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

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1. Introduction

A model of DNA -dependent RNA polymerase from *E. coli* has been developed on the basis of neutron and X-ray small angle scattering measurements [1–7]. These studies were performed in buffers of high ionic strength, in which the enzyme is monomeric. But, stable binding of RNA polymerase to DNA is restricted to low ionic strength, where the enzyme can aggregate. Core enzyme (M_r 395 000, subunit composition $\beta'\beta\alpha_2$) aggregates into oligomers consisting of up to 6 core molecules [8] and holoenzyme (M_r 487 000, subunit composition $\beta'\beta\alpha_2\sigma$) forms dimers [8]. Here, models of aggregated RNA polymerase, holoenzyme and core enzyme are presented. The aim is to get additional structural information in order to verify our model of holoenzyme.

2. Materials and methods

2.1. *Preparation of RNA polymerase*

Core enzyme and holoenzyme were purified as in [3,4]. The homodispersity, purity and activity of holoenzyme were checked as in [3]. For the measurements the proteins were dialysed overnight against a buffer containing 0.05 M Tris–HCl (pH 7.5) and 10^{-3} M mercaptoethanol.

2.2. *Small angle X-ray scattering*

The measurements were carried out with a Kratky camera with slit collimation system [12] using a copper tube (50 kV, 30 mA). Protein solutions were investigated at 4.5°C. Scattered intensities were recorded at 93 different angles from 0.0019–0.114 radians, using an entrance slit of 100 μm. Each scattering curve was recorded several times with a fixed number of pulses (10^5/angle) in order to minimize statistical errors. The experimental arrangement and the procedure used for data evaluation were as in [2].

3. Results and discussion

3.1. *Radius of gyration and maximum dimension*

A concentration series was measured using 5–17 mg/ml. The inner parts of the scattering curves were plotted according to Guinien ($\log I/c$ vs $(2\theta)^2$) and extrapolated to zero concentration. This plot should yield a straight line, the slope of which is proportional to the square of the radius of gyration. After desmearing [9] the radius of gyration was calculated to be $R = 7.96 \pm 0.1$ nm (holoenzyme dimer) and $R = 13.5 \pm 0.2$ nm (core enzyme oligomer). These values agree with the values computed from the $p(r)$ function [8]. The interparticle distance distribution function $p(r)$ was calculated with the evaluation program [9]. $p(r)$ becomes zero at values of r exceeding the maximum particle dimension D_{max}. From $p(r)$, D_{max} of dimeric holoenzyme is 28 ± 1 nm, and D_{max} of oligomeric core enzyme 50 ± 2 nm. In the case of core enzyme the interparticle effect, eliminated by the extrapolation to zero concentration is superimposed by the oligomer equilibrium. For that reason we had to consider a value of ±0.2 nm of the limit of error in the case of R and of ±2 nm in the case of D_{max}. Fig.1 and 2 show the desmeared scattering curves of dimeric holoenzyme and oligomeric core enzyme.

3.2. *Volume*

The invariant volume was calculated as in [2]. The

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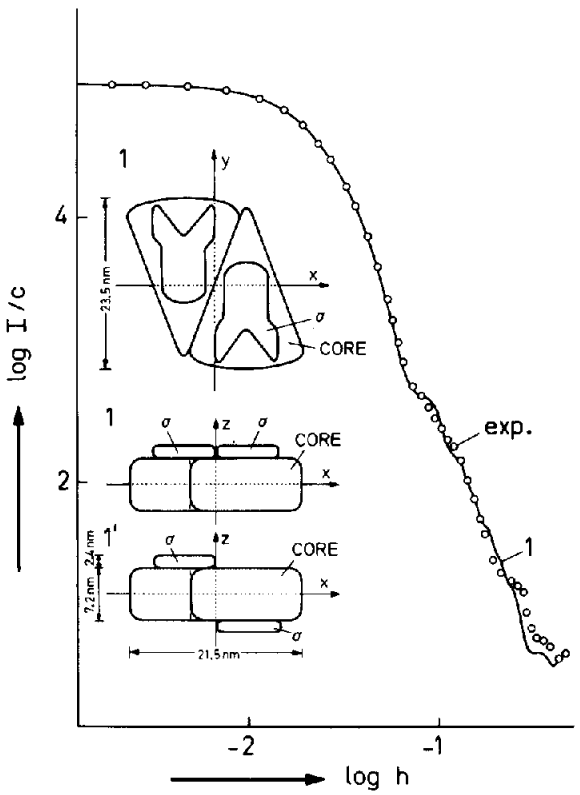

Fig.1. Comparison of the experimental scattering curve $I(h)$ of dimeric RNA polymerase holoenzyme $(E\sigma)_2$ (ooo) with the theoretical $I(h)$ of model 1 $(E\sigma)_2$ (——). I = scattered intensity; c = concentration, $h = (4\pi/\lambda) \cdot (\sin\theta)$, λ = wavelength of the CuK_α line, 2θ = scattering angle. Two schematic views of model 1 are shown in the xy and xz drawing plane. In model 1 the 2 holoenzyme models are arranged very close side-by-side to get an extent of 21.5 nm of the dimer.

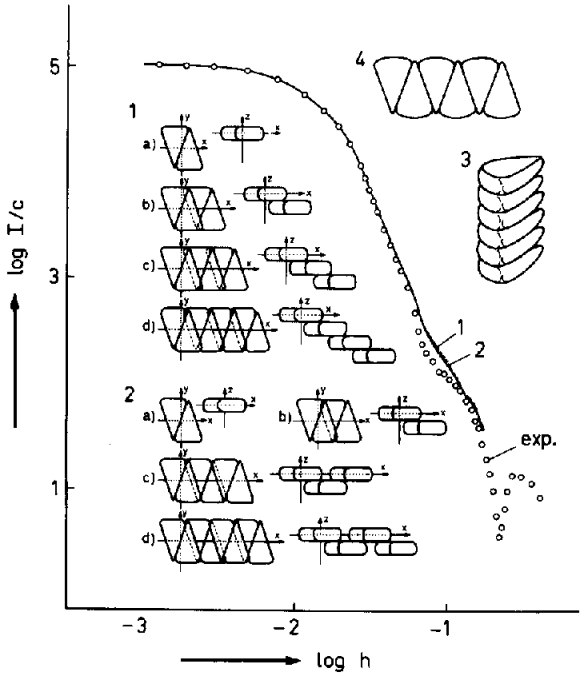

Fig.2. Comparison of the experimental scattering curve $I(h)$ of core oligomers (ooo) with the theoretical $I(h)$ of models: curves 1 (——) and 2 (– – –) represent mixtures of aggregates of models (a–d) and 2 (a–d) in the ratios 3:3:2:2 for a:b:c:d. Schematic view of the 2 tentative arrangements of core oligomers (model 1,2). Schematic view of model 3 and 4.

values obtained by this method are $V = 1600\ nm^3$ for dimeric holoenzyme (monomeric holoenzyme: $V = 790\ nm^3$ [7]) and $V = 3700\ nm^3$ for oligomeric core enzyme (monomeric core enzyme: $V = 675\ nm^3$ [4]). Experience shows that volumes calculated from the invariants are usually affected by errors $\geq 5\%$, presumably due to particle inhomogeneities which come into effect at large angles.

3.3. *Shape*

Model calculations were performed by means of a computer program which uses Debye's formula [10] to calculate the theoretical scattering curves of models composed of arbitrary spherical elements [11]. Models of aggregated RNA polymerase were built by different arrangements of models of monomeric RNA polymerase holoenzyme and core enzyme. These models were proposed in [4,7]. This method does not allow, a direct determination of the shape of the aggregates, but models which fit the experimental curves within the experimental error can be considered to have a high degree of reliability.

3.3.1. Holoenzyme

Holoenzyme exists at low ionic strength as a dimer in solution. This is shown by sedimentation measurements [8], and confirmed by our volume determination (see section 3.2). Monomeric holoenzyme has a triangular shape [7]. The subunits α_2 form the base of the triangle, and β, β' the sides [6]. In fig.3 a choice of plausible models of dimeric holoenzyme in different arrangements and the corresponding $p(r)$ functions are shown.

A comparison of the theoretical scattering curves in real space ($p(r)$ (fig.3) and in reciprocal space ($I(2\theta)$) (fig.1) with the experimental ones shows that the dimeric structure of holoenzyme is best approximated by model 1 (fig.1, fig.3a). This finding justifies our

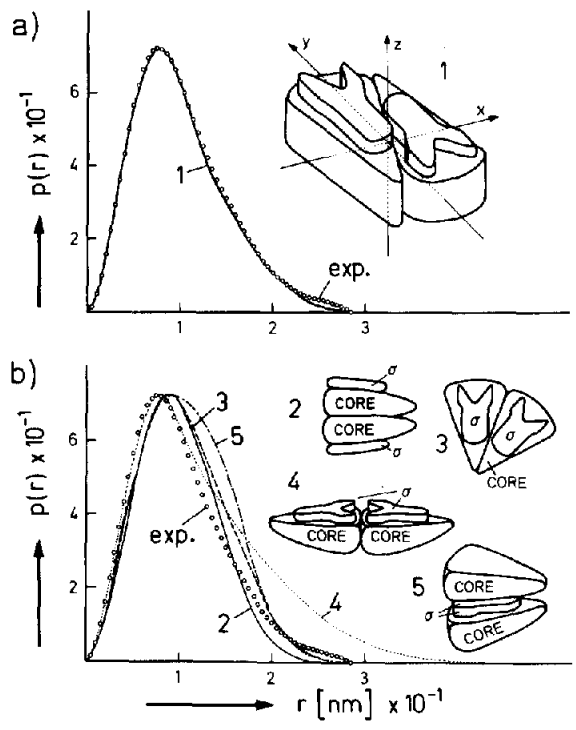

Fig.3. Comparison of the experimental distance distribution function $p(r)$ of $(E\sigma)_2$ (fig.3a,b) with the theoretical $p(r)$ of models. A small choice of the tested arrangements in models of dimeric holoenzyme is presented. Model 1 is shown in fig.1. All curves are normalized on equal areas. r = distance, ϕ = experimental data inducing propagated standard deviation.

assumption that there is no gross conformational change in holoenzyme from high to low ionic strength buffer. From our X-ray studies, it cannot be decided whether the holoenzyme molecules arranged side-by-side are bound via 2 equal subunits (β or β'), so that the σ subunit is placed on the same side of the protein surface (see model 1, fig.1) or are bound via β–β', so that the two σ-subunits are placed on opposite surfaces. We prefer the first arrangement. It explains without further assumptions about σ-induced conformational changes during aggregation, the stop of aggregation after dimerisation by occupation of the dimerisation site on β or β'. Distance determination of the subunits in the dimer by D-label triangulation measurements using neutron small angle scattering are in progress to decide which of the 2 models is valid in low ionic strength buffer.

3.3.2. Core enzyme

The large radius of gyration of core enzyme in low ionic strength buffer, the large D_{max} and the volume corresponding to the volume of penta-hexamer indicates aggregation. Due to the polydispersity of the oligomers, the interpretation of the scattering curve by model fitting is impossible without additional information or assumptions. By comparing the theoretical scattering curves with the experimental one it turned out that a large number of models are rather improbable like the arrangement where core enzyme molecules are stacked over each other (fig.2, model 3) or the arrangement where core molecules are bound side-by-side in a chain (fig.2, model 4). Theoretical scattering curves calculated for mixtures of dimers, trimers and higher oligomers with the same aggregation feature do not fit much better.

Building models for core oligomers we made 2 assumptions:

(i) At low ionic strength there is one strong binding site, which is the same as for holoenzyme. Therefore, core oligomers are composed of dimers as found in holoenzyme but without σ. This is suggested by fitting the $p(r)$ function evaluated from the experimental scattering curve without the inner part, to eliminate the contribution of higher aggregates.

(ii) We propose in the core enzyme a second weaker aggregation site via the vacant σ-binding site. This leads us to 2 tentative arrangements shown in fig.2, model 1 and model 2.

4. Conclusion

These considerations about the structure of oligomeric core enzyme are preliminary, but might be helpful for the interpretation of electron diffraction studies of core aggregates.

The structure of holoenzyme has a high degree of reliability since all the investigated structures of holoenzyme, subunits α_2 and σ, the partial complex $\beta\alpha_2$, the monomeric core enzyme and holoenzyme, and the dimeric holoenzyme are in line with a triagonal structure of RNA polymerase. The role of the dimeric form of holoenzymes in the RNA polymerase–promotor interaction is subject of investigation.

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