Preliminary crystallographic study on a low molecular weight form of bacterial plasminogen activator staphylokinase

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Abstract

Staphylokinase, a 17 kDa protein, produced by certain strains of *Staphylococcus aureus* functions as a fibrin-specific plasminogen activator. During its interaction with plasminogen, staphylokinase is converted into a low molecular weight form by loss of ten amino-terminal residues. This low molecular weight form of recombinant staphylokinase has been crystallized using the hanging-drop vapor-diffusion technique with polyethylene glycol 4000 as precipitant. Crystals belong to the orthorhombic space group $C222_1$ with unit-cell dimensions $a = 43.78$, $b = 59.86$ and $c = 103.25$ Å and one molecule in the asymmetric unit. These crystals diffract to about 2.4 Å resolution.

1. Introduction

Plasminogen activators are thrombolytic agents that convert plasminogen, an inactive proenzyme of the fibrinolytic system, to the active proteolytic enzyme plasmin. Plasmin dissolves the fibrin of a blood clot.

Biochemical and thrombolytic properties of staphylokinase have been summarized in a recent review by Collen & Lijnen (1994). Since 1948 it has been known that staphylokinase, an extracellular protein produced by *Staphylococcus aureus*, activates human plasminogen and can cause lysis of blood clots (Lack, 1948). Natural staphylokinase has been purified from *S. aureus* (Jackson, Esmon & Tang, 1981). The staphylokinase gene was sequenced and cloned more than a decade ago (Sako *et al.*, 1983; Behnke & Gerlach, 1987). A number of recombinant forms of staphylokinase have also been expressed, purified and characterized (Sako *et al.*, 1983; Sako & Tsuchida, 1983; Behnke & Gerlach, 1987; Schlott *et al.*, 1994). The staphylokinase gene encodes a protein of 163 amino acids of which the first 27 residues constitute a signal sequence. Thus, the mature form of staphylokinase consists of 126 amino acids. Four nucleotide differences have been found in the staphylokinase genes (*sakφC* and *sak42D*) that are isolated from the bacteriophages and the gene (*sak-STAR*) obtained from a lysogenic *S. aureus* strain. These differences result in some variations in residues 11, 34, 36 and 43 of the mature forms of staphylokinase (Sako *et al.*, 1983; Behnke & Gerlach, 1987; Collen, Zhao, Holvoet & Marynen, 1992).

Staphylokinase forms a 1:1 complex with plasminogen. This complex then converts other plasminogen molecules into the active serine protease, plasmin (Lijnen *et al.*, 1991). Activation of plasminogen–staphylokinase complex involves a peptide-bond cleavage in converting plasminogen to plasmin (Collen *et al.*, 1993). The interactions between plasminogen and staphylokinase are not well understood. However, it is known

that both naturally occurring forms, Glu and Lys forms, of plasminogen have comparable affinity for recombinant staphylokinase. Lysine-binding sites in kringles 1–4 of plasminogen are not required for the formation of the complex but they play an important role in determining the fibrin specificity of staphylokinase. The serine protease domain is in fact involved in binding staphylokinase (Lijnen, Van Hoef & Collen, 1993).

During activation of plasminogen in a purified system, a truncated version ($\Delta10$) of staphylokinase is generated by removal of ten amino-acid residues from the N-terminus of the mature form. This low molecular weight form ($\Delta10$, M_r 14 kDa) has similar fibrinolytic activity as full-length staphylokinase (Ueshima, Silence, Collen & Lijnen, 1993). Crystallization of full-length staphylokinase has been recently reported (Zhan *et al.*, 1996). Here we describe the crystallization and preliminary X-ray diffraction analysis of a recombinant $\Delta10$ form of staphylokinase.

2. Materials and methods

Purification of recombinant low molecular weight form will be described elsewhere. Briefly, the protein for crystallization was purified using a combination of immobilized metal affinity chromatography and hydrophobic affinity chromatography on butyl sepharose column. On sodium dodecyl sulfate poly-acrylamide gel electrophoresis, the purified staphylokinase protein migrated as a single band of approximate molecular mass of 14 kDa. The calculated molecular weight of this 126-residue protein is 14 336 Da.

The recombinant protein (25 mg ml^{-1}) was crystallized using the hanging-drop vapor-diffusion technique. The reservoir contained 25–35%(*w/v*) PEG 4000 in 100 m*M* Tris hydrochloride buffer, pH 8–9. Crystallization trials were carried out at 277 and 295 K.

A crystal of dimensions 0.1 × 0.1 × 0.04 mm was mounted and sealed in siliconized glass capillary. X-ray diffraction data were collected on a Siemens Hi-Star multiwire area detector using graphite-monochromated Cu $K\alpha$ radiation (40 kV, 100 mA) produced by a fine-focus rotating-anode generator. Each oscillation frame covered 0.25° and was measured for 300 s. The data were reduced using the *XENGEN*2.0 program (Howard *et al.*, 1987).

3. Results

Crystals of recombinant staphylokinase grew overnight, both at room temperature and at 277 K, and continued to grow for about a week to maximum dimensions of 0.2 × 0.2 × 0.1 mm.

However, crystals grown at room temperature were used for analysis. Fig. 1 shows a single crystal grown at room temperature. Crystals diffracted to 2.4 Å resolution. Among 12 016 recorded observations 5057 reflections were unique. This represented more than 89% of possible reflections (5 665) at 2.39 Å. The average I/σ was greater than 2.9 at the highest resolution range. R_{sym} was 6.6% and 91% of the data were recorded at least twice. Crystal cell parameters and systematic absences were consistent with orthorhombic space group $C222_1$. Refined cell parameters were $a = 43.78$, $b = 59.86$, $c = 103.25$ Å. Assuming one molecule in the asymmetric unit the specific volume was calculated to be 2.36 Å^3 Da^{-1}. The solvent content of 48% was within the range observed for protein crystals (Matthews, 1968). Since the structure of no homologous protein is known, the multiple isomorphous replacement method is being pursued for structure determination. Throm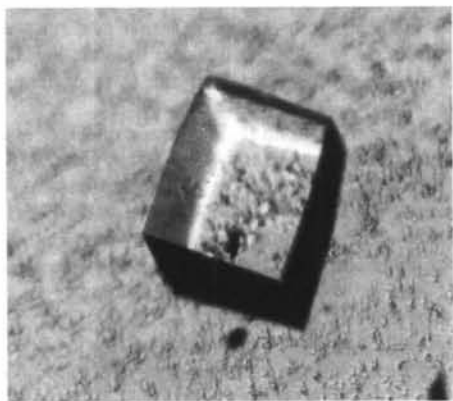bolytic therapy using plasminogen activators has become a routine treatment in patients with evolving myocardial infarction. However, the exact mechanism by which these agents function is not known. Structure determination of staphylokinase may help elucidate the molecular mechanism of plasminogen activation by this group of plasminogen activators.

Fig. 1. Crystal of staphylokinase (0.2 × 0.18 × 0.1 mm) grown at room temperature.

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