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Groel crystal growth and characterization

E. Pechkova, S. Tripathi, R. Spera, C. Nicolini*

Nanoworld Institute, CIRSDNNOB-University of Genova and Fondazione Elba, Rome, Italy

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ABSTRACT

Single crystals of ribosomal proteins obtained for the first time by Langmuir–Blodgett (LB) nanotemplate confirm earlier findings (Pechkova et al., 2008), pointing to a new generation of bionanomaterials of unique structure–function relationship. The ribosomal protein phage GroEL was overexpressed in *E. coli*. Since these protein's samples have some difficulties by classical vapour diffusion method to yield optimal diffraction quality and order (GroEL), the LB nanotemplate method has been applied and compared to the classical method. With the thin film nanotemplate method large phage GroEL crystals appeared in few days and were subsequently characterized by MALDI-TOF Mass Spectroscopy and by a very preliminary X-ray diffraction.

1. Introduction

Since some protein have some difficulties to be crystallized by classical crystallization method, the recently developed Langmuir–Blodgett nanotemplate crystallization method (Pechkova and Nicolini, 2004) can be applied. This method in principle could allow also to improve the crystal quality and radiation stability (Pechkova et al., 2004).

Phage growth λ E large (GroEL) in the *E. coli* cytoplasm, the first member of hsp60 family to be identified, was recognized as a chromosomally encoded product whose deficiency resulted in defective morphogenesis of bacteriophage and T4 head structures and T5 tail structures, suggesting a role in protein assembly (Horwich and Willison, 1993). Hsp60 is a well characterized chaperone mainly localized in mitochondria of eukaryotic cells (Cheng et al., 1989; Martin et al., 1993; Soltys and Gupta, 1996). Hsp60, also known as phage growth λ E large (GroEL) in bacteria, is involved in the folding and assembly of polypeptide chains into oligomeric complexes. Despite the protein was widely studied (Fersht, 1996), in some conditions the crystal possess low diffraction quality and appear to be quite disordered (M. Garber, personal communication).

Indeed, it is sometimes difficult and/or time-consuming to obtain crystals of suitable size for single crystal X-ray diffraction measurements. Whenever proteins are difficult to crystallize and to optimally diffract, powder diffraction technique serves indeed as an important tool to predict the space group of protein crystals and can give us a range of complementary information, which is difficult to get from single crystal X-ray diffraction techniques (Von Dreele, 2006; Margiolaki et al., 2005).

In our hands, LB nanotemplate method have shown prominent results and properties in protein crystallization in some cases, namely for human CK2 alpha kinase (Pechkova et al., 2003), Cytochrome P450scc (Nicolini and Pechkova, 2006) and lysozyme (Pechkova and Nicolini, 2001; Pechkova et al., 2005). Recently even the microcrystals of other ribosomal proteins (Pechkova et al., 2008) have been obtained and their diffraction analysis is being probed by synchrotron radiation (e.g. by microfocussed beamline, Riekel, 2000; Riekel et al., 2005) or at the powder diffraction beamlines (Margiolaki et al., 2005), Peaks obtained in powder diffraction analysis depend on the microstructure of materials and thus accurate unit cell information can be obtained even from poorly diffracting ribosomal proteins crystals (this issue discussed in details in the separate communication by Tripathi et al., 2008).

2. Materials and Methods

2.1. Expression and Purification Procedure

The phage GroEL protein was overproduced by M. Garber group in Protein Research Institute (Puschino, Russia) in bacterial expression system. *E. coli.* BL21(DE3) transformed with the plasmid carrying GroEL gene was grown at 37 °C to the cell density of 0.5–0.7 A_{600} in 2xTY medium supplemented with 100 μl/ml ampicillin. Expression of GroEL was induced by IPTG adding (final concentration 1 mM), followed by 3 h culture growth at 37 °C. Harvested cells (2 g) were suspended in 10 ml of buffer A (50 mM Tris–HCl, pH 7.8, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol), and sonicated. The cell debris was removed from the cell lysate by centrifugation (16,000 × *g* for 20 min). Ammonium sulfate powder was added to the supernatant up to 1.5 M and the protein solution was loaded onto Butyl-Toyopearl column (10 ml) preliminary equilibrated by buffer A containing 1.5 M ammonium sulfate. Ten column volumes of buffer A were used for impurities washing out. The protein was eluted by buffer A containing 200 mM NaCl and 25% ethanol. Fractions containing

* Corresponding author at: Nanoworld Institute, University of Genova, Corso Europa 30, 16132 Genova, Italy. Tel.: +39 010 35338220; fax: +39 010 35338217.
E-mail address: manuscript@ibf.unige.it (C. Nicolini).

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GroEL were combined, concentrated up to 20–30 mg/ml using "Vivaspin" concentrator 100 kDa and subjected to crystallization (M. Garber, personal communication).

2.2. Crystallization by Classical Vapour Diffusion and LB Nanotemplate Method

A thin film (Langmuir–Blodgett) LB nanotemplate method was applied (Pechkova and Nicolini, 2004).

Thin protein films of GroEL were prepared by LB Teflon trough with surface pressure control system. The protein solution were spread on the air-buffer interface using the Hamilton syringe, the volume of the purified and concentrated protein was about 100 μl. The protein monolayer was compressed by two Teflon barrier up to the surface pressure 20 mN/m and transferred to the cleaned glass cover slide by the modification of the Langmuir–Blodgett technique—Langmuir–Schaeffer method (horizontal lift). After that, the protein monolayer was dried in the nitrogen flux and the second monolayer was deposited onto the first one in the same way. Well-shaped isotherm (dependence of the surface pressure π from the barrier position (Pechkova and Nicolini, 2003) (not shown) were obtained and more than 20 template prepared at the chosen constant surface pressure conditions.

In the LB nanotemplate method, the droplet with protein solution and the crystallizing agent was placed on the thin protein film, deposited on the siliconized glass cover slide. The droplet was equilibrated against the reservoir solution contains the crystallizing agent with the concentration twice as much as in the droplet, as it is usually done by classical vapour diffusion method.

For crystallization the sample of GroEL with concentration 15 mg/ml was prepared in the following buffer: 50 mM Tris–HCl pH 8, 200 mM NaCl (buffer A). The preliminary screening for crystallization conditions was carried out using classical hanging drop vapour diffusion technique. Initially, crystalline precipitation were observed in solution containing 100 mM Hepes, pH 7.5, 10% PEG 8000, 8% ethylene glycol (Hampton research Screen II No. 37) (M. Garber, personal communication). The various trials were performed in order to optimize these conditions.

3. Mass Spectrometry

We used matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS, Bruker) to monitor the purity of proteins solutions of crystallographic interest. These solutions had been preventively dialyzed and/or the proteins had been precipitated in a solution of trichloroacetic acid to eliminate any trace of glycerol, salt and detergent that prevent the protein ionization.

The protein samples were diluted in a 0.1% (v/v) TFA solution. The matrix used for the mass spectrometric analysis was a saturated solution of acid (α-cyano-4-hydroxycinnamic acid for light proteins and sinapinic acid for heavy proteins, Bruker Daltonics) dissolved in 2/3 of 0.1% (v/v) TFA and 1/3 of acetonitrile. 1.5 μl of matrix solution was mixed with 1.5 μl of sample, then 1 μl of this mixture is spotted onto a suitable aluminium plate and air-dried.

MALDI-MS spectra were acquired in positive ion linear mode using an Autoflex mass spectrometer (Bruker Daltonics) externally calibrated using a solution of protein of known masses' resulting in a mass accuracy <100 ppm for intact proteins.

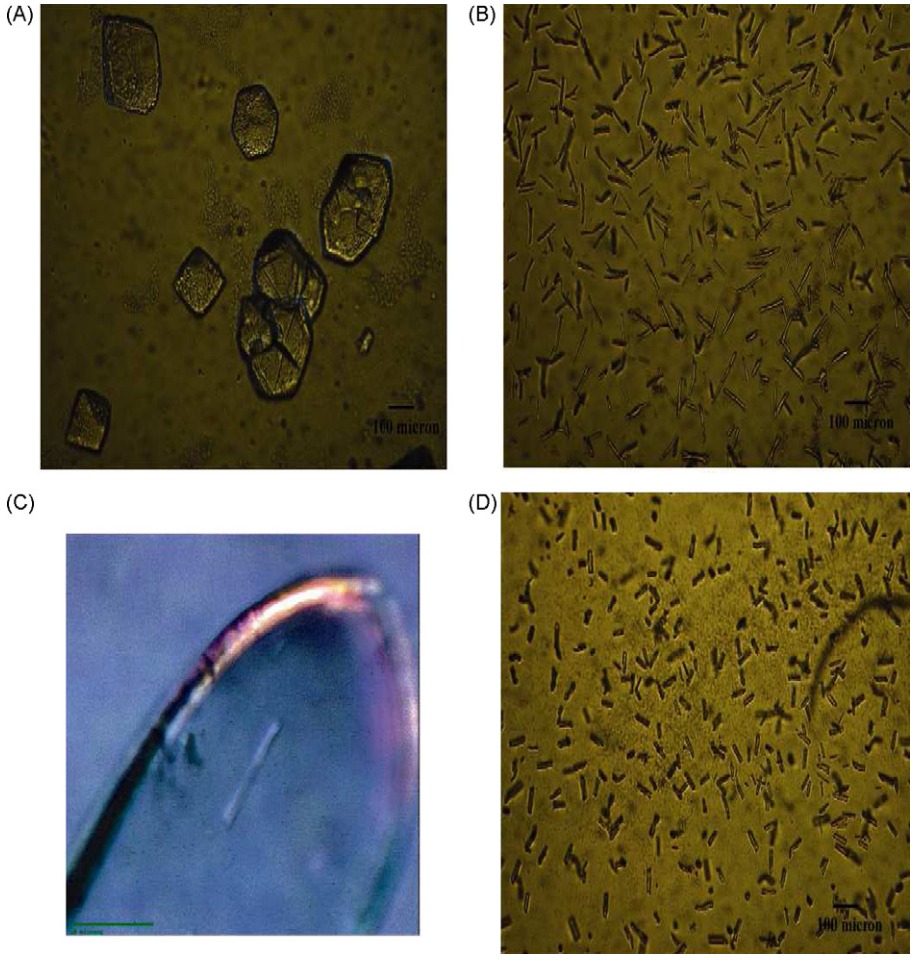

Fig. 1. (A) GroEL crystals, obtained by LB nanotemplate method under the light microscope (crystals dimensions are about 200 μm × 200 μm × 100 μm); (B) GroEL needle microcrystals, obtained by LB nanotemplate method under the light microscope (crystals dimensions are about 100 μm × 10 μm × 10 μm); (C) one of the needle microcrystal mounted in the cryoloop, bar corresponds to 50 μm); (D) GroEL crystals obtained by classical hanging drop method (crystals dimensions are about 50 μm × 5 μm × 5 μm).