Three-dimensional structure of ribonuclease from *Bacillus intermedius* 7P at 3.2 Å resolution

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1. INTRODUCTION

The description of enzyme-catalyzed cleavage RNA in terms of the structure–function relationship was limited to date by X-ray data on pyrimidine-specific bovine pancreatic ribonuclease and, to some extent, on a non-specific nuclease from *Staphylococcus aureus*. The knowledge of their three-dimensional structure gave ground for a plausible explanation of their catalytic properties [1–4].

At present, remarkable progress has been achieved in studying a number of guanyl-specific ribonucleases from microorganisms: bacterial RNases from *Bacillus amyloliquefaciens* (barnase, Ba) [5] and *Streptomyces erythreus* (St) [6], and fungal RNases from *Aspergillus oryzae* (T1) [7] and *A. clavatus* (C2) [8]. All these enzymes have a similar M_r-value and catalyze RNA cleavage via transphosphorylation to yield oligonucleotides and mononucleotides with the terminal 2′,3′-cyclophosphodiester groups which are hydrolyzed further by the enzyme to the respective 3′-phosphate, however, they differ from pancreatic RNase in specificity. The main purpose of studying the structure of these RNases was to answer the following questions: Is there any homology in the tertiary structure of guanyl-specific and pancreatic RNases? Are there any similarities in the active site structure of different RNases taking into account that chemical mechanism for the reaction catalyzed seems to be common for the RNases so far studied?

Here, we describe the results of an X-ray study of alkaline guanyl-specific RNase (binase, Bi) isolated from the sporogenous bacterium *Bacillus intermedius*, strain 7P [9]. The enzyme has an M_r of 12300 and consists of 109 amino acid residues with the known sequence [10]. This enzyme is unique in that it lacks sulfur-containing amino acid residues and has only one histidine, which is located in the enzyme active site [11,17].

2. MATERIALS AND METHODS

Crystals of RNase Bi were grown in test tubes from a solution containing 3 M CsCl, 6–8% saturated $(NH_4)_2SO_4$, 0.1 M Na-phosphate buffer (pH 7.0) and protein (10–12 mg/ml). The maximum dimensions of crystals appearing after 5–10 days were 0.3 × 0.6 × 0.8 mm. They belonged to the space group B2 with the following unit cell dimensions: a = 114.5 Å, b = 78.9 Å, c = 33.3 Å and γ = 119.2°. There were 2 protein molecules/asymmetric unit.

While looking for heavy-atom derivatives, we have found that the crystals crack when soaked in the presence of heavy-atom salts down to 10^{-5} M. Further work was done with crystals crosslinked by glutaraldehyde in a solution containing 4 M NaCl, 0.6 M Na-phosphate buffer (pH 7.0) and 0.1% glutaraldehyde. After 2–7 days of such treatment, the unit cell dimensions changed to a = 114.2 Å, b = 77.6 Å, c = 33.6 Å and γ = 118.7° [12].

X-ray intensities for crystals of the modified protein and its heavy-atom derivatives were col-

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lected with a 4-circle diffractometer Syntex $P2_1$. Anomalous scattering data were obtained for $Pt(NO_2)_4^{2-}$ and HgI_4^{2-} derivatives.

3. RESULTS

The main heavy-atom sites in the strongest $Pt(NO_2)_4^{2-}$ derivatives were localized using the difference Patterson map. For the derivatives containing HgI_4^{2-} and $PtCl_6^{2-}$ as well as for the iodinated protein, heavy-atom sites were found with the aid of the difference Fourier map.

After the heavy-atom refinement which was carried out with a NORD-100 computer according to [13] (table 1), an electron density map was calculated at a 3.2 Å resolution with the main figure of merit $m = 0.56$. One α-helix and 3 β-strands can be seen in the map, but its poor quality does not make its full interpretation possible.

The presence of two protein molecules in the asymmetric unit allows one to improve phases by molecular averaging [14]. Calculation of the rotation function [15] based on the intensities of the native protein shows that protein molecules in binase crystals are related by a non-crystallographic diad. The electron density map was then recalculated in an orientation perpendicular to the non-crystallographic diad, and similar features in the density pattern made it possible to find an approximate position of the diad and molecular borderlines. The position of the diad was refined on the bases of the R factor for density averaging, and 3 cycles of phase refinement were done. After each cycle phases set obtained from an averaged map by the Fourier inversion was combined with the observed structure amplitudes to calculate a new electron density map.

The final map was of a far better quality, and the course of the polypeptide chain was traced unequivocally. A correlation of this tracing with the primary sequence was facilitated by the positions of iodine sites in the iodinated protein which served as a marker for Tyr 16 in one protein molecule and for Tyr 89 and Tyr 102 in the other related by the non-crystallographic diad.

RNase Bi may be referred to as an $(\alpha + \beta)$-protein according to the globular protein classification suggested in [16]. This class includes also pancreatic RNase. The binase molecule contains 2 short α-helices (5–17, 25–33) and a twisted β-sheet of 5 anti-parallel strands (49–54, 69–74, 85–90, 92–100, 105–107) (fig.1).

There is a remarkable similarity in the tertiary structure of RNases Bi and Ba [4]. The similarity

Table 1

Statistics of heavy-atom refinement

Derivative	Resolution	No. refl.	R_c	F_H	E (E_{an})	R_c	R_K
$K_2Pt(NO_2)_4$	3.3	3645 (1819)[a]	0.240	3.05	1.92 (0.67)	0.62	0.13
Iodinated protein	3.2	4313	0.214	2.58	2.06	0.70	0.16
K_2HgI_4	4.0	939 (939)	0.124	2.52	1.64 (0.91)	0.64	0.08
K_2PtCl_6	5.0	517	0.147	1.62	1.52	0.77	0.11

[a] Number of anomalous pairs measured

F_H, root-mean-square heavy-atom scattering; $E(E_{an})$, root-mean-square isomorphous (anomalous) error; R_c, R_K, reliability factors [13]

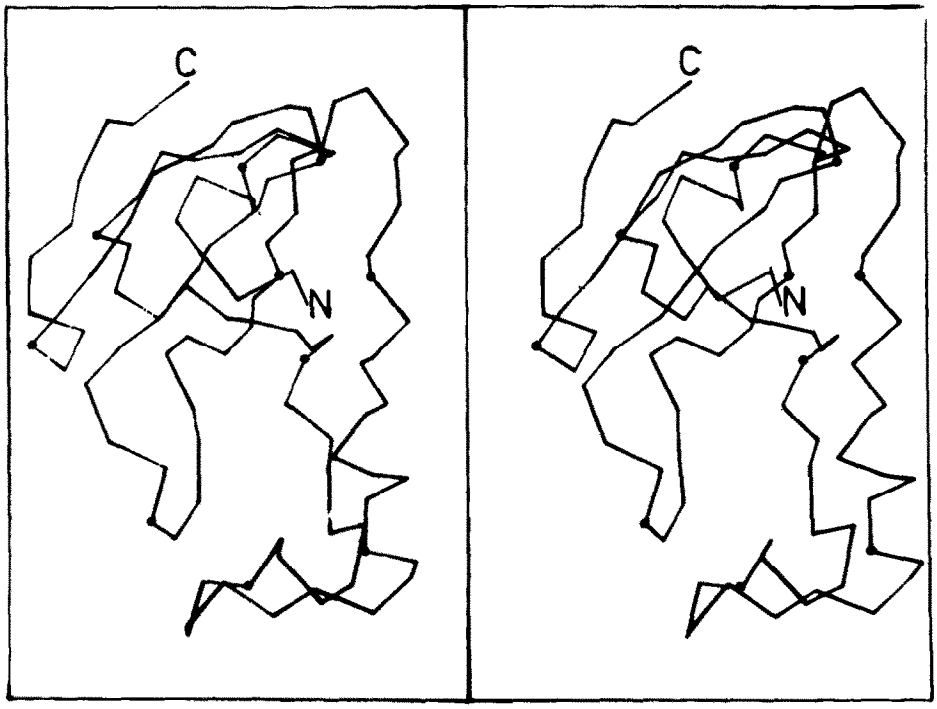

Fig.1. Stereodrawing of the course of polypeptide chain in the molecules of RNase Bi.

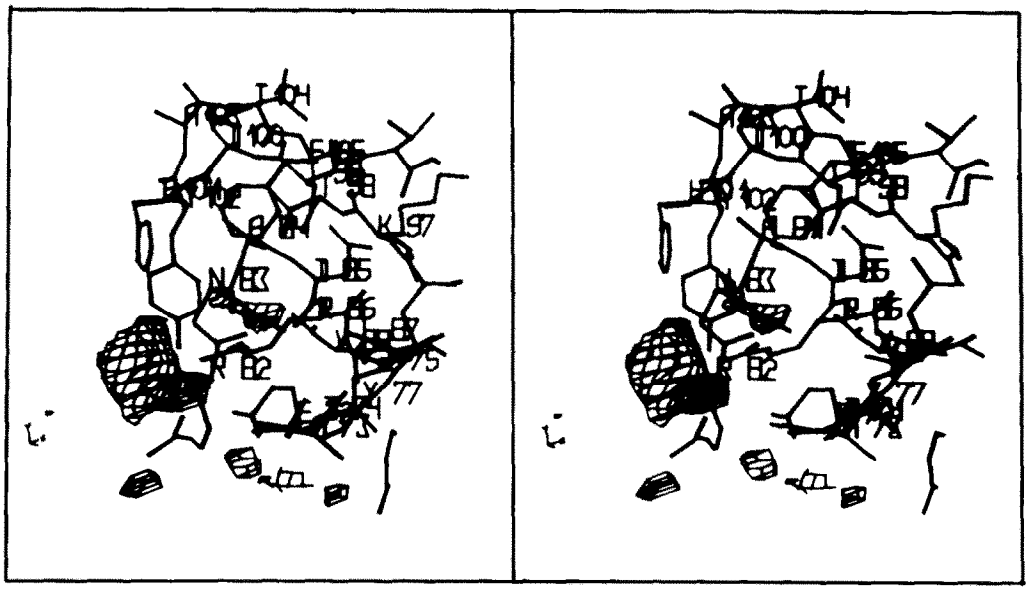

Fig.2. Stereodrawing of a part of the different Fourier map of the native protein *vs* that treated with glutaric aldehyde.

involves not merely the length and mutual orientation of secondary structure elements, but extends to the conformation of many irregular chain segments.

An isolated peak of positive electron density has been found on the Fourier map of the protein crosslinked with glutaric aldehyde. The peak was shown to be located in the C-terminal part of the protein molecule and it was identified tentatively as a phosphate ion. This site appeared to be more occupied in crystals not treated by glutaric aldehyde as illustrated by the difference Fourier map shown in fig.2. The sidechains of Arg 82, Arg 86, His 101 and Tyr 102 were found around the phosphate binding site situated 3.6–4.2 Å distance from the center of the phosphate ion. One may assume that these residues may be closely involved in the enzyme action. The conclusion agreed well with a solution study according to which the histidine residue of the enzyme was an essential component of the enzyme active site [17].

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