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Controlling Diffusion in Gel-Tube Method

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'Gel-Tube' is a method for a protein crystallization using a simplified counter-diffusion technique [1], based on gel-acupuncture method [2] which allows for higher possibility of crystallization in a single experiment than the conventional method such as vapour-diffusion or batch method. In Gel-Tube method, a gel in a silicon tube, through which protein and precipitant solution diffuse each other from opposite direction, is attached to the end of a capillary. If combined with 1-dimensional (1-D) simulation, it is possible to estimate the diffusion process inside the capillary and to design crystallization conditions in a short time. We have applied this method for space experiment since 2004, resulted in shortening time required for optimization of crystallization condition and yielding high quality crystals. In the practical point of view for space experiment, higher density of the crystallization device was required. We have developed a crystallization device of twice as much crystallization cells as original one, using gel-tube method. For obtaining crystals in space one to two weeks after the sample loading on ground, slower diffusion of protein and precipitant molecules are preferable. Therefore, we are developing new device for controlling diffusion in a capillary for better crystallization.
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Crystallographic and X-ray diffraction analysis of metalloenzymes from purple sulphur bacterium

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The cytochromes and the hydrogenases are ubiquitous proteins present in all living organisms and involved in a variety of intracellular processes that are essential for life. Most notable is their participation in electron transfer reactions, usually as components of a complex reaction pathway, necessary for the production of energy either through oxidation of metabolites or via photosynthesis [1]. The cytochromes consist of two heme molecules in single polypeptide chain with classical Cys-X-Y-Cys-His heme binding sites. It is the first cytochrome of its class that comes from an anaerobic organism. Due to their important function, it is of essential interest to study structural features of metalloenzymes using X-ray crystallography.
Cytochrome c_4 (cyt c_4) and hydrogenase from the purple sulphur photosynthetic bacterium *Thiocapsa roseopersicina* were isolated and purified according to [2]. Cyt c_4 was crystallized using standard crystallization methods based on vapor diffusion [3] and advanced crystallization method based on the counter-diffusion [4]. Crystallization trials were performed at 20°C. The most suitable concentration of protein 10 mg/ml was found. The first suitable crystal growth was observed at pH 6.0 [Figure 1] using the addition of metal ions - Cu^{2+}, Cd^{2+}, Co^{2+}, Ba^{2+} (Hampton Research Additive Screen).

Fig. 1

Colored crossbred plates of holoprotein crystals with dimensions of approximately 200 x 50 x 30 μm grew within 3-4 days under several conditions.
The monocrystals of cyt c_4 were tested at the home source diffractometer at LEC (University of Granada) and measured at synchrotron DESY (Hamburg), beamline X11. Structure of cyt c_4 will be solved using molecular replacement method.
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