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Crystallization and preliminary diffraction studies of firefly luciferase from *Photinus pyralis*

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Abstract

Firefly luciferase is a 62 kDa molecular weight enzyme which catalyzes a light-emitting reaction. Crystals of *Photinus pyralis* luciferase have been obtained by the microbatch technique, using polyethylene glycol as a precipitating agent. Firefly luciferase crystallizes as long needles which belong to the tetragonal space group $P4_12_12$, with cell dimensions $a = 119.5$, $b = 119.5$, $c = 95.4$ Å. One molecule is present in the asymmetric unit. Diffracted intensities beyond 2.0 Å resolution have been measured from frozen crystals using synchrotron radiation.

1. Introduction

Luciferase is the generic name for enzymes involved in the production of light from bioluminescent organisms, such as insects, bacteria and marine coelenterates. Luciferase enzymes catalyze the oxidation of a substrate to an excited-state product, which then decays to the ground state emitting a photon of light. The oxidation reaction is carried out in various species by different luciferase enzymes, requiring different substrates and cofactors and proceeding through different reaction pathways (Hastings, 1983).

Firefly luciferase (E.C. 1.13.12.7) is a 62 kDa molecular weight protein, which is functional in solution as a monomer (DeWet, Wood, Helinski & DeLuca, 1985). It is located in specialized peroxisomes present in the lanterns of fireflies (Keller, Gould, DeLuca & Subramani, 1987) and has evolved for their nocturnal mating behaviour. In the presence of MgATP and molecular oxygen, the enzyme converts its physiological substrate, luciferin, into an electronically excited species, which emits visible light (DeLuca & McElroy, 1978). The enzyme first activates luciferin (Luc-COOH) to form an enzyme-bound luciferyl adenylate (E:Luc—CO—AMP), which is then oxidized to produce yellow-green light ($h\nu$) and the enzyme-bound product oxyluciferin (E:Luc=O).

$$\text{E} + \text{Luc}-\text{COOH} + \text{ATP} \longrightarrow \text{E:Luc}-\text{CO}-\text{AMP} + \text{PP}_\text{i}$$

$$\text{E:Luc}-\text{CO}-\text{AMP} + \text{O}_2 \longrightarrow \text{E:Luc}{=}\text{O} + \text{CO}_2 + \text{AMP} + h\nu.$$

Firefly luciferase efficiently converts chemical energy into light with a quantum yield of 0.88 (McElroy & Seliger, 1960).

The formation of an enzyme-bound adenylate is analogous to the activation of amino acids and fatty acids catalyzed by aminoacyl-tRNA synthetases and fatty-acid CoA ligases, respectively. Firefly luciferase shows no sequence similarity with aminoacyl-tRNA synthetases, but shares extensive sequence homology with acyl-CoA synthetases such as fatty-acid CoA ligases (Suzuki *et al.*, 1990), acetic-acid CoA ligases (Toh, 1991) and 4-coumarate CoA ligases (Schroder, 1989). Sequence similarity with enzymes such as gramicidin S synthetase and tyrocidine synthetase involved in the synthesis of linear and cyclic polypeptides in fungi and bacteria has also been reported (Toh, 1990).

Firefly luciferase has had a long history of use as a tool in molecular and cell biology, especially for detection of ATP and as a reporter of genetic function, because of the convenience and sensitivity of its luminescence assay (Gould & Subramani, 1988). It has also been used as a model to study possible protein–anaesthetic interactions, being one of the few soluble proteins known to be competitively inhibited by a wide range of general anaesthetic molecules (Franks & Lieb, 1984).

2. Crystallization

Initial crystallization trials were carried out with firely luciferase purified from the lanterns of the North American firefly *Photinus pyralis*, by an affinity chromatography-based technique (Branchini, Marschner & Montemurro, 1980). Although salting-in crystallization conditions were previously reported (McElroy, 1960), the crystals were too small for diffraction analysis. Needle-like crytals of luciferase were obtained using polyethylene glycol (PEG) as precipitating agent. Luciferase aggregates at low ionic strength, and relatively high concentrations of salts such as phosphate or sulfate were necessary to reduce the amorphous precipitation.

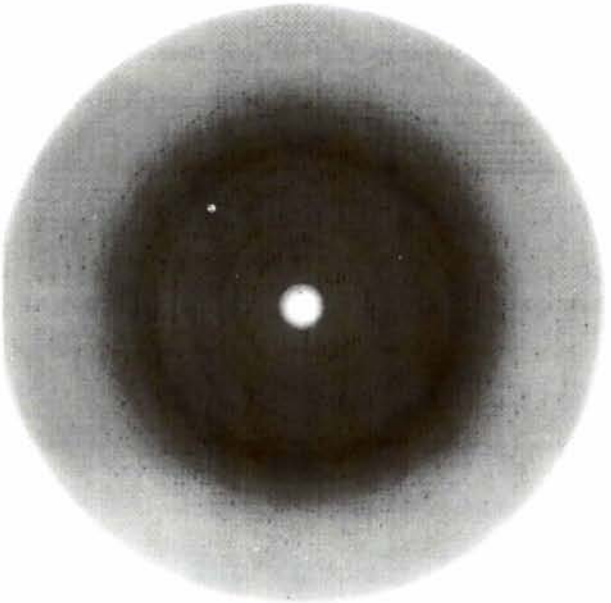
Crystallization experiments with recombinant protein purchased from Promega Corporation (DeWet *et al.*, 1985) resulted in bigger crystals and less precipitate. Since the enzyme isolated from firefly lanterns and the recombinant protein look indistinguishable on denaturing sodium dodecyl sulfate (SDS) gels, the improved quality of the crystals might be attributed to the presence of high concentrations of glycerol and ethylene glycol in the storage conditions of the recombinant protein. The use of glycerol or polyhydric alcohols as cosolvents in crystallization has been reported to increase protein solubility, and the crystal stability relative to the amorphous phase (Sousa & Lafer, 1990). The microbatch method proved to be advantageous for the stability of crystals. Crystals grown by batch under oil were stable for at least one month, whereas crystals grown by vapour diffusion disintegrated to an amorphous precipitate after a few days. Samples were dispensed and incubated at 277 or 283 K in microtitre plates filled with paraffin oil (Chayen, Shaw Stewart & Blow, 1992). The best crystals grew when 2 µl droplets of the recombinant protein at a concentration of 20 mg ml^{-1} in 200 m*M* ammonium sulfate, 1 m*M* EDTA, 1 m*M* DTT, 10% glycerol, 25% ethylene glycol, 25 m*M* Tris pH 7.8 were mixed with 2 µl of 500–540 m*M* lithium sulfate, 26%(*w*/*v*) PEG 8000, 100 m*M* Tris pH 7.8. All the materials were of the highest purity grade and the solutions were filtered through a 0.22 µl filter before use. Despite shock-nucleation problems associated with the microbatch technique, which resulted in

variable amount of nucleation, clusters of needles grew within a fortnight, usually with a cross-section of 0.04–0.08 mm and up to 1.5 mm long (Fig. 1). They were stable for a few days when transferred into a harvesting solution containing 100 m*M* lithium sulfate, 16%(*w*/*v*) PEG 8000, 5% glycerol, 12.5% ethylene glycol, 100 m*M* Tris pH 7.8 at 291 K. Larger crystals with cross-section of 0.15 to 0.20 mm could occasionally be obtained, but they were mechanically very fragile and easily cracked upon handling.

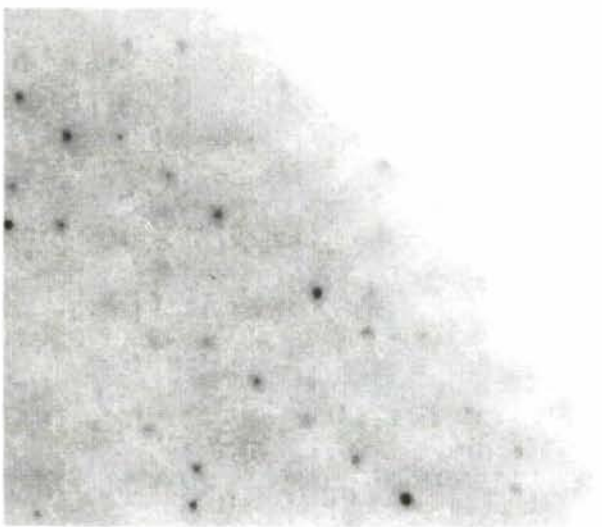
3. Crystal characterization

Firefly luciferase crystals give diffraction consistent with space group $P4_12_12$ (or its enantiomorph $P4_32_12$), with cell dimensions $a = b = 119.5$, $c = 95.4$ Å. Assuming one molecule per asymmetric unit, the specific volume $V_m = 2.8$ Å^3 Da^{-1} of protein corresponds to a solvent content of 56%(*v*/*v*) (Matthews, 1968). The crystals are extremely susceptible to X-ray radiation and room-temperature data collection did not prove feasible, the crystals lasting less than 30 min in the X-ray beam. To perform data collection at cryogenic temperatures, the crystals were transferred into the harvesting solution, allowed to equilibrate and then introduced for a few minutes into a cryoprotectant solution containing 8%(*w*/*v*) PEG 8000, 10% glycerol, 12.5% ethylene glycol and 100 m*M* Tris pH 7.8. Crystals were frozen using standard techniques (Teng, 1990) in a stream of nitrogen gas at 100 K produced by an Oxford Cryosystem. Only low-resolution (5 Å) data could be obtained from these small, weakly diffracting crystals using graphite-monochromated Cu $K\alpha$ radiation from an Enraf–Nonius FR671 rotating-anode source. Intense and highly collimated radiation from a synchrotron source gave a greatly improved signal-to-noise ratio and allowed higher resolution diffraction data to be measured. A complete data set to 2.7 Å resolution was collected at SRS (Daresbury) with low mosaicity and merging *R* factor of 5.8%. The images were evaluated using a modified version of *MOSFLM* (Leslie, personal communication) for processing image-plate data and the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994) was used in the data reduction. Diffracted intensities beyond 2.0 Å resolution (Fig. 2) were measured at DESY (Hamburg), using a large crystal frozen directly from the crystallization drop, thus avoiding the handling damage upon harvesting. Structure determination is in progress.

Fig. 1. Tetragonal crystals of firefly luciferase with a cross-section up to 0.06 mm, which have been grown by the microbatch technique under oil.



(*a*)



(*b*)

Fig. 2 (*a*) A 1° rotation image collected from a frozen firefly luciferase crystal on a 30 cm MAR Research image-plate system at DESY (Hamburg), using a wavelength of 0.86 Å and a crystal-to-detector distance of 320 mm. (*b*) An enlargement of the image in (*a*) showing the 2.0 Å diffraction at the edge of the plate.

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