SMALL-ANGLE X-RAY STUDY OF DNA-DEPENDENT RNA POLYMERASE SUBUNIT α_2 FROM *ESCHERICHIA COLI*

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1. Introduction

To obtain structural information about DNA-dependent RNA polymerase from *Escherichia coli* (subunit composition: $\beta'\beta\alpha_2\sigma$) by small angle X-ray scattering, we are investigating the structures of these subunits of RNA polymerase which can be obtained homodispersed in solution. We proposed a structure for the σ subunit in [1]. Here, we report studies of the structure of the α subunit, which is present in solution as a dimer α_2 [2,3]. The amino acid sequencing studies [4] indicate that both α subunits are chemically identical and each has M_r 36 500.

Few facts are available concerning the function of subunit α in the transcription process [2]: One or both α subunits are attached to the β subunit. ADP ribosylation of one α subunit after infection with *E. coli* phage T4 leads to a change in the specificity of the gene expression. Our aim is to use the structural information about the isolated α_2 subunit in order to evaluate a model of RNA polymerase. The aim is to obtain information about the function of the subunits and their interactions.

2. Materials and methods

2.1. *Preparation of α subunit*

The α subunit was prepared from RNA polymerase as in [6]. RNA polymerase was isolated from *E. coli* as in [7] with the slight modifications in [1]. For elimination of unspecific aggregates the α subunit was sedimented in a sucrose—glycerol gradient. The main fractions were pooled, concentrated by ammonium

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sulfate precipitation and dialysed overnight against a buffer containing 0.05 M Tris—HCl (pH 7.5), 0.55 M NH_4Cl and 10^{-3} M mercaptoethanol. This α fraction reconstituted with β', β, σ results in a fully active holoenzyme.

The purity of the α subunit was >95% as checked by SDS gel electrophoresis. The homodispersity of α was checked by sedimentation in an ultracentrifuge (Spinco model E). The α subunit ran as a single sedimenting material with an S-value corresponding to the dimeric form. The concentration of α factor was determined by the staining procedure developed [8], which was calibrated as in [9].

2.2. *Small-angle X-ray scattering*

The measurements were carried out with a Kratky camera with a slit collimation system [10] using a copper tube (50 kV, 30 mA). Protein solutions were investigated at 4°C. Scattered intensities were recorded at 93 different angles from 0.00216—0.123 radians, using an entrance slit of 120 μm. Each scattering curve was recorded several times with a fixed no. pulses (10^5)/angle in order to minimize statistical errors. The experimental arrangement and the procedures used for data evaluation were as in [1].

3. Results and discussion

3.1. *Radius of gyration and maximum dimension*

Two series of measurements were performed with freshly prepared α_2 samples. For each sample a concentration series was measured over 6—16 mg/ml. The inner parts of the scattering curves were plotted according to Guinier ($\log I/c$ vs $(2\theta)^2$) and extrapolated to

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zero concentration. This plot should yield a straight line whose slope is proportional to the square of the radius of gyration. After desmearing [11] the radius of gyration was calculated to be $R = 4.4 \pm 0.1$ nm. This value agrees with that computed from the $p(r)$ function [1].

The intraparticular distance distribution function $p(r)$ was calculated with the evaluation program [11]. The function $p(r)$ becomes zero at values of r exceeding the maximum particle dimension D_{max}. From $p(r)$ D_{max} results to 15 ± 0.5 nm. The desmeared scattering curve of α_2 is shown in fig.1, and the $p(r)$ function in fig.2.

From a plot Ih^2 vs h^2 (fig.3) the radius of gyration of the thickness was determined to be $R_D = 0.64$ nm which corresponds to an averaged thickness of the particle of 2.2 ± 0.1 nm [12,13]. Fig.3 shows a value of R_D which is consistent with model 1 for α_2.
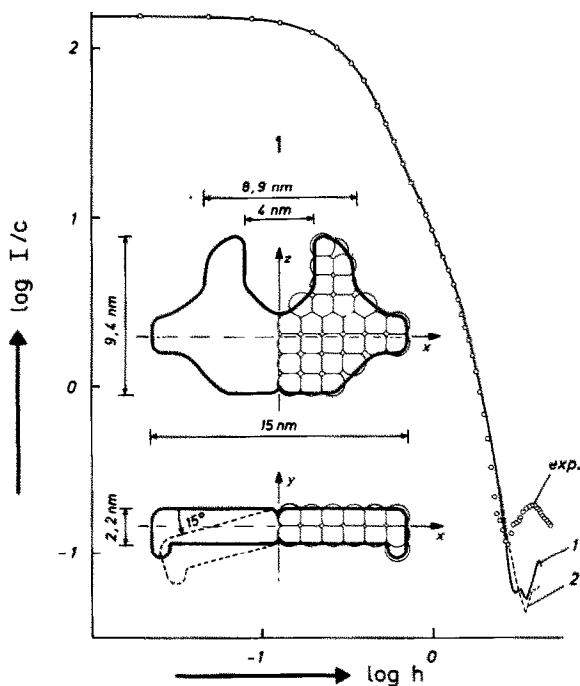
Fig.2. Comparison of the experimental distance distribution function $p(r)$ of α_2 (o o o) with the theoretical one of model 1 (———), model 2 (– – –), model 3 (. . .) and model 4 (—.—). r = distance; $\updownarrow$ = experimental data including propagated SD. The deviation of the theoretical curve of model 1 from the experimental one does not exceed the error band of the latter.

Fig.1. Comparison of the experimental scattering curve of α_2 (o o o) with the theoretical one of model 1 (———) and model 2 (– – –) (see fig.2): I = scattered intensity; c = concentration; $h = (4\pi/\lambda)\sin\theta$ (λ = wavelength of the CuK$_\alpha$ line, 2θ = scattering angle). Top-view and side-view of model 1. The right hand part of the picture shows the spherical subunits fit into model 1. Model calculations yielded an angle between the two α components of $180° \pm 15°$. The intensities at large angles of the model scattering curve are usually lower than the experimental one, because of a lower resolution in the model.
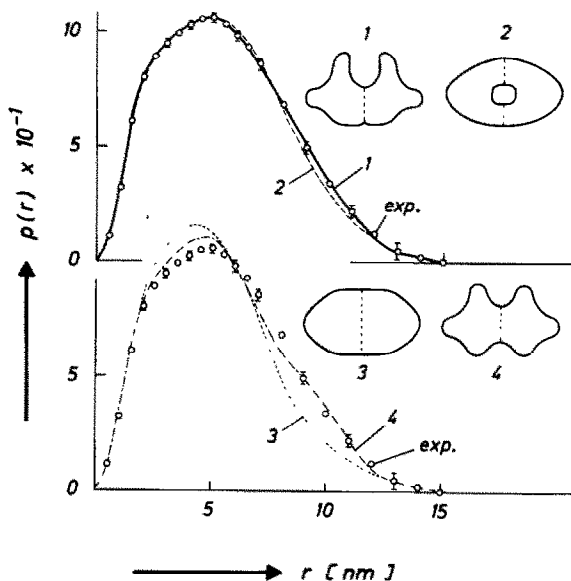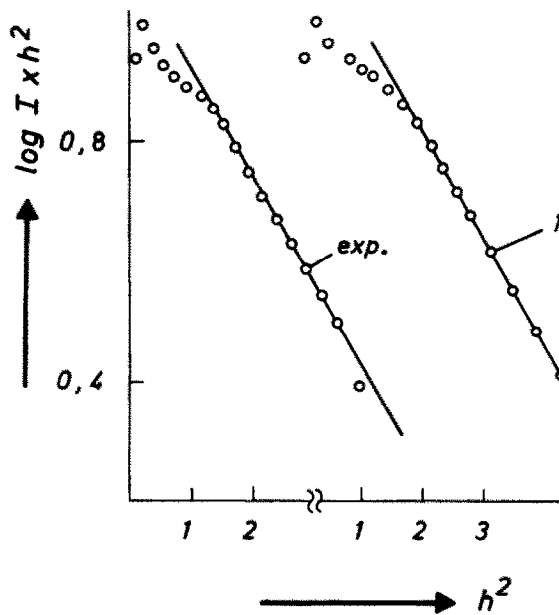
Fig.3. Plot for the determination of R_D. Comparison of the experimental curve with the theoretical one of model 1.

3.2. Volume

The volume of a hydrated macromolecule is proportional to its scattered intensity at zero angle and inversely proportional to its invariant Q [14]. The procedure used to determine Q is described in [1]. The volume of α_2 was found to be 146 nm^3 ± 5% by this method. Experience shows that the volume calculated from the invariant is usually affected by errors ≥5%, presumably due to particle inhomogenities which come into effect at large angles.

3.3. Shape

Small-angle X-ray scattering allows only an indirect determination of particle shape. The most common technique is to compare the experimental scattering curve or the $p(r)$ function with the theoretical curves of plausible models.

All model calculations were performed with a computer program which uses Debye's formula [15] to calculate the theoretical scattering curves of models composed of arbitrary spherical elements [1].

A large number of test-calculations were performed in order to find an α_2 model which agreed well with the experimental scattering data. Strictly speaking, model calculations can only exclude models which do not fit the experimental curve and thus produce a large number of possible solutions. To reduce this number, we had to consider the following limitations: the data of the models, such as R, D_{max}, V and R_D have to be the same as those determined experimentally. Since α_2 is a leaf-shaped particle the shape simulation can be reduced to a two-dimensional problem. Besides there are indications that there are no structural differences between the two subunits of α_2 [4]. Therefore an α_2 model may have an approximately symmetrical shape.

The simplest model which takes into account all these facts is an elongated disc with the dimensions $a:b:c$ = 15 nm:8.5 nm:2.2 nm (model 3, fig.2). Its $p(r)$ function clearly shows that its structure is too compact. Therefore the shape of α had to be approximated by models of increasing complexity. Just as in the case of subunit σ (1) a lot of plausible structures were tested. For example two crevices at the $\alpha-\alpha$ binding site were calculated in a series of models (model 4, fig.2). The experimental $p(r)$ function shows discrepancies with $p(r)$ of all models with this structural feature.

A disc-like model with one deep crevice in the middle (probably the $\alpha-\alpha$ binding site) was found to fit best the experimental scattering curve (model 1, fig.1) and the $p(r)$ function (fig.2). It consists of 116 spheres each with a radius of 0.67 nm. The average thickness of 2.2 nm corresponds to two layers of model spheres. R_D determined from model 1 agrees exactly with the experimental value, as is shown in fig.3. In this plot the slope of the straight line is proportional to R_D^2. It must be mentioned that models with a hole in the centre were also in good agreement with experimental data. The best fit of models with a hole yields model 2 (fig.2) with the dimensions $a:b:c$ = 15 nm:9 nm:2.2 nm and a hole diameter of 2.4 nm. However, model 1 is in better agreement with the experimental curves. By neutron small angle scattering the radius of gyration of α_2 in situ in complex with the other polymerase subunits β', β, σ was determined to be $R = 4.7 ± 0.2$ nm [16]. This R-value of α_2 agrees within experimental error with the R-value of isolated α_2. This indicates that there are only slight structural differences between α_2 in the isolated state and α_2 incorporated in the holoenzyme.

References

[1] Meisenberger, O., Pilz, I. and Heumann, H. (1980) FEBS Lett. 112, 39–41.
[2] Burgess, R. R. and Ravers, A. A. (1971) Methods Enzymol. 21D, 500.
[3] Ho, K. and Ishiama, A. (1973) J. Mol. Biol. 79, 115.
[4] Ovchinnikov, Yu. A. (1977) FEBS Lett. 76, 108–111.
[5] Zillig, W., Palm, P. and Heil, A. in (1976) Subunit Function and Reconstitution in RNA-Polymerase (Losick, R. and Chamberlin, M. eds) pp. 101–125, Cold Spring Harbor Laboratory NY.
[6] Stöckel, P., May, R., Stell, I., Cejka, Z., Hoppe, W., Heumann, H., Zillig, W., Cvespi, H., Katz, Z. Z. and Ibel, K. (1979) J. Appl. Cryst. 12, 176–185.

[7] Zillig, W., Zechel, K. and Halbwachs, H. I. (1970) Hoppe Seyler's Z. Physiol. Chem. 351, 221–227.

[8] Heil, A. and Zillig, W. (1970) FEBS Lett. 11, 165–172.

[9] Heumann, H., Stöckel, P. and May, R. (1980) in preparation.

[10] Kratky, O. (1958) Z. Elektrochem. 62, 66–73.

[11] Glatter, O. (1977) J. Appl. Cryst. 10, 415–421.

[12] Porod, G. (1948) Acta Phys. Austr. 2, 255–292.

[13] Kratky, O. and Porod, G. (1948) Acta Phys. Austr. 2, 133–147.

[14] Porod, G. (1951) Kolloid Z. 124, 83–114.

[15] Glatter, O. (1972) Acta Phys. Austr. 36, 307–315.

[16] Stöckel, P., May, R., Strell, I., Cejka, Z., Hoppe, W., Heumann, H., Zillig, W. and Crespi H. L. (1980) submitted.