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Small-Angle X-ray Studies of the Quaternary Structure of the *lac* Repressor from *Escherichia coli*†

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ABSTRACT: The quaternary structures of the *lac* repressor molecule from *Escherichia coli* and its tetrametic core, which can be derived from it by proteolytic cleavage, were studied in dilute solutions by small-angle X-ray scattering. The dimensions and general shape of the *lac* repressor and of the tetrameric core are reported. The core itself appears to be an elongated structure, and in the intact repressor the headpieces are located at its ends. The results are derived from model calculations and from the following molecular parameters determined from the scattering curve and the pair distance distribution function: for lac repressor, radius of gyration 5.30 ± 0.02 nm, radius of gyration of the cross section 2.20 ± 0.03 nm, maximum diameter 18.0 ± 0.5 nm, hydrated volume 329 ± 20 nm^3, relative molecular mass 149 000 ± 15 000; for tetrameric core, radius of gyration 4.92 ± 0.02 nm, radius of gyration of the cross section 2.24 ± 0.03 nm, maximum diameter 16.0 ± 0.5 nm, hydrated volume 278 ± 15 nm^3, relative molecular mass 120 000 ± 10 000.

The *lac* repressor is a regulatory protein of *Escherichia coli* originally isolated and characterized by Gilbert & Muller-Hill (1966). It has a molecular weight of 154 500 and is a tetramer of four identical subunits, each with a known sequence of 360 amino acids (Beyreuther, 1978; Farabaugh, 1978). Its function is to control the expression of the *lac* operon by forming a complex with the operator region of its DNA. Some of the physical properties of the repressor have been studied (Barkley et al., 1975), but little is known about its tertiary and quaternary structures. In the present paper we report the dimensions and the general shape of the repressor molecule and of the tetrameric core that can be derived from it by proteolytic cleavage, as determined by small-angle X-ray scattering.

Materials and Methods

The *lac* repressor (LR)[1] was prepared from cultures of the SQ mutant of *E. coli*, as previously described (Matthews et al., 1977). The tryptic core (T-core) and headpiece (HP) fragments of LR have been prepared according to the procedure of Geisler & Weber (1977). LR, at a protein concentration of 15 mg/mL in 1 M Tris-HCl buffer (pH 7.6), 30% glycerol, and 3×10^4 M dithiothreitol, was digested with 1.5% of its weight of *N,N*-dicyclohexylcarbodiimide-treated trypsin (Sigma Chemical Company) for 2 h at 20 °C. The trypsin was then inactivated by a threefold excess of soybean trypsin inhibitor (Sigma Chemical Company). Under these conditions, peptide bond hydrolysis at lysyl residue 59 goes to completion, while a partial cleavage occurs at arginyl residue 51. The monomeric HP (residues 1–51 and 1–59) and the tetrameric T-core (residues 60–347) have been purified at 5 °C by gel filtration on a column of Sephadex G-150 eluted with 0.2 M ammonium bicarbonate with 3×10^{-4} M dithiothreitol.

For diffraction studies LR, T-core, and HP 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 homogeneity of the LR and T-core preparations was checked by NaDodSO$_4$–gel electrophoresis, as shown in Figure 1.

A highly stabilized X-ray generator (Philips PW 1140) with a copper target tube (50 kV, 30 mA) and a camera with slit

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[1] Abbreviations used: LR, *lac* repressor; T-core, tryptic core; HP, headpiece; NaDodSO$_4$, sodium dodecyl sulfate.

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Table I: Molecular Parameters of the *lac* Repressor and T-Core

parameter	*lac* repressor	T-core
radius of gyration R (nm)		
derived from Guinier plot	5.30 ± 0.02	4.92 ± 0.02
derived from $p(r)$ function	5.31 ± 0.02	4.91 ± 0.02
radius of gyration of the cross section R_c	2.20 ± 0.03	2.24 ± 0.03
maximum diameter D_{max} (nm)	18.0 ± 0.5	16.0 ± 0.5
volume V (nm^3)	329 ± 20	278 ± 15
rel molecular mass M_r	149 000 ± 15 000	120 000 ± 10 000

collimation were used for data collection (Kratky, 1958). The *lac*-repressor solutions were placed in Mark capillaries at 4 °C. The scattered intensities were measured at 106 different scattering angles in the range between 0.002 and 0.13 radian using an entrance slit of 120 μm and an electronically programmable step scanning device (Leopold, 1965). A proportional counter with an X-ray analysis channel control was used for recording. A pulse height discriminator was focused on the Cu Kα line. Concentration series (from 4 to 44 mg of repressor per mL) were measured on five different LR preparations and extrapolated to zero concentration (Pilz et al., 1979). Each scattering curve was recorded several times; about 10^5 pulses were counted per scattering angle. Evaluation of the scattering data was performed by computer programs of Zipper (1972) and Glatter (1974), using the general procedures previously described in detail (Pilz et al., 1979; Kratky, 1963; Pilz, 1973). By a new method, the indirect Fourier transform of Glatter (1979), the pair distance distribution function $p(r)$ in real space can be calculated. This function gives the frequency 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.

Results

The radius of gyration R of a dissolved particle is one of the most important parameters obtained by small-angle X-ray scattering and can be determined by several different procedures. The most widely used method is based on the Guinier approximation, in which R is derived from the slope of the scattering curves at very small angles. The radius of gyration can also be determined from the distance distribution function $p(r)$. This method is particularly powerful when used in connection with the indirect Fourier transform (Glatter, 1979). It has the advantage that the whole scattering curve is used for the determination of the radius of gyration and not only its innermost portion, as in the Guinier method. In the present case, the values obtained using these two methods agree well; for the whole *lac* repressor R = 5.3 nm and for the T-core R = 4.9 (Table I).

The concentration dependence of the scattering curves is summarized in Table II for three samples each of *lac* repressor and T-core. For this comparison the immediately obtained slit-smeared and unsmoothed scattering curves were used. The apparent radius of gyration $\tilde{R}$ was calculated from the slope of the straight lines on the Guinier plot (log $\tilde{I}$ vs. $(2\theta)^2$), where $\tilde{I}$ is the slit-smeared scattered intensity and 2θ is the scattering angle. (Desmearing increases the value of the radius of gyration; thus, the final R values in Table I are higher than the apparent $\tilde{R}$ values in Table II; the tilde on $\tilde{R}$ and $\tilde{I}$ indicates the slit-smeared values.) Extrapolation to zero concentration

Table II: Concentration Dependence of the Scattering Curves of *lac* Repressor and T-Core[a]

T-core			*lac* repressor		
prepn no.	c (mg/g)	$\tilde{R}$ (nm)	prepn no.	c (mg/g)	$\tilde{R}$ (nm)
1	22.8	4.06	1	44.8	3.75
	14.1	4.17		34.9	3.94
	10.9	4.19		25.7	4.12
	9.1	4.23		13.8	4.38
	lim 0	4.35		lim 0	4.63
	$c\to0$			$c\to0$	
2	37.8	4.12	2	29.5	4.06
	30.0	4.17		23.0	4.21
	21.2	4.23		14.6	4.38
	15.0	4.28		12.4	4.42
	10.5	4.32		7.7	4.49
	lim 0	4.38		4.8	4.57
	$c\to0$			lim 0	4.66
				$c\to0$	
3	22.9	4.06	3	13.8	4.21
	12.0	4.19		12.4	4.26
	8.5	4.23		10.0	4.32
	7.5	4.26		7.8	4.40
	lim 0	4.35		4.7	4.45
	$c\to0$			lim 0	4.66
				$c\to0$	

[a] Apparent radii of gyration $\tilde{R}$ calculated from the unsmoothed, smeared scattering curves for the given concentrations c of three different preparations, extrapolated to zero concentration.

yields larger radii of gyration $\tilde{R}$ for both *lac* repressor and T-core, indicating that aggregation is not an important factor, since aggregation and hence $\tilde{R}$ in aggregating systems *increase* with increasing concentration. The possibility that the relatively large radii are an artifact of aggregation was also ruled out by examining the dispersity of the preparations in a Spinco Model E analytical centrifuge immediately following the X-ray measurements. No more than 2–3% aggregation was found, which would not make a significant contribution to the calculated radius.

Smaller radii of gyration (~4 nm) have been obtained by McKay et al. (personal communication) using the Guinier approximation in small-angle X-ray scattering and by Zaccai et al. (personal communication) using neutron diffraction. These radii are comparable to our *uncorrected* radii (Table II), and, in fact, in the study of McKay et al. no corrections were applied. We believe, on the other hand, that a slit width correction is necessary even if a monochromatic source is used and that the corrected values more closely reflect the actual dimensions of the molecule. In addition, extrapolation on a Guinier plot from larger scattering angles than those used in the present study may lead to R values that are too small.

The maximum diameter D_{max} determined from the $p(r)$ function was 18.0 ± 0.5 nm for the whole *lac* repressor and clearly smaller for the T-core, 16.0 ± 0.5 nm. These values are larger than the largest dimensions of the unit cell obtained by T. A. Steitz (personal communication), i.e., 15 nm for the T-core and 16.5 nm for LR. The comparison suggests that either the dimensions of these molecules are not the same in the crystal and in solution or, more likely, their shape cannot be properly approximated by an ellipsoid of revolution. The D_{max} obtained from the $p(r)$ function will correspond to the length of the longest molecular axis only for an ellipsoid of revolution. For a molecule whose shape is more nearly approximated by a prism, it will correspond to the diagonal. In fact, using the dimensions of the unit cells obtained by Steitz it is possible to calculate their diagonals to be 16.7 and 18.4 nm for the T-core and LR, respectively, in close agreement with the D_{max} value observed in the present study.

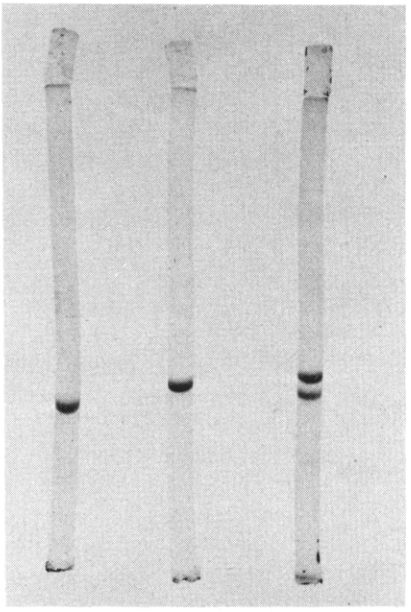

FIGURE 1: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 10% acrylamide gels (Laemmli, 1970): (a) *lac* repressor; (b) T-core; and (c) a mixture of *lac* repressor and T-core.

The volume (V) of the hydrated *lac* repressor and the T-core was calculated from the scattered intensity at zero angle and the invariant. It was found to be 329 ± 20 nm^3 for the *lac* repressor and 278 ± 15 nm^3 for the T-core. The invariant was determined by integration, using the Simpson rule in the angle of range 0 to 0.07 radian. At the tail ends the curves showed a course oscillating around $k/(2\theta)^4$, and the integration could be performed analytically from 0.07 radian to 0 after the determination of k.

The relative molecular mass M_r was determined from the absolute intensity (obtained using a calibrated Lupolen platelet; Kratky et al., 1966), the intensity at zero angle, and the partial specific volume (0.735 g/cm^3). Since the concentration and partial specific volume could not be determined with the necessary accuracy, the M_r values are subject to a relatively higher error.

The structural parameters discussed so far (cf. Table I) can be calculated directly from the scattering curve or its Fourier transform and are independent of any model. To obtain more detailed information on the shape of the protein, it is necessary to test specific models for agreement with experimental data. This type of analysis of small-angle data in terms of models describing molecular shape is only meaningful for structures known to be chemically homogeneous (see Figure 1). The basic strategy is to select models that have the experimentally determined structural parameters and to compare the experimental and the calculated scattering curves. This process can be carried out either in reciprocal space (scattering curve) or in real space (with the $p(r)$ function). All models which do not predict either curve correctly can be ruled out, but discrimination between different models giving a correct fit is not possible. Thus, agreement of the predictions of the model with experimental data does not constitute proof that the chosen model is correct. However, when a limited number of similar models predict the experimental scattering curve, the models can be considered to have a high degree of reliability.

Figure 2 shows the experimental scattering curves of the whole *lac* repressor and the T-core. Both curves are similar, and the slope at the principal maximum indicates that the

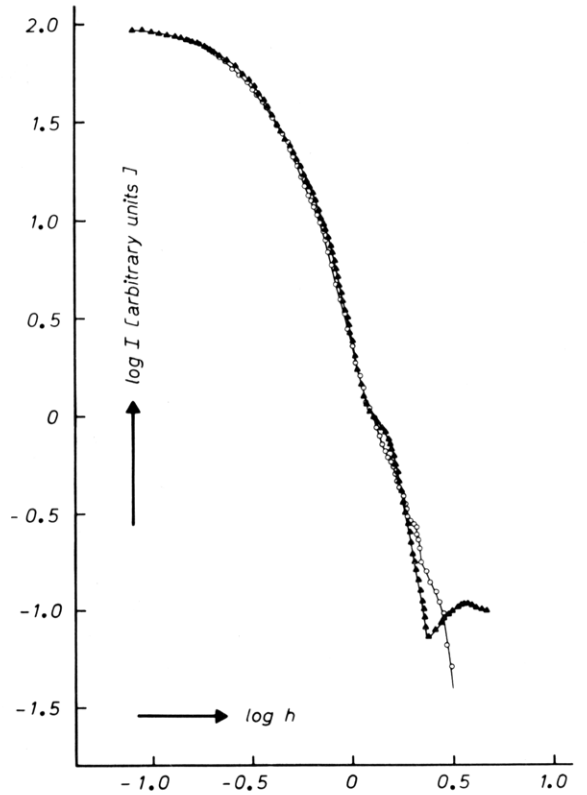

FIGURE 2: Scattering curve of the *lac* repressor (▲) and the T-core (○) in log–log plot: I = scattered intensity; $h = 4\pi \sin\theta/\lambda$; $\lambda = 0.154$ nm, wavelength used (Cu Kα line).

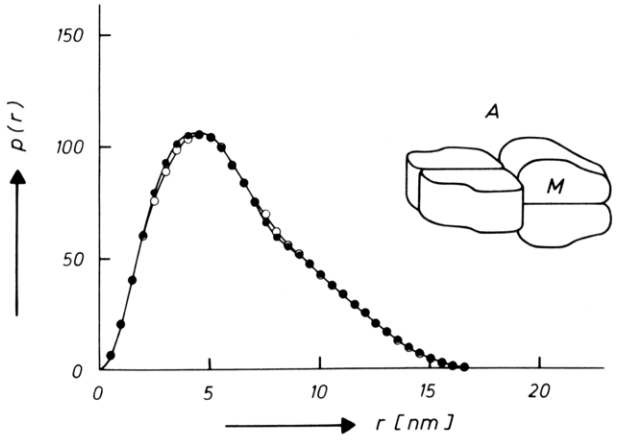

FIGURE 3: Distance distribution function $p(r)$ of the T-core (○) compared with that of model A (●). The model is identical with model B in Figure 3 without the headpieces; thus, the length of the monomer M is only 7.7 nm.

molecule is somewhat elongated with a main axial ratio on the order of 1:2. No fit could be obtained with simple triaxial bodies. Therefore, a large number of detailed models composed of spheres were calculated using the computer program of O. Glatter (unpublished results). The models were chosen, taking into account known features of the repressor structure: (1) the repressor is composed of four monomers and (2) each LR monomer contains a headpiece with a molcular weight of about 6000, which is absent in the T-core. X-ray measurements were also made on the headpiece, but the high error precludes any quantitative statement concerning their shape, beyond the conclusion that they are not spherical.

Generally, models assuming the repressor and the T-core to be nearly spherical or in the shape of an oblate ellipsoid of revolution can be ruled out. In contrast, models approximated by a prolate ellipsoid or a tetramer of prolate ellipsoids yield

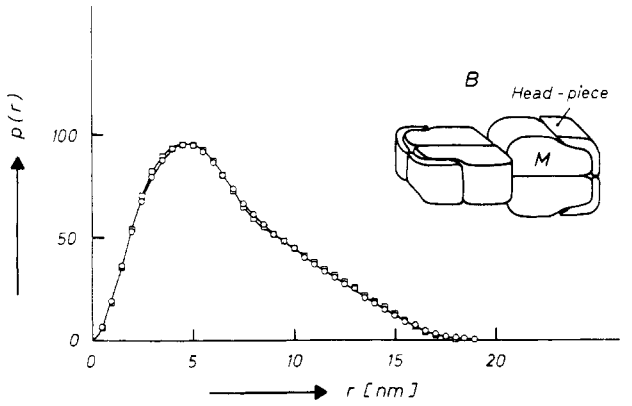

FIGURE 4: Distance distribution function $p(r)$ of the *lac* repressor (O) compared with that of model B (□). The model is a tetramer; the dimensions of one monomer M including the headpiece are 8.7 nm × 3.4 nm × 3.4 nm.

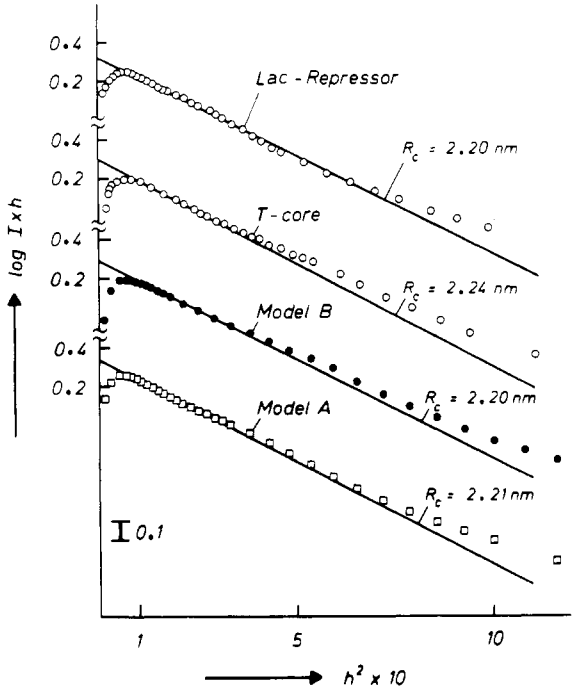

FIGURE 5: Cross section curves of *lac* repressor, T-core, model B, and model A in Guinier plot. The calculated radii of gyration R_c of the cross section are given in the figure; they are identical within experimental error.

better agreement with experimental data. Two such models for T-core and LR, respectively, are shown in Figures 3 and 4.

Model A (Figure 3) shows a good fit with the experimental $p(r)$ function and the molecular parameters of the T-core. It consists of 512 spheres each with a radius of 0.51 nm. Model B (602 spheres) in Figure 4 is in good agreement with the $p(r)$ curve of the whole *lac* repressor. The core itself thus appears to be an elongated structure. In the intact repressor the head-pieces are located at its ends. This conclusion follows *directly* from a comparison of D_{max} for LR and T-core and is merely confirmed by model calculations. It is consistent with a previous suggestion of Steitz et al. (1974) that the repressor is elongated and with the finding obtained by high-resolution NMR (Wade-Jardetzky et al., 1979), which indicates that HP exists as a separate, highly mobile domain in the intact repressor. A similar fit can be obtained using a model in which all four monomers lie in a single plane, rather than in the tetrahedral arrangement shown in the figures. Discrimination between different detailed models is not possible, as already noted, but the flat model may be in better agreement with the unit cell dimensions of the core crystals, i.e., 3.7 × 6.6 × 15.0 nm (T. Steitz, personal communication).

On the other hand, it is possible to obtain information on the cross section of particles whose length is only a few times larger than the other dimensions (Mittelbach, 1965). The elongated shape of both the *lac* repressor and the T-core allows us to determine a cross section, R_c, in both cases (Table I). The cross section curves of the models agree well with the experimental curves, as seen in Figure 5. The radii of gyration calculated for the models also agree reasonably well with those derived from the experiment. We therefore conclude that models of this general type represent the shape of the repressor and the T-core to a good approximation. It deserves reemphasis, however, that the principal conclusions of this study, i.e., that the T-core is an elongated structure and that the headpieces are located at its ends, do *not* depend on any specific model, but follow directly from the experimental data.

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