Available online at www.sciencedirect.com
ScienceDirect
Journal of Structural Biology 157 (2007) 437–442
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

Crystallization note

Two-dimensional crystallization of human vitamin K-dependent γ-glutamyl carboxylase

Ingeborg Schmidt-Krey [a,*], Winfried Haase [b], Vasantha Mutucumarana [c], Darrel W. Stafford [c], Werner Kühlbrandt [b]

[a] *Georgia Institute of Technology, School of Biology, 310 Ferst Drive, Atlanta, GA 30332-0230, USA*
[b] *Max-Planck-Institute of Biophysics, Department of Structural Biology, Max-von-Laue-Str. 3, 60438 Frankfurt am Main, Germany*
[c] *The University of North Carolina at Chapel Hill, Department of Biology and Center for Thrombosis and Hemostasis, Chapel Hill, 27599-3280, USA*

Received 15 June 2006; received in revised form 8 August 2006; accepted 8 August 2006
Available online 15 August 2006

Abstract

Planar-tubular two-dimensional (2D) crystals of human vitamin K-dependent γ-glutamyl carboxylase grow in the presence of dimyristoyl phosphatidylcholine (DMPC). Surprisingly, these crystals form below the phase transition temperature of DMPC and at the unusually low molar lipid-to-protein (LPR) ratio of 1, while 2D crystals are conventionally grown above the phase transition temperature of the reconstituting lipid and significantly higher LPRs. The crystals are up to 0.75 μm in the shorter dimension of the planar tubes and at least 1 μm in length. Due to the planar-tubular nature of the crystals, two lattices are present. These are rotated by nearly 90° in respect to each other. The ordered arrays exhibit $p12_1$ plane group symmetry with unit cell dimensions of $a = 83.7$ Å, $b = 76.6$ Å, $\gamma = 91°$. Projection maps calculated from images of negatively stained and electron cryo-microscopy samples reveal the human vitamin K-dependent γ-glutamyl carboxylase to be a monomer.

Keywords: Human vitamin K-dependent γ-glutamyl carboxylase; Two-dimensional crystallization; Electron crystallography; Blood coagulation; Anti-coagulation

1. Introduction

The vitamin K-dependent γ-glutamyl carboxylase is a membrane protein that catalyzes the post-translational carboxylation of the vitamin K-dependent proteins. This conversion of several glutamic acid residues to γ-carboxyglutamic acid (Gla) is essential for the activity of these proteins (Furie and Furie, 1988; Vermeer, 1990). The carboxylation was found to occur via a processive mechanism in the presence of vitamin K, where the carboxylase modifies multiple residues while bound to the substrate (Morris et al., 1995; Stenina et al., 2001).

The vitamin K-dependent proteins play essential roles in a number of diseases linked to blood coagulation and anti-coagulation, bone mineralization/metabolism, cell proliferation, and signal transduction (Dahlback, 2000; Lamson and Plaza, 2003; Price, 1988; Manfioletti et al., 1993; Zittermann, 2001). In blood coagulation, the presence of the γ-carboxyglutamic acid enables these proteins to form a protein-membrane binding site in the presence of calcium.

The human liver vitamin K-dependent γ-glutamyl carboxylase is an 87 kDa membrane protein located inside of the endoplasmic reticulum (ER) (Tie et al., 2000). The membrane location allows the carboxylase to convert the vitamin K-dependent proteins as they are secreted from the ER. *In vitro* translation/cotranslocation could determine a membrane topology with 5 transmembrane domains (Tie et al., 2000). The same study also showed the N-terminus to be facing the cytoplasm and the C-terminus, with a large hydrophilic domain, to be in the lumen of the ER.

* Corresponding author. Fax: +1 404 894 0519.
E-mail address: Ingeborg.Krey@biology.gatech.edu (I. Schmidt-Krey).

1047-8477/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2006.08.002

The human carboxylase can be purified in sufficient amounts (Wu et al., 1991, 1997) for two-dimensional (2D) crystallization and electron crystallography. 2D crystallization generally requires far smaller amounts of purified protein than three-dimensional (3D) crystallization, and thus it is a particularly suitable method for the structure determination of eukaryotic membrane proteins (Kühlbrandt, 2003). The most commonly used approach for 2D crystallization is dialysis, where the detergent-solubilized membrane protein is reconstituted into a phospholipid bilayer (Kühlbrandt, 1992, 2003; Jap et al., 1992; Walz and Grigorieff, 1998; Hasler et al., 1998; Stahlberg et al., 2001; Mosser, 2001; Schmidt-Krey, 2006). The phospholipid bilayer can have different morphologies in the form of vesicles, sheets, and narrow tubes with helical arrays, or wide planar-tubular (flattened, barrel-shaped vesicular) crystals. Eukaryotic membrane proteins, which are often highly fragile when purified, are thereby kept under conditions that are close to their native lipid environment rather than in a detergent micelle. Synthetic lipids frequently also have this effect of stabilizing the membrane protein, sometimes in combination with co-purified native lipids (Wang et al., 1993). After 24 h of storage, the stability of the detergent-solubilized carboxylase decreases to 78% of its original level at 4 °C in comparison to 35% at room temperature (Presnell et al., 2001). This makes the 2D crystallization approach with the stabilizing reconstitution preferable as both 2D and 3D crystallization usually necessitate prolonged incubation.

Here we describe the two-dimensional crystallization of the human liver vitamin K-dependent γ-glutamyl carboxylase grown by reconstitution via dialysis. An initial projection map reveals the quarternary structure of the carboxylase.

2. Crystallization conditions

The purified and solubilized protein in buffer A (25 mM Tris–HCl at pH 7.2, 500 mM NaCl, 0.3% CHAPS (3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulphonate), 15% glycerol, 2 μM proFIX 19, 0.5 ng/ml leupeptin, 0.7 ng/ml pepstatin, 2.0 ng/ml aprotinin, and 2 mM DTT) at a concentration of 0.6–1 mg/ml was mixed with either dioleoyl phosphatidylcholine (DOPC), dimyristoyl phosphatidylcholine (DMPC), or bovine liver lecithin at molar ratios in the range of 1–30. The lipid was solubilized in 0.3% Na-deoxycholate. Aliquots of 100 μl of the protein-detergent-lipid mixture were dialysed against 125 ml detergent-free dialysis buffer (25 mM Tris–HCl at pH 7.2, 20% glycerol, 250 mM NaCl, and 10 mM β-mercaptoethanol), which was a simplified version of buffer A. A lower salt concentration was used in the dialysis buffer since higher salt was only necessary for the purification, and the glycerol concentration was slightly increased due to previous successes with other membrane proteins in obtaining larger proteoliposomes with 20% glycerol (Schmidt-Krey et al., 1998, 2004).

Despite the low phase transition temperature of DMPC of 23 °C and the common practice to dialyze samples at least at 1–2 °C above the phase transition temperature of the reconstituting lipid, the samples were dialysed in a crystallization room at 20 °C. To verify that the 2D crystals were not composed of ordered phospholipids, freeze-fracture experiments were performed (Fig. 1), and controls of lipid without protein were dialysed under identical conditions. Surprisingly, the resulting liposomes also formed square vesicles reminiscent of planar-tubular crystals (not shown), and ordered lipid was observed under these protein-free conditions as well.

The standard crystallization was also successful at a temperature of 32 °C, or additional incubation at 32 °C, but did not show an improvement over the crystalline arrays grown at 20 °C. Due to the temperature sensitivity of the carboxylase, 20 °C was used as the standard temperature.

Crystallization trials were sampled after 1–4 days, and the samples were negatively stained with 1% uranyl acetate and screened with FEI CM12 and CM120 electron microscopes equipped with 1K×1K CCD cameras. Due to the large hydrophilic domain of the carboxylase, ordered protein arrays could be observed on the small fluorescent screen of the electron microscope, greatly facilitating the screening for crystals.

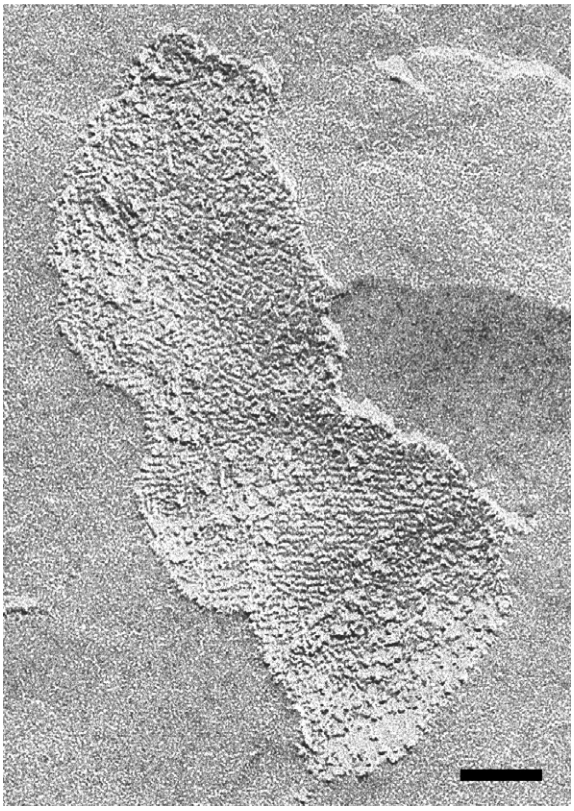

Fig. 1. A freeze-fracture image of a mosaic carboxylase 2D crystalline sheet grown below the phase transition temperature of the added phospholipids (DMPC). The freeze-fracture results demonstrate that the carboxylase crystals are indeed constituted of ordered protein. The scale bar is 100 nm.