THE STRUCTURE OF PHAGE T4 LYSOZYME IN SOLUTION NOTICEABLY DIFFERS FROM ITS CRYSTALLINE STRUCTURE

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1. Introduction

In the preceding paper [1] it was shown by the method of large-angle diffuse X-ray scattering [2,3] that the structure of bovine pancreatic ribonuclease and that of its complex with an inhibitor in solution coincide with their crystalline structures (on a distance scale $\geqslant 10$ Å). Also it was shown [2] that hen egg-white lysozyme structure in solution differs from its crystalline structure by a small opening of the substrate-binding cleft, the binding of the competitive inhibitor stabilizing the closed state of the cleft observed in the crystalline structure. Sperm whale myoglobin structure in solution also differs from its crystalline structure, the differences being interpreted as changes of the GH hairpin position relative to the remaining part of the molecule [4]. In all probability in both cases we are dealing with fluctuations in relative positions of large masses of a protein molecule which are frozen in the crystalline structure but which manifest themselves in solution. The possibility of such fluctuations was demonstrated by direct model calculations for hen egg-white lysozyme [2].

Here the above-mentioned method has been used to study phage T4 lysozyme. The crystalline structure of this protein is characterized by the closed substrate-binding cleft, therefore 'in order to allow the substrate to enter, the enzyme would have to undergo a fairly substantial conformational change' [6]. Our results provide evidence of a noticeable difference between the structure of this protein in solution and in crystal showing for the first time that the enzyme structure in solution can substantially differ from its crystalline structure.

2. Materials and methods

Phage T4 lysozyme was isolated as in [7]. Two samples (A and B) were investigated, obtained from independent preparations. Protein homogeneity was tested by electrophoresis and N-terminal analysis. The preservation of enzymatic activity in the course of X-ray measurements was determined by measuring the turbidity decrease in a suspension of *E. coli* or *M. lysodeikticus* cells.

The disaccharide-tetrapeptide, *N*-acetylglucosaminyl-*N*-acetylmuramic-L-alanyl-γ,D-glutaminyl-meso-diaminopimelyl-D-alanine, was used as a concurrent inhibitor. As a source for the preparation of inhibitor a peptidoglucan was used which was isolated from the intact *E. coli* cells by treating them with hot sodium dodecylsulphate [8,9]. Treatment with proteases was excluded. After isolation the peptidoglucan was digested by hen egg-white lysozyme in 0.03 M NH_4HCO_3, the digest was dialyzed against water and the inhibitor purified from the concentrated dialyzed liquid by gel filtration on BioGel P-4 and two successive paper chromatographies using the butanol/acetic acid/water (4:1:5, upper phase) system [10].

Inhibitor protein binding was tested for by fluorescence spectra and ultraviolet difference spectra; the binding constant $K_{ass} = (1.2 \pm 0.1) \times 10^4$ mol^{-1} [11]. All solutions were measured in Na–K-phosphate

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buffer (pH 7.4, $I = 0.05$) containing 5×10^{-3} M dithioerythritol. These conditions are close to the optimum for enzymatic activity [12] and correspond to good protein–inhibitor binding [11,13]. The scattering curves were obtained by the equipment in [14]. The maximal statistical error in the determination of the intensity did not exceed 1% and 5% at minimal and maximal scattering angles, respectively. The scattering curves for the protein and its complex with the inhibitor were measured three times for two different protein species.

The theoretical scattering curve was calculated by a modified 'cube method' [15,16] from the X-ray atomic coordinates of the protein [17]. To obtain the inhibitor coordinates, a molecular model of the protein–inhibitor complex was built in which the centers of both saccharide rings coincided with their positions calculated in [18], and the peptide tail was set between the F and G helices in the most complementary way [11]. The inhibitor atomic coordinates obtained with the help of the molecular model were then refined taking into account bond lengths and bond angles. For a more strict comparison of theoretical and experimental curves, theoretical curves were 'smeared' using real parameters of the slits used [1]. To obtain gyration radii and molecular weights, a collimation correction of the experimental curves was made as in [19].

3. Results and discussion

The scattering curves were measured at concentrations from 5–41 mg/ml and did not depend on the concentration (calculating per molecule) at $\mu \geqslant 0.1$ Å^{-1}. ($\mu = (4\pi/\lambda)\mathrm{Sin}\Theta$ where $\lambda = 1.54$ Å is an X-ray wavelength and 2Θ is the scattering angle.) For phage T4 lysozyme and its complex with the inhibitor extrapolation to zero concentration gave the molecular weights and the gyration radii shown in table 1. The molecular weight values are in good agreement with those calculated from the chemical composition, but both for the protein and for its complex with the inhibitor the experimental R_g values are 13% greater than those calculated from the X-ray atomic coordinates [17] (table 1; fig.1). Already this circumstance testifies to a difference between the protein structures in solution and in crystal.

Figure 2 represents a comparison of the experimental scattering curves of phage T4 lysozyme with the curve calculated from its X-ray atomic coordinates. Both experimental and theoretical curves are represented on an absolute intensity scale calculated per

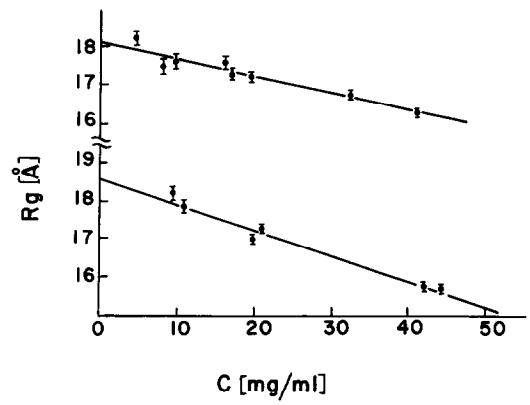

Fig.1. Concentration dependence of the gyration radii R_g for phage T4 lysozyme (upper) and its complex with the inhibitor (lower).

Table 1
Values of molecular weights and gyration radii

	Molecular weight ($M \times 10^{-3}$)		Gyration radius R_g (A)	
Sample	Exp.	Calc.	Exp.	Calc.
Phage T4 lysozyme	18 ± 1.0[a]	18.7	18.6 ± 0.6	16.4
Lysozyme–inhibitor	21 ± 1.0[a]	19.6	18.1 ± 0.6	16.2

[a] The M values were determined according to the partial specific volume $\bar{v} = 0.737$ cm^3/g calculated from the amino acid composition of the protein [20]

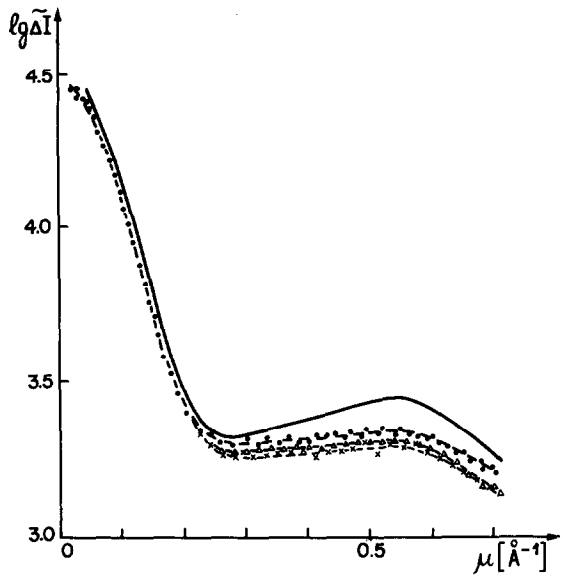

Fig.2. Comparison of the theoretical scattering curve for phage T4 lysozyme (——) with the experimental curves (signs (×, •) correspond to two independent experiments for sample A, and (△–△) for sample B). $\tilde{\Delta I}$ is the scattering intensity (impulses per second) per molecule extrapolated to zero concentration.

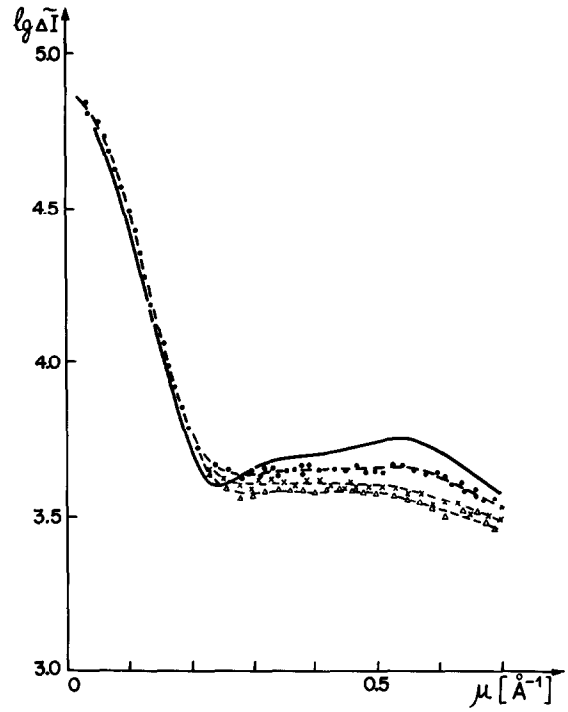

Fig.3. Comparison of the theoretical scattering curve for the complex of phage T4 lysozyme + inhibitor (——) with the experimental curves (signs (×) correspond to sample A, and signs (•,△–△) to two independent experiments for sample B).

macromolecule and are not displaced relative to each other for better coincidence. It is clear from fig.2 that starting from $\mu \approx 0.3$ Å^{-1} (Bragg's distance $d = \lambda/2\,\mathrm{Sin}\Theta \approx 20$ Å) the experimental curves considerably differ from the theoretical one. The basic maximum on the theoretical scattering curve at $\mu \approx 0.55$ Å^{-1} ($d \approx 10$ Å) is almost fully smeared on the experimental ones. Figure 3 represents an analogous comparison for the complex of phage T4 lysozyme with the inhibitor and fig.3 also shows the great differences between the experimental and the theoretical curves, starting, in this case, from $\mu \approx 0.25$ Å^{-1} ($d \approx 25$ Å). There is no maximum on the experimental curve at $\mu \approx 0.55$ Å^{-1} which is characteristic for the theoretical one.

For comparison it should be noted that for ribonuclease and its complex with an inhibitor the experimental curves obtained were practically the same as the theoretical ones at $\mu \leqslant 0.5$ Å^{-1} [1] and for hen egg-white lysozyme only small differences were observed in this region, which practically disappeared after binding of the inhibitor [2]. This circumstance allows one to conclude that discrepancies between experimental and theoretical curves both for phage T4 lysozyme and its complex with the inhibitor reflect essential differences in the structure of this protein and its complex with the inhibitor in solution and in crystal.

Figure 4 represents a comparison of the theoretical scattering curves for the crystalline structure of phage T4 lysozyme and the complex of the same structure with the inhibitor. It is seen that as with ribonuclease [1], binding of the inhibitor without any changes in the protein structure causes noticeable changes in the scattering curve. However, in contrast to ribonuclease, the difference between the experimental scattering curves is not great as was predicted by calculations (fig.5). This can mean that either the inhibitor does not in practice contribute to the scattering curve of protein with the structure which it has in solution, or this contribution is an essential one, like in the case of crystalline structures, as seen in fig.4, but it is

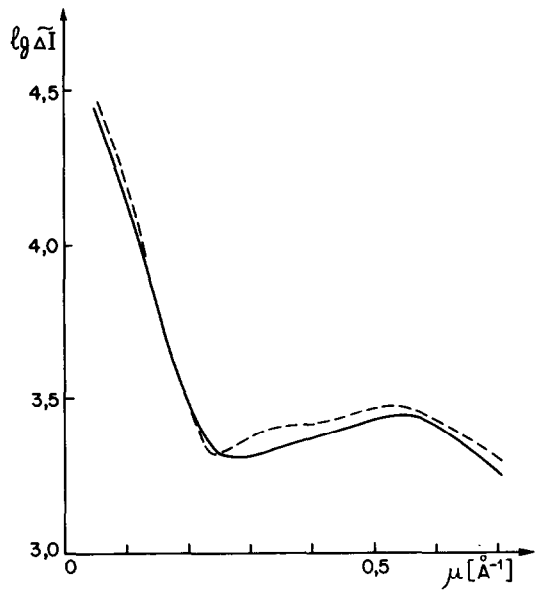

Fig.4. Comparison of the theoretical scattering curves for phage T4 lysozyme (——) and its complex with the inhibitor (– – –).

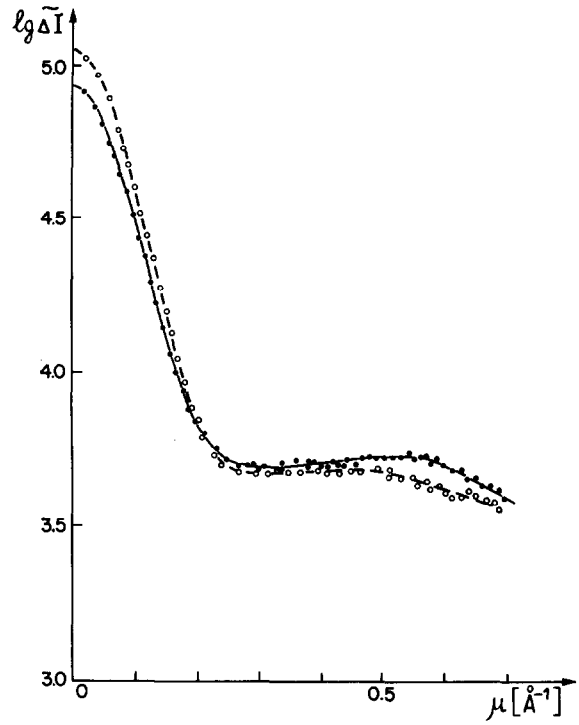

Fig.5. Comparison of the experimental scattering curves for phage T4 lysozyme (–●–●–) and its complex with the inhibitor (–○–○–). Both experimental curves present mean values of three independent experiments for each curve.

compensated by a change in the protein conformation at the site of fitting of the inhibitor. The choice between these alternatives is our further problem.

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