CRYSTALLIZATION AND PRELIMINARY X-RAY DATA OF WELL-ORDERED CRYSTALS FROM PIG HEART CITRATE SYNTHASE

Georg WIEGAND

Max-Planck-Institut für Biochemie, 8033 Martinsried/BRD und Physikalisch-Chemisches Institut der Technischen Universität, München, West Germany

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1. Introduction

Citrate synthase catalyzes the first reaction of the citric acid cycle and fills a prominent role in cellular regulation. The enzyme catalyzes the enolase, ligase and hydrolase activities and forms with the two substrates oxalacetate and acetyl-CoA citrate. The question of whether citryl-CoA is an intermediate is not completely settled. However, the work of Eggerer et al. makes it a likely intermediate [1], and the stereochemical course of the reaction has been brilliantly elucidated [2].

Oxalacetate forms with citrate synthase a binary complex, demonstrated by ultraviolet difference spectra, and protects the citrate synthase activity against urea denaturation [3]. Pig heart citrate synthase requires no cofactors or metal ions for activity [4]. Until now there have been no definitive investigations which amino acid residues are essential for the catalytic activity. But the enzyme activity is inhibited by reagents that modify amino groups. Sulfhydryl groups do not participate in the active site [5,6]. However, histidine residues seem to be involved in the enzymatic citrate synthesis [7]. ATP is a competitive inhibitor of acetyl-CoA at least in vitro [8].

Although the primary structure of citrate synthase is not known, the investigation of its tertiary and quaternary structure by X-ray crystallography seems feasible. The first crystalline preparation of pig heart citrate synthase was described by Ochoa and Stern [9].

This communication describes the growing of large crystals of pig heart citrate synthase suitable for X-ray crystallography and their crystallographic data.

2. Materials and methods

Citrate synthase (EC 4.1.3.7) from pig heart, oxalacetic acid and ATP were purchased from

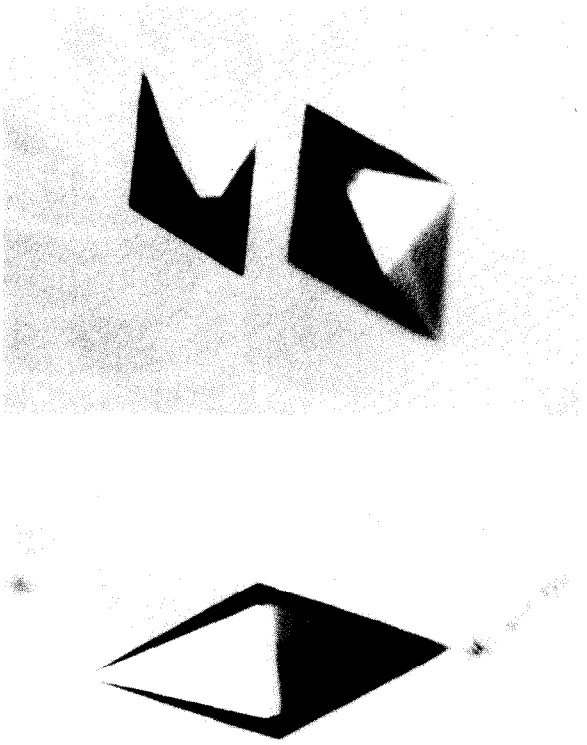

Fig.1. Photomicrograph of the pig heart citrate synthase crystals. The length of the tetragonale bi-pyramids is approximately 2 mm.

Boehringer Mannheim, potassium and sodium phosphate from Merck Darmstadt, Sephadex G-100 from Deutsche Pharmacia. The protein concentration was determinated photometrically at 280 nm with $E_{1\,cm}^{1\%} = 17.8$ [4]. The citrate synthase was separated from higher aggregates on a Sephadex G-100-column (2 X 100 cm) in a 0.125 M potassium–sodium phosphate buffer pH 7.4.

The main fraction was concentrated in a Minicon-B-dialysis chamber (Amincon) to 7.5 mg/ml. This protein solution was dialyzed against a potassium–sodium phosphate buffer (1.35 M pH 7.4) with 2×10^{-3} M oxalacetate or 2×10^{-3} M ATP and concentrated in vapor-diffusion chambers over 1.55 M potassium–sodium phosphate buffer pH 7.4 at 22°C. After 1–3 weeks the citrate synthase crystallized.

3. Results and discussion

The citrate synthase crystallizes into large tetragonal bi-pyramids up to 2 mm in length (fig.1).

X-ray precession photographs of the hkO reciprocal lattice section of citrate synthase crystals shows 4-mm symmetry, demonstrating point group 422. Systematic absences were observed on the 10° precession photograph of the h01 reciprocal lattice section (fig.2) for the h00 class of reflections ($h \neq 2n$) and the 001 class of reflections ($1 \neq 4n$). Together with cone axis photographs and screenless rotation photographs the space group was established as $P\,4_1 2_1 2$ or enantiomorph with eight general positions.

The dimensions of the unit-cell were found to be:

a = b = 77.1 Å c = 196.8 Å

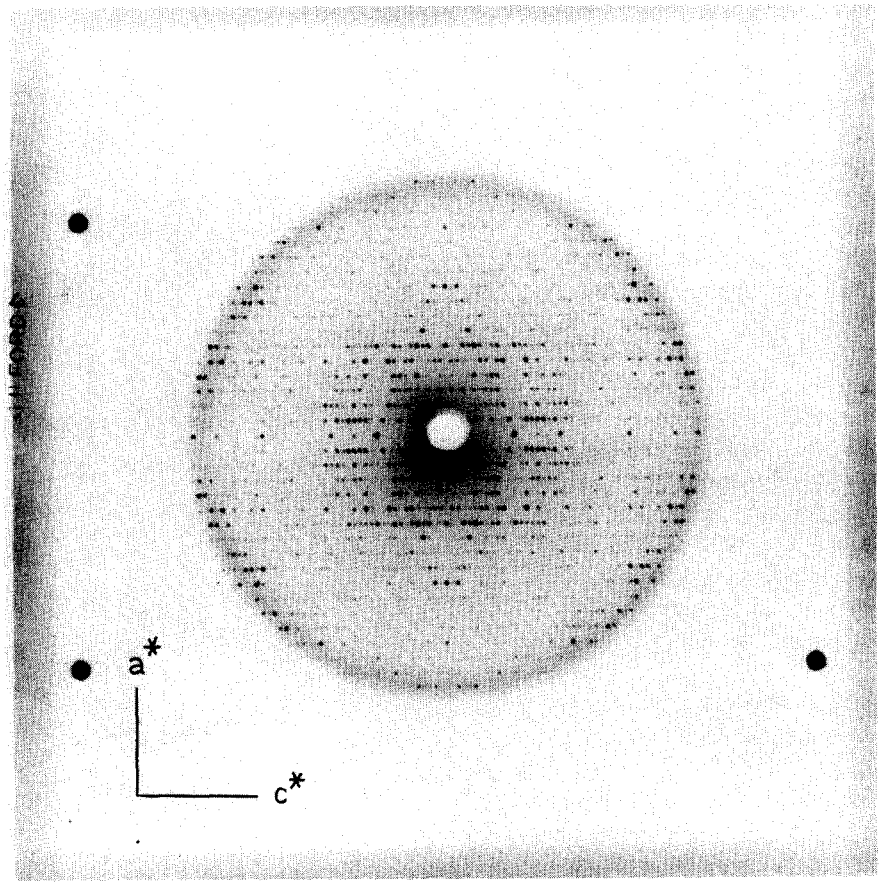

Fig.2. A 10° precession photograph of the h01 reciprocal lattice section of citrate synthase.

Volume of the unit cell

$V = 1.17 \times 10^6$ Å^3

Determinations of the density of the crystals have been made by the method of Low and Richards [10] and F. Cramer et al. [13].

We have determinated the mol. wt./assymmetric unit to 37 000–66 000. The crystal cell contains, therefore, 4 citrate synthase molecules of molecular weight 100 000 [11]. Pig heart citrate synthase therefore consists of two identical subunits related by a two-fold axis of symmetry. This is in agreement with results obtained by tryptic maps, SDS gel electrophoresis and sedimentation equilibrium studies [4,11].

For citrate synthase crystals the values 2.9 Å^3/dalton and 59% solvent content are typical of that found in crystals of other proteins with molecular weights of 100 000 daltons or higher [12].

Native crystals diffract to about 2.8 Å resolution and intensity measurements of the native protein are in progress.

Acknowledgement

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