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Preparation and preliminary study of crystals of the recombinant calcium-regulated photoprotein obelin from the bioluminescent hydroid *Obelia longissima*

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Crystals of recombinant obelin, the Ca^{2+}-regulated photoprotein from the marine hydroid *Obelia longissima*, have been grown from sodium citrate solutions. Crystals grow as hexagonal light-yellow rods ($0.1 \times 0.1 \times 1.0$ mm) which diffract to beyond 1.8 Å with synchrotron radiation of 1.0 Å wavelength. The crystals have a primitive hexagonal lattice with unit-cell parameters a = 81.55, c = 86.95 Å. The asymmetric unit contains two molecules. This represents the successful preparation of single crystals of a photoprotein obelin which have promising diffraction properties.

1. Introduction

The term 'photoprotein' was introduced to designate a certain class of self-contained bioluminescent systems that do not fit the classical pattern, in which the enzyme luciferase reacts with a diffusible substrate, the luciferin (Shimomura & Johnson, 1966). For the majority of photoproteins, only the addition of calcium ions is required to generate the bioluminescence and they are therefore referred to as 'calcium-activated photoproteins' (Hastings & Morin, 1969). Later, the term 'calcium-regulated photoproteins' was suggested – firstly because calcium regulates the function of these proteins but is not essential for it and secondly because these proteins are similar in many ways to calcium-regulated effector proteins such as calmodulin and troponin C (Blinks, 1986).

The first photoprotein to be discovered was aequorin, isolated from the jellyfish *Aequorea forskalea (victoria)* (Shimomura *et al.*, 1962). Aequorin apparently has a tightly bound ligand, coelenterazine peroxide, and the addition of calcium allows an oxidative decarboxylation to generate the product in its first excited singlet state, from which the radiative emission occurs (Shimomura & Johnson, 1978; Musicki *et al.*, 1986). This product, coelenteramide, readily dissociates from the protein on removal of the calcium. The apoprotein can be 'recharged' by incubating it with synthetic coelenterazine in the presence of molecular oxygen and a sulfhydryl reagent (Shimomura & Johnson, 1975).

Similar but distinct photoproteins have been identified in a variety of marine organisms, mostly coelenterates (Morin, 1974). The one under study here is obelin from the marine hydroid *Obelia longissima*. Obelin consists of a single polypeptide chain of 22.2 kDa (Bondar *et al.*, 1992; Illarionov *et al.*, 1995). The primary sequences of obelin, aequorin and other photoproteins are highly homologous and they presumably generate bioluminescence by a common chemical mechanism (Tsuji *et al.*, 1995; Ohmiya & Hirano, 1996). There is also sequence homology in regions corresponding to the EF-hand structures of calcium-binding proteins such as calmodulin and troponin C (Tsuji *et al.*, 1995), suggesting that some resemblance in three-dimensional structures might also ensue. Obelin, along with a number of other photoproteins, is available in an efficient expression system (Illarionov *et al.*, 1999).

The main application of photoproteins has been for the detection of calcium in biological systems, and aequorin was the first really successful intracellular calcium indicator of any kind. Photoproteins have now been used successfully in a great many different types of living cells both to estimate the intracellular calcium concentration under steady-state conditions and to study the role of calcium transients in the regulation of cellular function (Blinks *et al.*, 1982). Recently, it has also been shown to be possible to measure free calcium in different cell compartments by the expression of the cDNA of the photoprotein along with some appropriate target sequence (Rizzuto *et al.*, 1998; Maechler *et al.*, 1999).

In spite of extensive applications of bioluminescence systems in biomedical research and diagnostics, little protein structural information is available. The three-dimensional crystal structures of the bacterial luciferase (Fisher *et al.*, 1995) and firefly luciferase (Conti *et al.*, 1996) have been published. The structure of a calcium-regulated photoprotein is a very desirable goal because it represents a stable enzyme intermediate and would be relevant to the reaction mechanism. Recently, crystals of recombinant aequorin were grown and

diffracted beyond 2.2 Å (Hannick *et al.*, 1993). However, no structural analysis has appeared so far.

Here, we describe the preparation and some characteristics of crystals of the photoprotein obelin which have promising diffraction properties.

2. Materials and methods

High-purity recombinant obelin was obtained according to the previously described procedure (Illarionov *et al.*, 1999) with small modifications. Apo-obelin was converted to obelin with synthetic coelenterazine (Prolume Ltd). For crystallization, the protein was exchanged into a buffer containing 10 m*M* potassium/sodium phosphate, 1 m*M* EDTA pH 7.4 by gel filtration on a BioGel P2 column and was concentrated to approximately 10 mg ml^{-1} using Amicon Centricon-10 tubes. Protein used for crystallization was homogeneous according to LC–electrospray ionization mass spectrometry. Crystals were grown by the hanging-drop vapor-diffusion technique using equal volumes of protein (5 µl) and precipitant (5 µl) solutions in the drop. The best precipitant was 1.4 *M* sodium citrate at pH 6.0. Crystals grow as light-yellow hexagonal rods (Fig. 1) of approximate dimensions 0.1 × 0.1 × 1.0 mm after 7–30 d at 277 K.

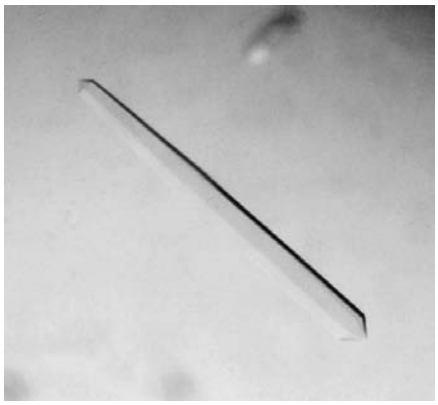

Figure 1
Crystal of the photoprotein obelin, grown from 1.4 *M* sodium citrate. Approximate dimensions are 0.1 × 0.1 × 1.0 mm.

3. Results and discussion

For the preliminary X-ray analysis, a cut crystal measuring 0.1 × 0.1 × 0.2 mm was suspended in a fiber loop containing a minimal amount of mother liquor (Teng, 1990) and flash-cooled (Hope, 1988) to 100 K with no cryo-protectant. The crystal diffracted to beyond 1.8 Å resolution as recorded on an Area Detector System Corporation Quantum 4 CCD detector (Fig. 2) at beamline 5.0.2, Advanced Light Source (ALS), Berkeley National Laboratory using 1.0 Å X-rays. The crystal-to-detector distance was 150.0 mm and measurements were taken over a 180° rotation of the crystal in 1° steps. Data processing was carried out using *HKL* 1.9.1 (Otwinowski & Minor, 1997) and the results indicated that the crystals belong to a primitive hexagonal lattice with a = 81.55, c = 86.95 Å. Examination of the distribution of intensities for the 00l reflections indicated the space group to be $P6_2$ or $P6_4$. The Matthews coefficients (V_m; Matthews, 1968) are calculated to be 3.79 or 1.90 Å Da^{-1} for one or two molecules per asymmetric unit, which correspond to 68 or 35% solvent content, respectively. The structure determination is currently in progress.

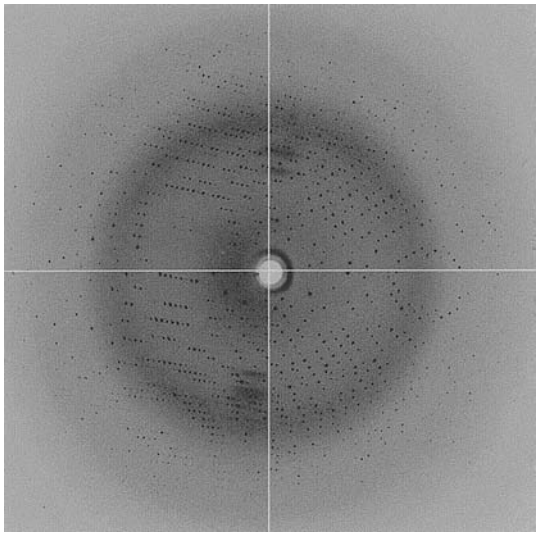

Figure 2
The diffraction pattern for the obelin crystals recorded on a Quantum 4 CCD detector at beamline 5.0.2 of the Advanced Light Source (ALS), Berkeley National Laboratory. The crystals diffract to beyond 1.8 Å.

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References

Blinks, J. R. (1986). *The Sarcoplasmic Reticulum in Muscle Physiology*, Vol. II, edited by M. L. Entman & W. B. Van Winkle, pp. 73–107. Boca Raton, FL: CRC Press.
Blinks, J. R., Wier, W. G., Hess, P. & Prendergast, F. G. (1982). *Prog. Biophys. Mol. Biol.* **40**, 1–114.
Bondar, V. S., Trofimov, K. P. & Vysotski, E. S. (1992). *Biochemistry (Moscow)*, **57**, 1020–1027 (in Russian).
Conti, E., Franks, N. P. & Brick, P. (1996). *Structure*, **4**, 287–298.
Fisher, A. J., Raushel, F. M., Baldwin, T. O. & Rayment, I. (1995). *Biochemistry*, **34**, 6581–6586.
Hannick, L. I., Prasher, D. C., Schultz, L. W., Deschamps, J. R. & Ward, K. B. (1993). *Proteins*, **15**, 103–107.
Hastings, J. W. & Morin, J. G. (1969). *Biochem. Biophys. Res. Commun.* **37**, 493–498.
Hope, H. (1988). *Acta Cryst.* B**44**, 22–26.
Illarionov, B. A., Bondar, V. S., Illarionova, V. A. & Vysotski, E. S. (1995). *Gene*, **153**, 273–274.
Illarionov, B. A., Frank, L. A., Illarionova, V. A., Bondar, V. S., Vysotski, E. S. & Blinks, J. R. (1999). In the press.
Maechler, P., Kennedy, E. D., Sebo, E., Valeva, A., Pozzan, T. & Wollheim, C. B. (1999). *J. Biol. Chem.* **274**, 12583–12592.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Morin, J. G. (1974). *Coelenterate Biology: Reviews and New Perspectives*, edited by L. Muscatine & H. M. Lenhoff, pp. 397–438. New York: Academic Press.
Musicki, B., Kishi, Y. & Shimomura, O. (1986). *J. Chem. Soc. Chem. Commun.* **21**, 1566–1568.
Ohmiya, Y. & Hirano, T. (1996). *Chem. Biol.* **3**, 337–347.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rizzuto, R., Pinton, P., Carrington, W., Fay, F. S., Fogarty, K. E., Lifshitz, L. M., Tuft, R. A. & Pozzan, T. (1998). *Science*, **280**, 1763–1766.
Shimomura, O. & Johnson, F. H. (1966). *Bioluminescence in Progress*, edited by F. H. Johnson & Y. Haneda, pp. 495–521. Princeton University Press.
Shimomura, O. & Johnson, F. H. (1975). *Nature (London)*, **256**, 236–238.
Shimomura, O. & Johnson, F. H. (1978). *Proc. Natl Acad. Sci. USA*, **75**, 2611–2615.
Shimomura, O., Johnson, F. H. & Saiga, Y. (1962). *J. Cell Comput. Physiol.* **59**, 223–240.
Teng, T. Y. (1990). *J. Appl. Cryst.* **23**, 387–391.
Tsuji, F. J., Ohmiya, Y., Fagan, T. F., Toh, H. & Inouye, S. (1995). *Photochem. Photobiol.* **62**, 657–661.