which can transfer its energy to any energy acceptor including IV, LP and YFP (6). Other proposal includes the formation of 3-membered dioxirane intemediate which behaves as a energy carrier although it is not electronically excited species itself (7,8). To test this proposal, novel fluorescent fatty aldehyde (parinaric aldehyde) are developed as mechanistic probes for the

mechanism of bacterial bioluminescence reaction. The parinaric aldehyde has a fluorescence emission maximum at around 430 nm which was expected to be obseved if fatty acid was formed as a primary excited product. Two type of parinaric aldehydes were prepared (cis-parinaric aldehyde and trans-parinaric aldehyde) and although both were good substrate of bacterial luciferase, neither of them shows any 430 nm peak or shoulder neglecting the proposal of fatty acid as a primary excited product (9).

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