SMALL-ANGLE X-RAY STUDY OF DNA-DEPENDENT RNA POLYMERASE CORE ENZYME AND PARTIAL COMPLEX $\beta\alpha_2$ FROM *ESCHERICHIA COLI*

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1. Introduction

DNA-dependent RNA polymerase has to fulfill different complicated functions during transcription. For a detailed understanding of these functions, it is necessary to know the structure of this enzyme. The eubacterial RNA polymerase has the general composition formula $\beta'\beta\alpha_2$ (core enzyme) and $\beta'\beta\alpha_2\sigma$ (holoenzyme), respectively. The best characterized RNA polymerase is that of *E. coli*, which was used in our studies.

Since protein crystals of RNA polymerase are not available, other methods were used for analysing the structure, such as electron microscopy [1], neutron [2,3] and X-ray small angle scattering [4]. Since, at that time the preparation and small angle scattering technique had not been developed as it is today, we describe here a new approach using X-ray scattering to study the structure of core enzyme (M_r 395 000) and partial complex $\beta\alpha_2$ (M_r β 155 000; M_r α_2 73 000).

For a detailed model of core enzyme it is necessary to know the structure of its subunits. The structure of isolated α_2 has been investigated in [5]. Isolated subunits β and β' are not suitable for X-ray scattering experiments, because of their tendency to aggregate.

The study of the structure of the partial complex $\beta\alpha_2$, which can be homo-dispersly distributed in solution, will give information about the structure of β.

The aim of this paper is to verify the model of RNA polymerase core enzyme developed by neutron small angle scattering and to refine this model using information about the structure of isolated α_2 and $\beta\alpha_2$.

2. Materials and methods

2.1. *Preparation of the partial complex $\beta\alpha_2$ and core enzyme*

RNA polymerase was isolated from *E. coli* by the procedure in [6] with slight modifications [7]. The complex $\beta\alpha_2$ and core enzyme have been isolated from purified RNA polymerase by phosphocellulose chromatography [8,9]. For elimination of unspecific aggregates, core enzyme and $\beta\alpha_2$ were sedimented in a sucrose–glycerol gradient. The main fractions were pooled, concentrated by ammonium sulphate precipitation and dialysed overnight against a buffer containing 0.05 M Tris–HCl (pH 7.5), 0.55 M NH_4^+ and 10^{-3} M mercaptoethanol. The $\beta\alpha_2$ fraction reconstituted with β' and σ results in fully active holoenzyme. Core enzyme was stimulated 12-fold by addition of σ using T7-DNA as template. The purity of core enzyme and $\beta\alpha_2$, respectively, was >95% as checked by SDS gel electrophoresis. The molecular homo-dispersity of the proteins was checked by sedimentation in an ultracentrifuge (Spinco model E). Both core enzyme and $\beta\alpha_2$ ran as single sedimenting material, but $\beta\alpha_2$ contained ~5% faster sedimenting material. The protein concentration was determined by the staining procedure developed [10], calibrated as in [7].

2.2. *Small-angle X-ray scattering*

The measurements were carried out using a Kratky camera with slit collimation system [11] on a copper tube (50 kV, 30 mA). Protein solutions were investigated at 4°C. Scattered intensities were recorded at 96 different angles over 0.00216–0.123 radians, using an entrance slit of 120 μm. Each scattering curve was recorded several times with a fixed number (10^5) of pulses per angle in order to minimize statistical errors.

The experimental arrangement and the procedures used for data evaluation were as in [5,12].

3. Results and discussion

3.1. *Radius of gyration and maximum dimension*

A concentration series was measured using 5–22 mg/ml. The inner parts of the scattering curves were plotted according to Guinier (log I/c vs $(2\theta)^2$) and extrapolated to zero concentration. This plot should yield a straight line whose slope is proportional to the square of the radius of gyration. After desmearing [8] the radius of gyration was calculated to be $R = 6.5 \pm 0.1$ nm (core enzyme) and $R = 6.86 \pm 0.1$ nm ($\beta\alpha_2$). These values agree with the values computed from the $p(r)$ function [12]. The intraparticle distance distribution function $p(r)$ was calculated with the evaluation program [13]. $p(r)$ becomes zero at values of r exceeding the maximum particle dimension D_{max}. From $p(r)$, D_{max} of core enzyme amounts to 24 ± 1 nm, and D_{max} of $\beta\alpha_2$ to 30 ± 1 nm. Fig.1 and fig.3 show the desmeared scattering curves of $\beta\alpha_2$ and core enzyme. The $p(r)$ functions are shown in fig.2.

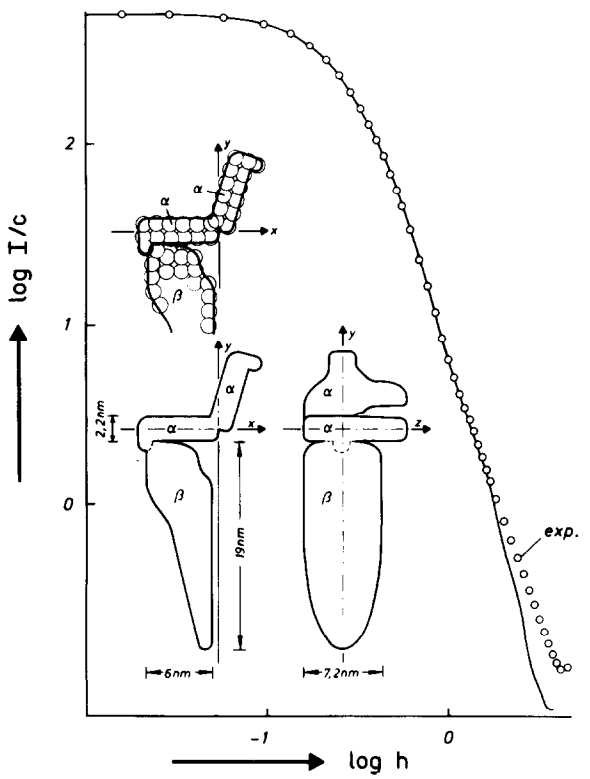

Fig.1. Comparison of the experimental scattering curve $I(h)$ of the partial complex $\beta\alpha_2$ (o–o) with the theoretical $I(h)$ of model $\beta\alpha_2$ (——). I = scattered intensity; c = concentration; $h = (4\pi/\lambda)\sin\theta$ (λ = wavelength of the Cu K_α line, 2θ = scattering angle). Two views of the model $\beta\alpha_2$ are shown in the xy and yz drawing plane. One picture shows that the model is composed of spherical elements.

3.2. *Volume*

The invariant volume was calculated as in [12]. The values obtained by this method are $V = 675$ nm^3 (core enzyme) and $V = 410$ nm^3 ($\beta\alpha_2$), respectively. Experience shows that the volumes calculated from the invariants are usually affected by errors ⩾5%, presumably due to particle inhomogenities which come into effect at large angles.

3.3. *Shape*

Model calculations were performed by means of a computer program which uses Debey's formula [14] to calculate the theoretical scattering curves of models composed of arbitrary spherical elements [12]. This method does not allow a direct determination of the particle shape, but models which fit the experimental

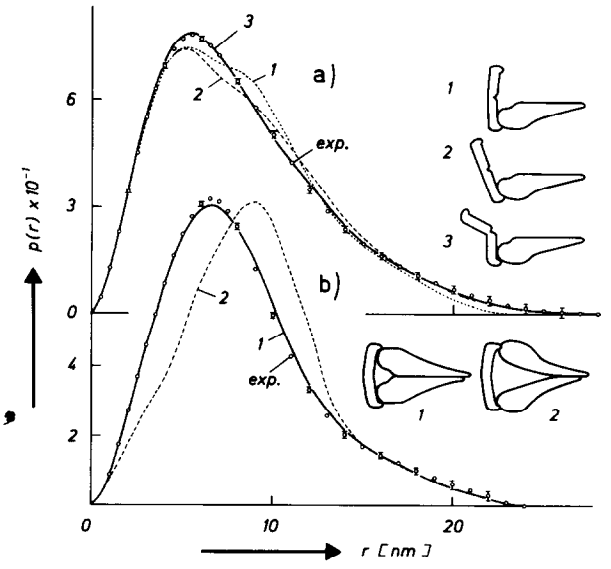

Fig.2. (a) Comparison of the experimental distance distribution function $p(r)$ of $\beta\alpha_2$ (o–o) with the theoretical $p(r)$ of the $\beta\alpha_2$ model 1 (· · ·), model 2 (– – –) and model 3 (——). (b) Comparison of the experimental function $p(r)$ of core enzyme (o–o) with the theoretical one of model 1 (——) and model 2 (– – –). r = distance; Φ = experimental data including propagated standard deviation.

curves well can be considered to have a high degree of reliability, if other results are in agreement with the proposed structure.

3.3.1. Partial complex $\beta\alpha_2$

The structure of the isolated subunit α_2 has already been investigated [5]. The best fit was obtained by a disc-like model of α_2 having one deep crevice as model 1 in [5]. Here, the same model of α_2 is used for constructing models of $\beta\alpha_2$ and core enzyme. We assumed that the structure of isolated α_2 is the same as that of α_2 in complex with the other subunits β,β'. The validity of this assumption is discussed in [5]. The startpoint for our model calculations was the spatial arrangement of $\beta\alpha_2$ within RNA polymerase as determined by neutron small angle scattering [2,3]. Many $\beta\alpha_2$ models were calculated, varying the shape of subunit β and in addition the angle between the subunits β and α_2. The theoretical scattering curves of all these models were compared with the experimental one in reciprocal space ($I(2\theta)$) and in real space ($p(r)$). It turned out that the structure of β is best approximated by an elongated model with a D_{max} = 19 ± 1 nm and R = 5.13 nm. The bulk of mass is located near the β–α_2 binding site, forming an approximately conical structure. A good arrangement of the subunits β and α_2 was given by a somewhat larger angle (110°) between β and α_2 (model 2, fig.2a) as it was found in the final core enzyme model 1 (~90°) (model 1, fig.2a,b). The used structure of isolated α_2 was formed by a more or less straight arrangement of the two leaf-shaped α particles (180°). It turned out that the fit with the experimental scattering curve (fig.1) and the $p(r)$ function (fig.2a) became much better, if the two α subunits were arranged not straight but angular. This fact can not be proved by model 3, suggested here, since many parameters could be changed by calculating $\beta\alpha_2$ models. For example, a mixture of structures with different angles between β and α_2 and between α and α could also be possible. Because of the number of uncertain parameters model 3 in fig.1 appears to be too detailed. It is pictured here because it shows the best fit of all tested models. It consists of 325 spheres with a radius of 0.67 nm.

3.3.2. Core enzyme

It was found by neutron small angle scattering studies [2] that the subunits β, $\beta'\alpha_2$ are elongated and arranged within the core enzyme forming a trigonal molecule. A trigonal overall shape of RNA polymerase was obtained by electron microscopy [1]. To check the compatibility of our experimental data with a trigonal model, the volume of core enzyme was approximated by a small number of spheres (75) forming models of any shape, but taking into account experimental parameters, like R, V and D_{max}. Indeed, models with trigonal structure showed the best agreement with the experimental scattering curve.

Considering the trigonal structural feature of core enzyme and the already determined structure of α_2 and β, we were able to construct a detailed model of core enzyme (model 1, fig.2b, fig.3). Model 1 consists of 536 spheres with a radius of 0.67 nm. Subunit β' is

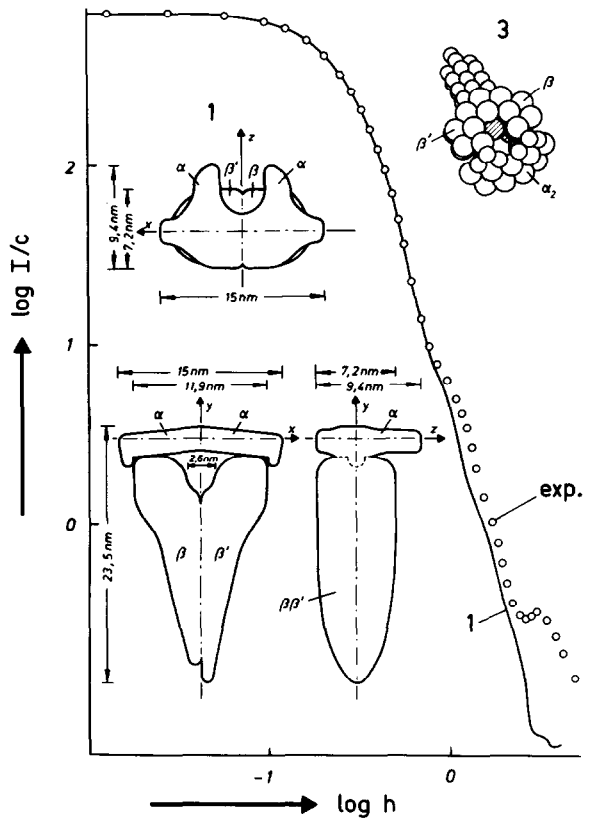

Fig.3. Comparison of the experimental scattering curve of core enzyme (o–o) with the theoretical one of model 1 (——). Three views of model 1 in the xy, yz and xz drawing plane are shown, and a perspective view of model 3, in which the structure of model 1 is approximated by only 75 spheres. The intensities I at large angles of the model scattering curve are usually lower than the experimental I, because of lower resolution in the model. The model is calculated as a homogeneous body. If arbitrary inhomogenities were included in the model, the calculation would be much better, but the resolution of the method would remain unchanged.

approximated by a structure similar to that of subunit β. Only the value of D_{max} is somewhat larger (21 ± 1 nm, $R = 5.62$ nm). The angle between α_2 and β is $\sim 90°$. All subunits are arranged in such a manner that a central cavity is left with a diameter of ~ 2.6 nm. An intersubunit centre-to-centre distance between β and β' of 5 nm fits optimally the experimental data (fig.1, model 1). The corresponding value of neutron scattering experiments is 7 nm. This value is realized in model 2 (fig.2b). The corresponding *p(r)* function shows however the maximum at larger distances. Model 3 in fig.3 shows a perspective view of a simple core enzyme model built up by 75 spheres which fits the experimental scattering curve (fig.3) and the *p(r)* function (fig.2b) almost as well as model 1.

Although it can never be excluded that other models exist which would also fit the experimental curves model 1 for core enzyme has a high degree of reliability: It is in agreement with the model developed by neutron small angle scattering [2,3] and electron microscopy [1]. Further investigations of RNA polymerase concerning the structure of holoenzyme are in progress.

Acknowledgements

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