Amino Acid Composition, Crystallization and Preliminary X-ray Diffraction Studies of Guanyloribonuclease from *Streptomyces Aureofaciens*

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Abstract. The amino acid composition and preparation of crystals for X-ray diffraction analysis of guanyloribonuclease isolated from *Streptomyces aureofaciens* is described. Single crystals have been grown from ammonium sulphate solutions. The crystals are orthorombic, space group $P\,2_12_12$, cell dimmensions $a = 6.47$ nm, $b = 7.89$ nm, $c = 3.93$ nm. There are two molecules of a molecular weight of 10 600 in the asymmetric unit. The solvent content of the crystal is 50 %.

Key words: Crystallization — Guanyloribonuclease — X-ray diffraction studies — Amino acid composition

Introduction

The guanyloribonuclease was isolated from the culture medium of *Streptomyces aureofaciens* (RNase Sa), a chlortetracycline producing strain (Bačová et al. 1971). Later, the method of isolation and purification was modified in order to increase the yield (Gašperík et al. 1982). The enzyme is an endonuclease splitting the bond between the guanosine 3′-phosphate and the —OH group at 5′ position of the adjoining nucleotide (Zelinková et al. 1971). The active site components were identified by NMR spectroscopy and kinetic measurements and its model was proposed (Both et al. 1982). Molecular weight determination based on amino acid composition gave the result of $10\,600 \pm 350$.

The mechanism of specific reactions which are catalysed by guanyloribonucleases cannot be explained satisfactorily without knowing their tertiary structure. Therefore, the tertiary structure of guanyloribonucleases as well as that of the guanyloribonuclease complex with a substrate are studied in several laboratories (Martin et al. 1981; Pavlovsky et al. 1982; Polyakov et al. 1982; Yamamoto et al. 1981; Heinemann et al. 1980). Our aim is to define the tertiary structure of RNase Sa and thus to contribute to the elucidation of the mechanism of its action and to general principles of biocatalysis.

Materials and Methods

Materials

The RNase Sa for determination of the amino acid composition and for crystallization experiments was isolated and purified in the department of experimental production of biopreparates in our Institute (Gašperík et al. 1982). All chemicals used were of analytical grade and were used without further purification.

Amino acid composition

Amino acid composition (Table 1) was determined according to Spackman et al. (1958). All amino acid analyses were performed using an amino acid analyser 6020 A, produced by the Czechoslovak

Table 1. Amino acid composition

Amino acid	Number of residues
Lys	1
His	2
Arg	5
Asp	10—11
Thr	11
Ser	7—8
Glu	11—12
Pro	6
Gly	7—8
Ala	5—6
1/2 Cys	4
Val	5
Met	1
Ile	3—4
Leu	4—5
Tyr	7
Phe	3
Trp	0

Academy of Sciences. Cysteine and methionine content was determined as cysteic acid and methionine sulphone, respectively (Moore 1963). Tryptophan was determined by the spectrophotometric method, as described by Messineo et al. (1972).

Crystallization

The crystals of RNase Sa (Fig. 1) were prepared by the vapour equilibration technique (Davies et al. 1971) and its modification, the hanging drop method. The crystallization experiments were performed in plastic Petri dishes sealed by chloroform. This arrangement prevented contamination of samples. Chloroform vapours do not play any role in the crystallization of the enzyme.

Lyophilized enzyme of the specific activity of about 250 000 units/mg (Gašperík et al. 1982) was dissolved in phosphate buffer, 0.1 mol.l^{-1}, pH 7.2, containing 3% (v/v) of dioxane and 8% (v/v) of saturated ammonium sulphate solution to give a 3% (w/v) concentration of the enzyme. The solution

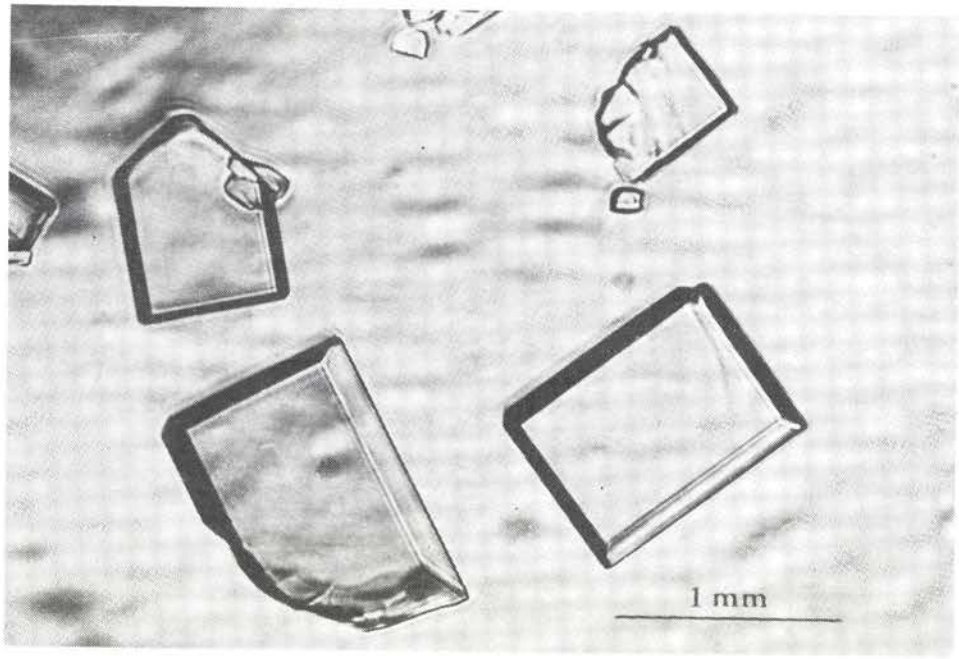

Fig. 1. Crystals of RNase Sa

was centrifuged to remove undissolved material and from the supernatant crystallization samples of 10 μl were pipetted. After one or two days small crystals were obtained by leaving the droplets to equilibrate with 10 % (v/v) ammonium sulphate solution. These crystals were used as seeds in 10 μl droplets of a 3% protein solution in 0.1 mol.l^{-1} phosphate buffer, pH 7.2, containing 22% (v/v) of saturated ammonium sulphate solution and cesium chloride in a concentration of 3 mol.l^{-1}. Then the droplets were equlibrated with 36% (v/v) ammonium sulphate solution. After three weeks, the size of the largest crystals reached about $1.3 \times 0.5 \times 0.4$ mm. All crystallization experiments were carried out at a temperature of about 20°C.

X-ray examination

For X-ray crystallographic investigations the crystals were mounted in glass capillaries in the usual way. X-ray precession photographs were taken at room temperature using unfiltered CuK_α radiation from a fine-focus sealed tube operated at 40 kV, 18 mA, (source Micrometa 2). Crystal-to-film distance given by the geometry of the precession camera (Hanic et al. 1956) was 60 mm. Reflections on the precession photographs extend to 0.21 nm resolution.

Results and Discussion

X-ray precession photographs (Fig. 2) indicate that the space group is $P\,2_12_12$ (m, m, m diffraction symmetry and systematic absences of h, 0, 0 for h odd and

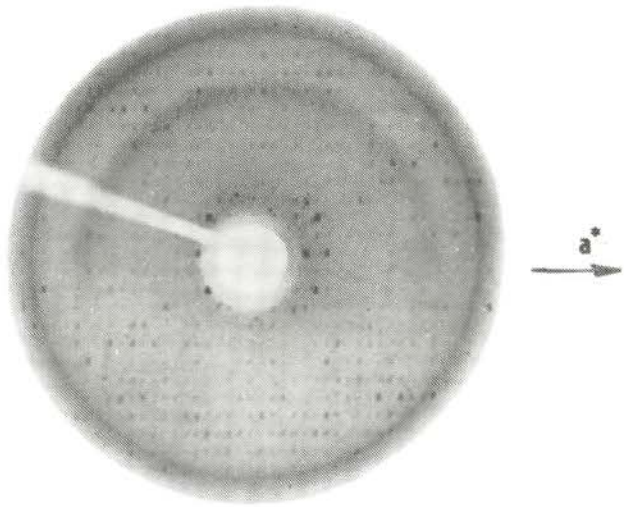

Fig. 2. X ray precession photograph of the h, 0, l zone

0, k, 0 for k odd). The unit cell parameters, which were determined from precession photographs, are a = 6.47 nm, b = 7.89 nm, c = 3.93 nm. The corresponding unit cell volume V = 200.5 nm^3. The volume of the asymmetric unit is 50.1 nm^3, and on the assumption that it contains two molecules of molecular weight of 10 600, the ratio of volume to unit protein mass may be calculated to be 2.36×10^{-3} nm^3/dalton protein. The value is in agreement with the observed average value for protein crystals by Matthews (1968). The partial specific volume, $\bar{v} = 0.707$ $cm^3.g^{-1}$, was determined from amino acid composition according to Cohn et al. (1943). The fractional volume of the crystal occupied by the solvent, $V_{solv} = 50$ % (v/v). The crystallographic data for RNase Sa are summarized in Table 2.

Table 2. Crystallographic data for RNase Sa

Space group	$P2_12_12$
Cell constants	a = 6.47 nm ± 0.02
	b = 7.89 nm ± 0.02
	c = 3.93 nm ± 0.02
Cell volume	V = 200.5 nm^3
Observed maximum resolution	d = 0.21 nm
Molecules/asymmetric unit	2
Partial specific volume	$\bar{v} = 0.707$ $cm^3.g^{-1}$
Solvent content of the crystal	$V_{solv} = 50\%$

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