Small-angle X-ray studies on structure of the subunits of the *lac* repressor from *Escherichia coli*

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1. INTRODUCTION

In [1] we reported a small angle X-ray scattering study of the *lac* repressor and its tetrameric core obtained by proteolytic cleavage, showing both to be highly asymmetric, oblate structures with maximum diameters of 18.0 ± 0.5 nm and 16.0 ± 0.5 nm, respectively. Here, we report an extension of this study to T-core monomers and dimers, which indicates that the T-core monomers are elongated particles with an axial ratio of ~ 1:2. Dimers are formed by a side-to-side, rather than end-to-end apposition of the 2 monomers and tetramers by an end-to-end apposition of 2 dimers.

2. MATERIALS AND METHODS

Repressor and T-core were prepared as in [1,2]. To obtain the subunits, T-core preparations were treated with carboxy peptidase A and B and the fragments separated by gel-filtration on a Sephadex G-150 column and further purification on a G-50 column, eluted with 0.2 M ammonium bicarbonate with 3×10^{-4} M dithiothreitol, at 5°C. The dimer preparation represents the more rapidly, and the monomer preparation the more slowly, moving peak on the G-50 column.

For the small angle studies the T-core subunits were extensively dialyzed against a 0.3 M potassium phosphate buffer containing 0.2 M KCl, 1×10^{-4} M dithiothreitol, and 1×10^{-4} M ethylenedinitrilotetraacetic acid (pH 7.7).

The small angle X-ray scattering measurements on the repressor subunits were made as in [1]. Concentration series (from 3–30 mg protein/LM) were measured on 2 different subunit preparations and extrapolated to zero concentration [3]. Each scattering curve was recorded several times; ~ 10^5 pulses were counted/scattering angle. Evaluation of scattering data was performed by computer programs from [4,5] using the general procedures detailed in [6,7]. As in [1] the indirect Fourier transform method [8], was used to obtain the pair distance distribution function $p(r)$ in real space. This function describes the frequency distribution of the distances r within a macromolecule by combining any volume element with any other volume element and taking into consideration the difference in electron density. From the $p(r)$ function the radius of gyration R and the maximum diameter $D_{\max}$ can be calculated; $p(r)$ becomes zero at values of r equal to or greater than the maximum diameter $D_{\max}$ of the particle. Thus the principal structural parameters are obtained without recourse to any specific model. The shapes of the monomer and dimer were approximated by a large number of closely packed small spheres (radius 0.5 nm); 132 spheres packed in an asymmetric array with an approximate axial ratio of 1:2 were required to obtain a fit to the experimental curves for T-core monomer.

3. RESULTS AND DISCUSSION

The radii of gyration and maximal diameters of particles in the two subunit preparations (I,II) are summarized in table 1. In fig.1 the pair distance distribution function $p(r)$ of the model suggested for the monomer is compared with the experimental

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Table 1

Radii of gyration R and maximum diameters D_{max} of T-core subunits

Parameter	Prep. I	Model a (dimer)	Prep. II	Model M (monomer)
R [nm]	3.00±0.05	3.1	2.80±0.05	2.6
D_{max} [nm]	10.0 ±0.5	10.0	9.5 ±0.5	9.0

Experimental values obtained for preparation I and II compared with those calculated for model *M* (monomer, fig.1) and model *a* (dimer, fig.2)

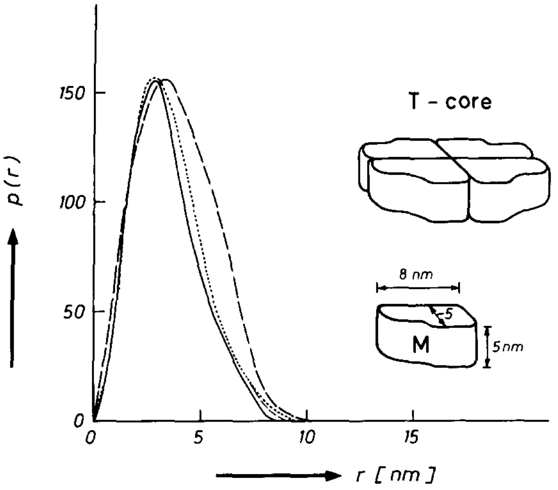

Fig.1. Comparison of the distance distribution *p(r)* – functions of model M (monomer T-core) (——) with preparation I (- - -) and II (. . .) respectively (- - -) without innermost part of the scattering curve (eliminating some aggregates).

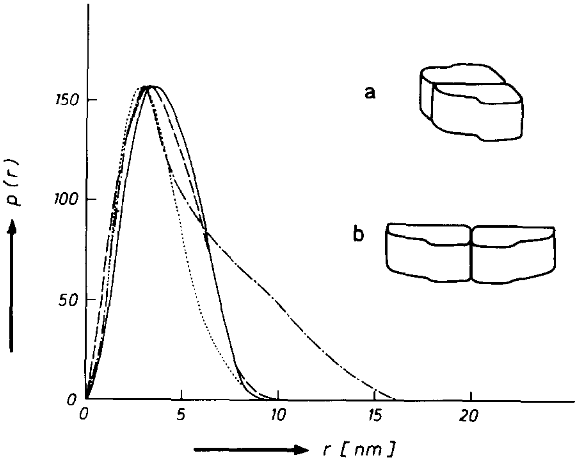

Fig.2. Comparison of the distance distribution functions of the dimer models a (——) and b (—•—••—) with the preparation I (- - -) and II (• • •).

curves of preparation I and II. In fig.2 the experimental curves of both preparations are compared with the theoretical curves of dimers obtained by arranging the monomers in the direction of the longest axis (*b*) or side-by-side (*a*).

The figures show that preparation II fits fairly well the *p(r)* function of the monomer, preparation I that of the dimer obtained by arranging 2 monomers side-by-side (fig.2). The radius of gyration (R = 2.8 ± 0.05 nm) and the maximum distance (D_{max} = 9.5 ± 0.5 nm) calculated for the particles of preparation II corresponds within the uncertainties of the inhomogeneities (probably small amounts if dimers) to the values of the monomer (R = 2.6 nm, D_{max} = 9 nm). The values obtained for preparation I on the other hand (R = 3.0 ± 0.05 nm, D_{max} = 10 ± 0.5 nm) are very similar to those calculated for dimer *a* (R = 3.1 nm, D_{max} = 10 nm). The curve for dimer *b* does not correspond to any of the measured *p(r)* functions, indicating that *b* can be ruled out as a possible dimer structure. In spite of the fact that the presence of some inhomogeneities on the samples precludes an exact evaluation of the data, the accuracy is sufficient to draw 2 important conclusions:

(1) The monomers of the T-core of the *lac* repressor are somewhat elongated particles (main axial ratio 1:2).

(2) The dimers are formed by arranging the monomers side-by-side and not end-to-end.

The tetramers can be viewed as a pair of dimers attached end-to-end.

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