THE STRUCTURE OF RIBONUCLEASE IN SOLUTION DOES NOT DIFFER FROM ITS CRYSTALLINE STRUCTURE

A. A. TIMCHENKO, O. B. PTITSYN, D. A. DOLGIKH and B. A. FEDOROV
Institute of Protein Research, USSR Academy of Sciences, 142292 Poustchino, Moscow Region, USSR

Received 27 January 1978

1. Introduction

A traditional question confronting investigators of a protein structure is to what extent may we transfer X-ray data for proteins in crystal to their structures in solution. To answer this question, a method of large-angle diffuse X-ray scattering was proposed in our laboratory [1,2] having high sensitivity to details of an internal structure of globular proteins [1–3]. The use of this method for a direct comparison of protein structures in crystal and in solution is based on the calculation of a scattering curve from protein X-ray atomic coordinates (taking into account solvent influence) [4,5] and on a comparison of the obtained theoretical scattering curve with the experimental one in solution. The region of scattering angles, in which there are differences between these curves, allows to form an opinion about the scale of distances affected by these differences.

In this paper the above method was used to compare the structures of ribonuclease and its complex with the inhibitor in crystal and in solution. It was shown that on a distance scale $\geqslant 10$ Å the structures of both ribonuclease and its complex with the inhibitor in solution do not differ from their crystalline structures.

2. Materials and methods

Bovine pancreatic ribonuclease A was isolated in the laboratory of M. Ya. Karpeisky (Institute of Molecular Biology, USSR Academy of Sciences) and was kindly provided for our investigations. The concurrent inhibitor was isolated from a mixture of 2′-CMP and 3′-CMP as in [6]. The solutions of the enzyme and its complex with the inhibitor were investigated in a 0.2 M Na-acetate buffer at 21.0 ± 0.5°C, pH 5.4 (at which the protein–inhibitor binding constant is close to the maximal one [7]). The binding of the inhibitor with the enzyme was tested by ultraviolet difference spectra at $\lambda = 260$ nm. The preservation of enzymatic activity before and after X-ray measurements was checked by the increase of ultraviolet absorbance at $\lambda = 260$ nm on splitting of RNA. The scattering curves were obtained with the equipment in [8]. The maximal statistical error in the determination of intensities did not exceed 1% at minimal scattering angles and 5% at maximal ones. The curve for ribonuclease was measured 4 times for two different species, the curve for ribonuclease–2′-CMP complex was measured 3 times; fig.1–3 represent averaged curves.

For a more strict comparison of the experimental curves with the theoretical ones we did not undertake a slit desmearing but compared the experimental curves with the theoretical ones which were 'smeared' taking into account the parameters of our collimation slits (the distribution of an incident beam intensity along the height has a trapezium-like shape with bases of 32.2 mm and 58.4 mm, the receiver slit length is 16.7 mm and the sample–receiver distance is 204 mm).

3. Results and discussion

To investigate the influence of a possible association of molecules as well as intermolecular interference on scattering curves, the latter were measured at 5 concentrations from 39.0–13.8 mg/ml. The scattering

Table 1
Values of molecular weights and gyration radii

Sample	Molecular weight ($M \times 10^{-3}$)		Gyration radius R_g (A)	
	Exp.	Calc.	Exp.	Calc.
Ribonuclease	13.3 ± 1.0	13.7	15.9 ± 0.5	15.0
Ribonuclease–2′-CMP	15.5 ± 1.0	14.0	16.4 ± 0.6	14.8

curves (calculated per molecule) did not depend on concentration at $\mu \gtrsim 0.1$ Å^{-1}, where

$$\mu = (4\pi/\lambda) \cdot \mathrm{Sin}\,\theta$$

($\lambda = 1.5$ Å is an X-ray wavelength, 2θ is the scattering angle.) For both ribonuclease A and its complex with 2′-CMP an extrapolation of small-angle scattering data to zero concentration gave the values of molecular weights M and gyration radii R_g close to those calculated from the chemical composition and the X-ray atomic coordinates, respectively (table 1).

As X-ray atomic coordinates of ribonuclease A are not yet available, we compared our experimental curves with those calculated from X-ray atomic coordinates of ribonuclease S [9] and its complex with 2′-CMP [10] (the same X-ray atomic coordinates were used for R_g calculation in table 1). Such a comparison is meaningful because the main chain course of ribonuclease S [9] differs from that of ribonuclease A [11] only in a small region of the molecule (from residues 18–23) close to the rupture of the bond between residues 20 and 21. Moreover, our calculations have shown that the model displacement of the S-peptide in the ribonuclease S [9] relative to its position in the ribonuclease A [11] did not in practice influence the scattering curve.

The scattering curve calculation was made by the 'cube method' [3,4] taking into account solvent influence. The method was modified to take into consideration more exactly cavities and clefts in a protein molecule accessible to the solvent. The scattering curves for ribonuclease S and its complex with 2′-CMP calculated by this method are plotted in fig.1 as

$$\log \widetilde{\Delta I}(\mu) \text{ versus } \mu$$

where $\widetilde{\Delta I}$ is a scattering intensity of one molecule,

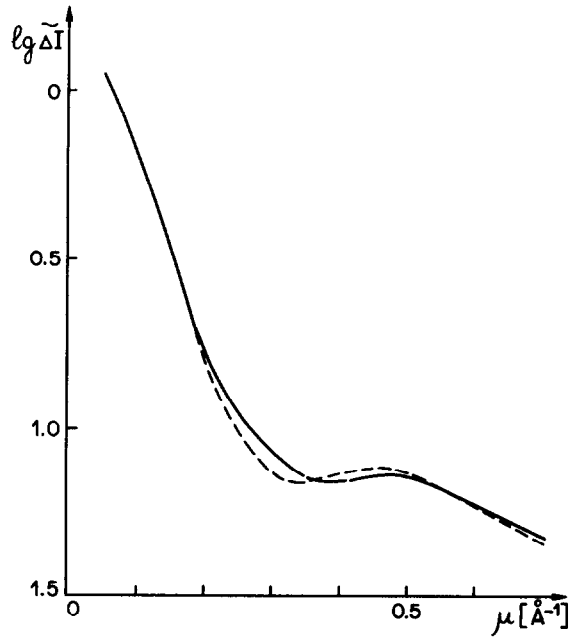

Fig.1. Comparison of the theoretical scattering curves for ribonuclease S (——) and its complex with 2′-CMP (– – –).

'smeared' taking into account real geometry of the slits used. According to the data of paper [10] the inhibitor binding does not cause noticeable changes of ribonuclease structure, therefore any differences between the curves can be due only to the contribution of the inhibitor altering the molecular weight to which it contributes only ~ 2% of the molecular weight of the complex. Figure 1 shows that the inhibitor binding without any alterations in the protein structure noticeably changes the calculated curve, which provides evidence once again about the high sensitivity of the method (cf. [1–3]).

In fig.2 the experimental scattering curve for ribo-

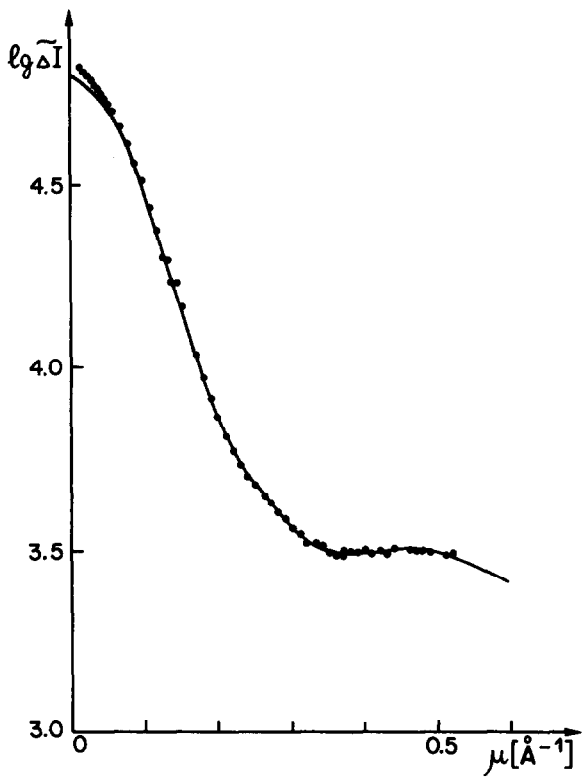

Fig.2. Comparison of the theoretical scattering curve (——) with the experimental one for ribonuclease (· · ·). $\Delta\tilde{I}$ is a scattering intensity extrapolated to zero concentration (impulses per second) calculated per macromolecule.

Fig.3. Comparison of the theoretical scattering curve (——) with the experimental one for ribonuclease–2′-CMP complex (· · ·).

nuclease A is compared with the scattering curve calculated from X-ray atomic coordinates of ribonuclease S. The comparison of the curves was done at an absolute intensity scale calculated per macromolecule. The divergence between experimental and theoretical values did not exceed a systematic error in the calculation of the absolute scale (~ 10%) which allowed one to superpose the absolute values to facilitate comparison of the curves. Figure 2 shows that the curves coincide well at all the angle intervals measured ($\mu \leqslant 0.5$ Å^{-1}) corresponding to Bragg's distances

$$d = \lambda/(2 \operatorname{Sin} \theta) \gtrsim 10 \text{ Å}$$

This shows that the ribonuclease structure in solution practically coincides with its crystal structure at a distance scale $\gtrsim 10$ Å. A comparison of the laser Raman scattering spectra of ribonuclease A in crystal and in solution [12] has already indicated the absence of noticeable changes in internal rotation angles of the main chain (averaged over the whole molecule). Our results indicate the absence of noticeable displacements of large masses of the molecule caused by changes of angles in short segments of the chain.

Figure 3 represents an analogous comparison for the scattering curves of the 'ribonuclease–2′-CMP' complex. Figure 3 shows that the structure of the complex in solution practically coincides with its crystalline structure. This means, in particular, that in the given case (in contrast to hen egg-white lysozyme [1]) binding of the inhibitor does not result in a noticeable change of the protein structure, not only in crystal but also in solution.

Acknowledgements

The authors would like to express their sincere gratitude to Dr I. N. Serdyuk for numerous discussions of the progress and the results of the work and to Professor M. Ya. Karpeisky for putting ribonuclease A at our disposal.

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