Korean J. Cryst., Vol. 5, No. 1, pp. 20~23, 1994.

대장균 염색체 복제 개시 저해제, IciA 단백질의 결정화

송 현규, 차 훈, 유 순지*, 정 진하*, 황 덕수**, 서 세원
서울대학교 자연과학대학 화학과
*서울대학교 자연과학대학 분자생물학과
**서울대학교 유전공학연구소

Crystallization of *Escherichia coli* IciA Protein, An Inhibitor of Initiation of Chromosomal Replication

Hyun Kyu Song, Hoon Cha, Soon Ji Yoo*, Chin Ha Chung*, Deog Su Hwang**, and Se Won Suh
Department of Chemistry, College of Natural Sciences, Seoul National University
*Department of Molecular Biology, College of Natural Sciences, Seoul National University
**Institute for Molecular Biology and Genetics, Seoul National University

요 약

대장균의 IciA 단백질은 DnaA 단백질의 작용장소에 결합하여 DNA의 복제가 개시되는 것을 막는다. 따라서 IciA 단백질은 세포주기의 주요 단계에서 결정적인 역할을 한다. 이러한 IciA 단백질의 구조와 기능간의 관계를 연구하기 위하여 X-선 결정학을 이용하여 삼차원 구조를 결정하고자 한다. 그 첫 단계로 IciA 단백질 결정화를 시도하였다. sodium formate 를 침전제로 이용하여 결정을 얻을 수 있었다.

Abstract

Specific binding to the *oriC* region of *E. coli* chromosome by IciA protein inhibits initiation of chromosomal replication *in vitro* by blocking the opening of this region effected by the initiator DnaA protein. The IciA protein has been suggested to play a critical role in a key stage of the cell cycle. In order to study the structure-function relationship of IciA protein, we are determining the three-dimensional structure of IciA protein by X-ray crystallography. As a first step toward its structure determination, *E. coli* IciA protein has been crystallized using sodium formate as a precipitant.

1. Introduction

Recognition of nucleotide bases in DNA by proteins is of central importance in molecular biology. Several different motifs have been found in many of the DNA-binding proteins. IciA protein from *E. coli* binds specifically to the 13-mers in the origin of the minichromosome (*oriC*) and thus

inhibits initation of replication *in vitro* by blocking the opening of this region effected by the initiator DnaA protein. Therefore, the IciA protein has been suggested to play a critical role in a key stage of the cell cycle[1,2].

The IciA protein consists of two identical subunits, each with 298 amino acid residues (molecular mass of 33,471 Da)[3]. The encoded IciA protein contains a helix-turn-helix motif which may be involved in the DNA binding and also possesses a sequence homology to the large LysR family of transcriptional regulators[2,4]. The helix-turn-helix motif is near the N-terminus of IciA protein and 27kDa C-terminal fragment produced by protease Do can no longer bind to the *oriC* region containing the tandom 13-mer repeats[5].

In order to provide a detailed structural basis for understanding the function of IciA protein, we have initiated its structure determination by X-ray crystallography. As the first step toward its structure determination, crystalliztion experiments have been performed. We report here the results of these experiments.

2. Experimental Procedure

2.1. Purification

The gene encoding the IciA protein from E. coli was cloned into *E. coli* and the protein was overproduced. IciA protein was purified according to the methods previously reported[3]. Homogeneity of the purified enzyme was determined by discontinuous electrophoresis in 12.5 % (w/v) polyacrylamide gel in the presence of 0.1 % (w/v) sodium dodecyl sulfate[6]. The gel was stained by the silver staining procedure[7]. The enzyme concentration was estimated by measuring the absorbance at 280nm, assuming that the concentration of 1.0 mg/ml corresponds to the unit absorbance at 280 nm for the path length of 1.0 cm.

2.2.Crystallization

Purified protein was concentrated to 10 mg/ml by ultrafiltration using YM 30 membrane (Amicon) and then dialyzed against 50 mM Tris-HCl (pH 8.00), 1mM DTT (1,4-dithiothreitol), 150 mM sodium chloride, 1mM EDTA (ethylenediamine tetraacetic acid), 0.02% (w/v) sodium azide, 5% (v/v) glycerol. Crystallization was achieved by the hanging drop vapor diffusion method at room temperature (22 ± 2 ℃) using 24-well tissue culture plates (Flow Laboratories). Initial crystallization conditions were established by the incomplete factorial method[8] and sparse matrix sampling[9]. Each hanging drop was prepared by mixing equal volumes (3 μl) of the protein and the reservoir solutions. The cover glass with a hanging drop was placed over the 1 ml reservoir solution in each well of the tissue culture plate and an air-tight seal was made with grease.

2.3.X-ray studies

A crystal was mounted in a thin-walled glass capillary for X-ray experiments. Both ends of the capillary were filled with the mother liquor and then sealed with wax. X-ray experiments were carried out on the FAST diffractometer system (Enraf-Nonius) using graphite-monochromatized Cu Kα X-rays from a rotating anode generator (Rigaku RU-200BH), running at 40 kV and 70 mA with a 0.3 mm focus cup and a 0.6 mm collimator.

3. Results and Discussion

Microcrystals of *E. coli* IciA protein have been obtained when the reservoir solution contained 100mM acetate (pH 4.61), 2.2 M sodium formate, 50mM amonium sulfate. These crystals grew in two months but they were too small to be subject to

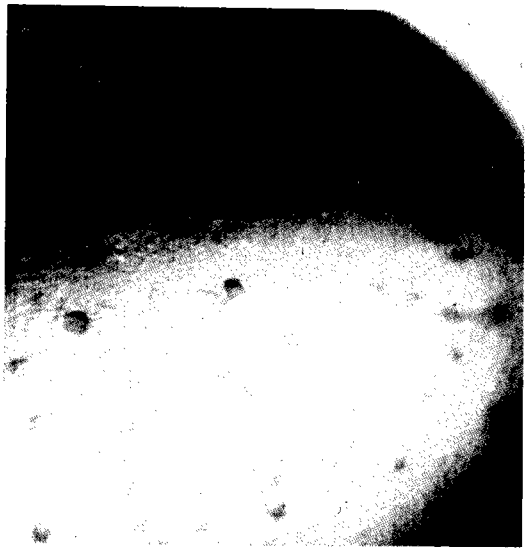

Figure 1. Photograph of microcrystals of IciA protein.

Figure 2. Photograph of a crystal of IciA protein. Approximate dimensions are 0.5 mm x 0.2 mm x 0.1 mm.

X-ray analysis (Fig. 1). Further experiments established the following condition to grow larger crystals. The reservoir soultion [100 mM acetate, 2.3M sodium formate, 5 % (v/v) glycerol] was prepared by mixing appropriate volumes of 1.0 M acetate (pH 4.61), 5.0 M sodium formate, 50 % (v/v) glycerol and adjusting the final volume with water. The condition of IciA protein solution was as follows: 10 mg/ml IciA protein, 50 mM Tris-HCl (pH 8.00), 1mM DTT, 150 mM sodium chloride, 1mM EDTA, 0.02 % (w/v) sodium azide, 5 % (v/v) glycerol. A crystal grew to approximate dimensions of 0.5 mm x 0.2 mm x 0.1 mm in three months. (Fig. 2). However, the crystal showed no measurable diffraction intensities upon exposure to X-rays. Further crystallization efforts are necessary to obtain better-diffracting crystals. In addition, it may be worth trying to crystallize the fragments of the protein, which can be produced by gene manipulation, in view of the difficulty in growing well-diffracting crystals of the intact protein. This may be tried either in the presence or absence of the DNA oligomer containing the 13-mer sequence.

Acknowledgments

This work was supported by the Korea Ministry of Education, Genetic Engineering Research Fund. We thank the Inter-University Center for Natural Science Research Facilities for providing X-ray equipments.

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