SMALL-ANGLE X-RAY STUDY OF DNA-DEPENDENT RNA POLYMERASE HOLOENZYME FROM *ESCHERICHIA COLI*

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Received 17 November 1980

1. Introduction

RNA polymerase holoenzyme (Eσ) of *E. coli* is a multisubunit enzyme composed of the subunits $\beta'\beta\alpha_2\sigma$. The dissociable subunit σ is responsible for specific initiation [1,2]. After the synthesis of an RNA chain of a few nucleotides [3], σ factor dissociates from the transcription complex (core RNA polymerase ($\beta'\beta\alpha_2$), DNA and RNA), and can be used again as initiation factor.

Here, the structure of holoenzyme (M_r 487 000), the arrangement of σ on core enzyme, is investigated by small-angle X-ray scattering, considering the results in [4,5] on the structure of core enzyme and σ factor.

2. Materials and methods

2.1. *Preparation of RNA polymerase holoenzyme*

Core enzyme and σ factor were purified as in [4,5]. Holoenzyme was prepared by mixing stoichiometric (1:1) amounts of core enzyme and σ factor, as in [6]. The homodispersity, purity and activity of holoenzyme were checked as in [4,5].

2.2. *Small-angle X-ray scattering*

Measurements were carried out with a Kratky camera with slit collimation system [7] on a copper tube (50 kV, 30 mA). Enzyme solutions were investigated at 25°C and 5°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 [4].

3. Results and discussion

3.1. *Radius of gyration and maximum dimension*

In solution, holoenzyme Eσ is partially dissociated into core enzyme and σ factor. However, the complex become more stable, at higher temperatures. Thus, two series of measurements were performed at 25°C with freshly prepared holoenzyme. For each sample, concentration series were measured over 5–22 mg/ml, and the inner parts of the scattering curves were extrapolated to zero concentration. Moreover, the scattering contribution of dissociated core enzyme and σ factor was subtracted from the experimentally obtained scattering curves. At 25°C and c = 22 mg/ml, ~4% of Eσ is dissociated, using a binding constant $K = 1.6 \times 10^7$ (M^{-1}) in 0.5 M KCl at 25°C, yielded by fluorescence titration experiments [8]. After desmearing, the radius of gyration [4], determined from this corrected scattering curve was calculated to be R = 6.67 ± 0.1 nm. This value agrees with the value computed from the $p(r)$ function [4]. The intraparticular distance distribution function was calculated by use of the evaluation program [9]. $p(r)$ becomes zero at values of r exceeding the maximum particle dimension D_{max}. Thus determined, D_{max} amounts to 24 ± 1 nm. It was found that the change of the values of R and D_{max} by the above-mentioned correction lies within the limit of error. The desmeared scattering curve of Eσ is shown in fig.1, the $p(r)$ function in fig.2.

Additionally, two series of measurements were performed at T = 5°C. In this case the radius of gyration increases (R = 7.2–7.4 nm) and the scattered intensity at zero angle decreases (1–5%). The reason of this unexpected behaviour of Eσ could be explained perhaps by conformational changes and aggregation

Elsevier/North-Holland Biomedical Press

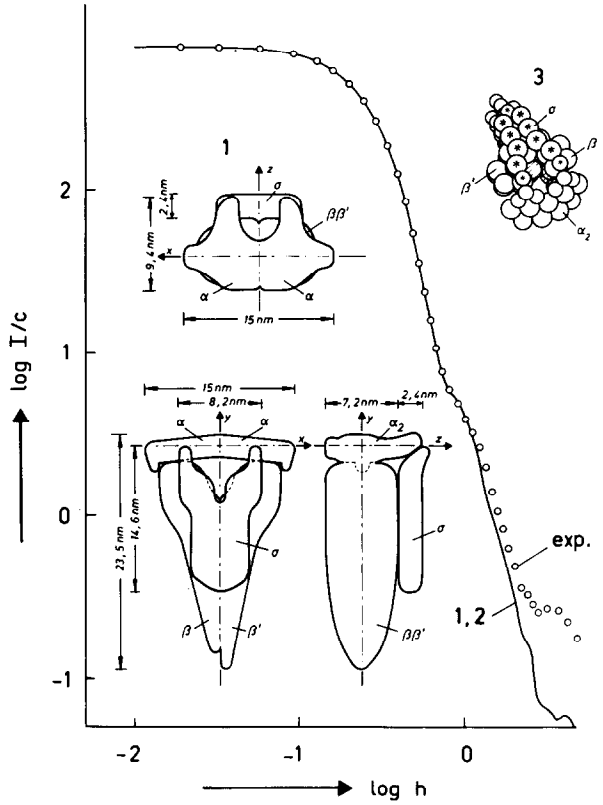

Fig.1. Comparison of the experimental scattering curve of RNA polymerase holoenzyme (o——o) with the theoretical one of model 1 (identical with model 2) (——). I = scattered intensity; c = concentration; $h = (4\pi/\lambda)\sin\theta$ (λ = wavelength of the CU Kα line, 2θ = scattered angle). Three views of model 1 in the xy, yz and xz drawing plane. Model 1 consists of 629 spheres, but in consideration of the general view, the outlines of the single spheres are not drawn in. General perpective view of model 3, consisting of 86 spheres. The intensities at large angles of the model scattering curves are usually lower than the experimental values, because of lower resolution in the models [5].

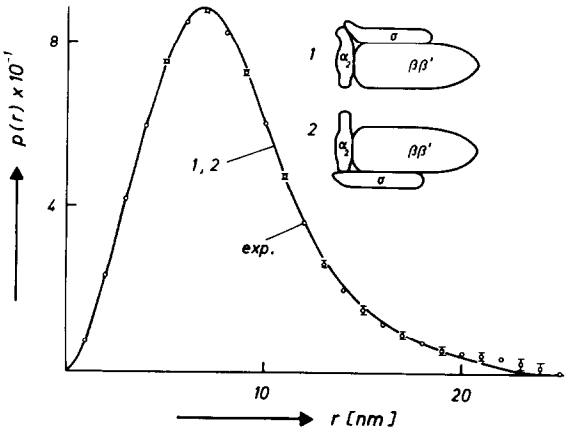

Fig.2. Comparison of the experimental distance distribution function $p(r)$ of Eσ (o——o) with the theoretical one of model 1 (identical with model 2) (——). r = distance; ϕ = experimental data, including propagated standard deviation.

phenomena. This temperature-dependent scattering of Eσ is the subject of further investigation.

3.2. *Volume*

The invariant volume was calculated as in [4]. Using this method, the volume of Eσ was calculated to be $V = 790$ nm^3. Experience shows that the volume calculated from the invariant is usually affected by errors $\geqslant 5\%$, presumably due to particle inhomogenities which come into effect at large angles.

3.3. *Shape*

Holoenzyme (Eσ) consists of core enzyme and σ factor. The structures of σ [4] and core enzyme [5] have already been investigated. Here, the proposed models (core enzyme model 1 (in [5]) and σ model 3 (in [4]) were composed in different ways, resulting in models of Eσ. The model calculations were performed as in [4,5,10] by means of a computer program, which used Debye's formula [11] to calculate the theoretical scattering curves of models composed of arbitrary spherical subunits.

Many possible locations of σ factor on core enzyme were tested, by comparing the theoretical scattering curves of the different models with the experimental one. The best fit is given by the arrangements in model 1 and 2 (fig.1, fig.2). A characteristic feature is the central position of σ on the core enzyme. There is good agreement with the data obtained from measurements at 25°C. However, the resolution of this method allows no distinction between models 1 and 2 of Eσ. In model 1, the crevice of σ factor is positioned above the crevice of α_2. Both models consist of 629 spheres with a radius of 0.67 nm. Fig.1 shows the calculated scattering curve of model 1 (or 2) together with the experimental one and fig.2 the comparison of the $p(r)$ functions. Model 3 in fig.1 shows a general perspective view of a simple Eσ model built up by only 86 spheres, which fits the experimental data almost as well as models 1 and 2.

The deviation of the theoretical $p(r)$ function of model 1 from the experimental one exceeds only insignificantly the error band of the latter. However,

the large distances at the tail end of the experimental $p(r)$ function (fig.2) could not be fitted very well by any model of Eσ, representing all possible locations of σ on core enzyme and considering even a structural alteration of core enzyme. If these discrepancies come from effects mentioned in section 3.1., the proposed model 1 (or 2) might represent the real structure of Eσ. In that case, the radius of gyration would be a little smaller (~3%) than the experimental value (section 3.1.).

Although this method allows only an indirect determination of particle shape, the proposed models 1 and 2 of Eσ have a high degree of reliability: All investigated structures, such as those of σ factor [4], α_2 [10], $\beta\alpha_2$ and core enzyme [5], and at least that of Eσ here, yield a uniform picture of RNA polymerase structure.

The trigonal concept of the subunit arrangement, as proposed by neutron scattering studies, was confirmed [12,13]. Electron micrographs also show a triangular shape in ~50% of the pictured holoenzyme particles [14], whereas the remainder appear to be globular. The proposed Eσ model 1 [2] can indeed show globular and triangular projections, depending on its orientation (fig.1).

Acknowledgements

I. P. and O. M. thank the Österreichischen Fonds zur Förderung der wissenschaftlichen Forschung for generous support. We also thank B. Müller for drawing the figures. H. H. thanks the Deutsche Forschungsgemeinschaft for generous support of his work, P. Stöckel for valuable discussion and G. Baer for excellent technical assistance.

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