SHORT COMMUNICATIONS

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Crystallization and preliminary X-ray diffraction studies of recombinant staphylokinase

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Abstract

Staphylokinase, a fibrin-specific plasminogen activator, was highly expressed in *Escherichia coli* and purified by ion-exchange and gel-filtration chromatography. The purified recombinant staphylokinase was fully active and readily crystallized against 1.2 *M* sodium citrate in 100 m*M* Tris–HCl buffer at pH 8.0 using the hanging-drop method. Crystals of staphylokinase diffract to better than 2.2 Å resolution. The crystal belongs to the tetragonal space group $P4_12_12$ or its enantiomorph with unit-cell parameters $a = b = 67.5$, $c = 150.1$ Å. There are two molecules in the asymmetric unit. In this paper, we described the first crystallization of a kind of plasminogen activator and present the results of preliminary X-ray diffraction data from the native protein.

1. Introduction

In the fibrinolytic system, an inactive proenzyme, plasminogen, is converted by plasminogen activators to plasmin, an enzyme which degrades fibrin. Staphylokinase (Sak),† a $M_r = 15.5$ kDa protein produced by the lysogenic phase of *Staphylococcus aureus* has profibrinolytic properties (Lack, 1948) and has been found to be a thrombolytic agent with a potency comparable to that of streptokinase (Sk) (Kowalska-Loth & Zakrzewski, 1975). Sak, like Sk, is not an enzyme; it forms a stoichiometric complex with plasminogen. Plasminogen bound in such a complex may be altered in conformation to yield a form capable of converting another plasminogen molecule to the proteolytic enzyme, plasmin, which promotes the dissolution of fibrin filaments in blood clots (Ericson, 1977). However, the molecular interaction of the human plasma fibrinolytic system with Sak differs in many essential aspects from those regulating the non-fibrin specific activation with Sk (Collen *et al.*, 1993; Lijnen *et al.*, 1991).

Thrombolytic therapy, consisting of the intravenous administration of plasminogen activators, has become an established treatment for thromboembolic complication of cardiovascular disease (Collen & Lijnen, 1991). The thrombolytic agents that are presently available for clinical use are, however, not uniformly effective in recanalizing occluded coronary arteries, and their administration has been associated with the occurrence of bleeding complications, which are caused by the degradation of fibrinogen, and with antibody formation with some agents (de Bono, 1989). Recently, the fibrinolytic properties of Sak were studied and it has been clear that Sak induces fibrinolysis specifically without degrading fibrinogen (fibrin-specific) and has higher fibrinolytic activity compared with other plasminogen activators such as Sk, urokinase and t-PA (Sakai, Watanuki & Matsuo, 1989; Matsuo *et al.*, 1990). In order to investigate the three-dimensional structure of Sak at atomic level and to explore further the molecular mechanism of the activation of plasminogen by Sak, we have developed recombinant staphylokinase (r-Sak), cultured the X-ray diffraction quality crystals, and initiated preliminary X-ray diffraction studies.

2. Materials and methods

Sak was overexpressed and purified as described elsewhere (Tang, Gu, Zhang & Song, 1996*a*,*b*). Briefly, a strain of *S. aureus* secreting natural Sak was screened and cultured on a large scale. The natural Sak was purified from the culture. We determined the amino-acid composition and carboxyl terminal amino-acid sequence of natural Sak, and designed a pair of amplification primers. The *Sak* gene was successfully amplified by the polymerase chain reaction (PCR), with the chromosome of the same strain as template. The complete nucleotide sequence of the *Sak* regulatory gene was determined in both orientations. The expression plasmid pSTE-Sak, which contained the *Sak* gene and regulatory factors such as the PRPL promoter, was transformed and maintained in a special *E. coli* strain. r-Sak was found within the bacteria and accounted for 45% of the total bacterial protein. r-Sak was purified by ion-exchange and gel-filtration chromatography, yielding 400–500 mg pure product with a molecular weight of 15.5 kDa, 98–99% purity and a specific activity of 10^5 IU mg^{-1} from a 1 l culture (Tang *et al.*, 1996*a*,*b*). It is fully active in an animal thrombolytic model and in the clinical treatment of acute myocardial infarction (Tang *et al.*, 1996*a*,*b*).

Initial crystallization trials on the r-Sak were performed based on sparse-matrix approaches (Jancarik & Kim, 1991). After nearly 5000 trials, microcrystals of Sak were obtained using sodium citrate or PEG 4000 as precipitant. The screening of additives was carried out in 1.2 *M* sodium citrate, 0.1 *M* Tris–HCl (pH 8.0), which generated the largest crystals. About 30 additives such as β-octylglucoside (β-OG), cadmium sulfate, nickel chloride, cobalt chloride, $MgCl_2$ and $Zn(CH_2COOH)_2$ were tested. Divalent metal salts, in particular $MgCl_2$ and $ZnCl_2$ were useful. β-OG has some effects on obtaining good crystals. The conditions were improved by varying the protein and salt concentration as well as the pH.

† Abbreviations used: Sak, staphlokinase; Sk, streptokinase; r-Sak, recombinant staphlokinase; t-PA, tissue-type plasminogen activator; PEG, polyethylene glycol; β-OG, β-octylglucoside.

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3. Results and discussion

X-ray quality crystals were obtained at 277 K by the vapor-diffusion method using sodium citrate as precipitant. The reservoir solution contains 1.2 *M* sodium citrate, 100 m*M* Tris–HCl buffer (pH 7.5), 0.02% NaN_3, 20 m*M* $MgCl_2$, 0.5% β-OG. The purified Sak was dissolved in 50 m*M* Tris–HCl buffer (pH 8.0) at 20 mg ml^{-1} and mixed with the reservoir solution in 1:1 ratio. After 4 d, a microcrystal appeared, and grew to dimensions of 1.0 × 1.0 × 0.6 mm in about a month (Fig. 1). Owing to the tendency of crystals to crack because of their sensitivity to temperature and salt concentration, handling and mounting them were possible by using a stabilization solution with a higher concentration (about 1.4 *M*) of sodium citrate.

The Sak crystals diffract typically to 2.2 Å resolution, but diffraction to 2.0 Å has been observed on still X-ray photographs. Procession photography reveals that these crystals are tetragonal, space group $P4_12_12$ or its enantiomorph with unit-cell dimensions $a = b = 67.5, c = 150.1$ Å (Fig. 2). Assuming a dimer per asymmetric unit, the specific volume of the protein is 2.8 $Å^3$ Da^{-1}, which corresponds to 56%(*v*/*v*) solvent content. This value is within the commonly observed ranges (Matthews, 1968). The X-ray diffraction analysis of the crystals of Sak was performed on a Siemens XRD-100 area detector mounted on a Ru-200B rotating-anode X-ray generator. The source was operated at 50 kV and 200 mA using a focal spot size of 0.3 × 3 mm. The area detector was set at 160 mm from the crystal; and the θc angle was set to 21.5° (Xuong, Nielsen, Hamlin & Anderson, 1985). Using a $\Delta\varphi = 0.15°$ and an exposure time of 100 s per frame, 1200 frames of data were collected. The data were processed using the *XENGEN* program package (Howard *et al.*, 1987). The data set contains 69 406 observations and 18 905 unique reflections. It is 94.1% complete to 2.2 Å with $R_{merge} = 6.7\%$. Screening of potential heavy-atom derivatives is in progress.

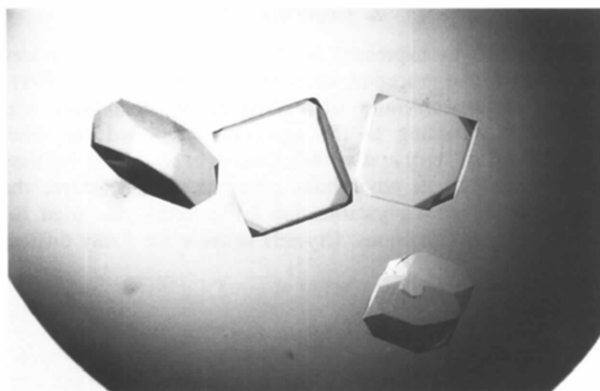

Fig. 1. A single crystal of staphylokinase (Sak) grown from 1.2 *M* citrate, 0.02% NaN_3 20 m*M* $MgCl_2$, 0.5% β-OG and 100 m*M* Tris–HCl buffer (pH 8.0).

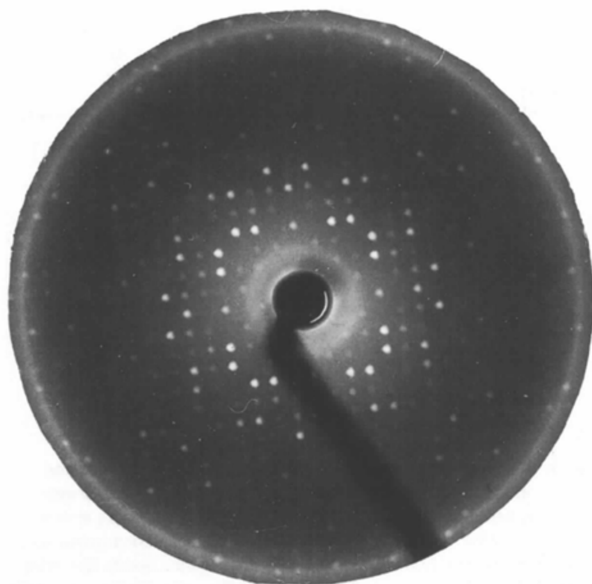

Fig. 2. Procession photograph of the *hk*0 layer along the *c* axis of Sak. The space group is $P4_12_12$ or its enantiomorph.

In the past, few results have been obtained on the crystallization of plasminogen activators such as Sk, urokinase and t-PA. Despite this, the crystal structure of some of their domains have been determined, such as the kringle 2 domain of t-PA (de Vos *et al.*, 1992). The results presented above show great promise for the rapid solution of the three-dimensional structure of Sak. The X-ray structure determination of Sak will provide for the first time a high-resolution view of a plasminogen activator. Furthermore, this structure will provide considerable insight into the biochemical mechanism of the activation of plasminogen to an active form, plasmin, which is the first and crucial step in the fibronilytic process.

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References

de Bono, D. (1989). *Problems in thrombolysis. Thrombolysis in cardiovascular disease*, edited by D. Julian, W. Kubler, R. M. Norris, H. J. C. Swan, D. Collen & M. Verstraete, pp. 279–292. New York: Marcel Dekker Inc.

Collen, D. & Lijnen, H. R. (1991). *Blood*, **78**, 3114–3124.

Collen, D., Schlott, B., Engelborghs, Y., Van Hoef, B., Hartmann, M., Lijnen, H. R. & Behnke, D. (1993). *J. Biol. Chem.* **268**, 8284–8289.

Ericson, R. (1977). *Chem Abstr.* **86**, 202.

Howard, A. J., Gilliland, G. L., Finzel, B. C., Poulous, T. L., Ohlendorf, D. H. & Salemme, F. R. (1987). *J. Appl. Cryst.* **20**, 383–387.

Jancarik, J. & Kim, S. H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Kowalska-Loth, B. & Zakrzewski, K. (1975). *Acta Biochem. Pol.* **22**, 327–339.

Lack, C. H. (1948). *Nature (London),* **161**, 559–560.

Lijnen, H. R, Van Hoef, B., De Cock, F., Okada, K., Ueshima, S., Matuso, O. & Collen, D. (1991). *J. Biol. Chem.* **266**, 11826–11832.

Matsuo, O., Okada, K., Fukao, H., Tomioka, Y., Ueshima, S., Watanuki, M. & Sakai, M. (1990). *Blood*, **76**, 925–929.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Tang. Q., Gu. Y., Zhang, X. & Song, H. (1996*a*). *Chin. J. Biotechnol.* In the press.

Tang, Q., Gu. Y., Zhang, X. & Song, H. (1996*b*). *Blood.* In the press.

Sakai, M., Watanuki, M. & Matsuo, O. (1989). *Biochem. Biophys. Res Commun.* **162**, 830–837.

Vos, A. M. de, Ultsch, M. H., Kelley, R. F., Padmanabhan, K., Tulinsky, A., Westbrook, M. L. & Kossiakoff, A. A. (1992). *Biochemistry,* **31**, 270–279

Xuong, N. H., Nielsen, C., Hamlin, R. & Anderson, D. (1985). *J. Appl. Cryst.* **18**, 342–350.