The ionic strength-dependent aggregation of DNA-dependent RNA polymerase from *Escherichia coli*

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DNA-dependent RNA polymerase   *Aggregation*   *Light-scattering*

1. INTRODUCTION

Stable binding of DNA-dependent RNA polymerase to promoter sites of DNA templates is restricted to low ionic strength when the enzyme can aggregate. It was found by sedimentation measurements [1,2] that with decreasing ionic strength (<0.3) core enzyme E (M_r 385 000) aggregates into oligomers, containing up to 6 promoters, whereas holoenzyme Eσ (M_r 455 000), the complex of core enzyme and σ factor (M_r 70 000) forms only dimers. At high ionic strength (<0.3) both types of polymerase exist as monomers [2]. The knowledge of the association constants for holoenzyme at different ionic strengths is of interest for several reasons:

(i) The formation of the preinitiation complex between promoters and RNA polymerase should be different for polymerase molecules in the monomeric and the dimeric state.

(ii) The binding of factor σ to core enzyme might be influenced by the state of aggregation of the polymerase;

(iii) Only under conditions where the polymerase is monodispersed in solution can structural studies by small angle X-ray and neutron scattering measurements be performed.

The aim of this work is the determination of the apparent association constant for holoenzyme at different ionic strengths.

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2. MATERIALS AND METHODS

2.1. *Preparation of RNA polymerase*

RNA polymerase was isolated from *Escherichia coli* as in [3]. The concentration was determined using the extinction coefficients $E_{280}^{1\%}$ for core enzyme 6.3, for holoenzyme 6.3 and for σ factor 6.2.

2.2. *Light scattering*

The light scattering experiments were performed in:

(i) A Sofica instrument for the angle-dependent measurements;

(ii) A fluorescence spectrometer ($\gamma = 350$ nm, $\alpha = 90°$C) equipped with a completely sealed automatic titration device (cuvette vol. 1 ml). This device allows a series of measurements to be made without the incorporation of dust during the mixing of substances and it also consumes much less material compared to the Sofica instrument. However, measurement can be performed only at one fixed angle: $\alpha = 90°$.

The buffer was 0.05 M Tris–HCl (pH 7.9) and 10^{-3} M mercaptoethanol with an ionic strength adjusted by addition of KCl. All solutions were membrane (Sartorius) filtered and degased. The refractive index increments (Chromatix) dn/dc of core enzyme, holoenzyme and σ factor are equal within experimental error (dn/d$c = 0.19$, $\gamma = 633$ nm in 0.4 M KCl). The scattering data are corrected for the increase of dn/dc with decreasing ionic strength.

2.3. Theoretical background

The molar concentration c of the scattering molecules with molecular weight M was calculated from the scattering intensity I by the equation:

$$I = k \cdot c \cdot M^2 \qquad (1)$$

with

$$k = k' \cdot \left(\frac{dn}{dc}\right)^2 \cdot \frac{P(\lambda,\alpha)}{1 + M^2 \cdot P(\lambda,\alpha) \cdot (2A_2 c + 3A_3 c)}$$

k' = a proportionality factor
P = the form-factor
A_2, A_3 = the virial coefficients

Equation (1) was derived from the Debye formula using the following approximations:
(i) The form-factor $P(\gamma = 350\ nm,\ \alpha = 90°) = 1$;
(ii) The virial coefficients are = 0.

These approximations are justified from the data in fig.1:
(i) The scattering intensities show no significant dependence on the observation angle α. Therefore the experiments can be performed at one fixed angle ($\alpha = 90°$);
(ii) Under conditions when the RNA polymerase, core enzyme and σ factor, are monodispersed, the scattering intensity is proportional to the concentration (mg/ml).
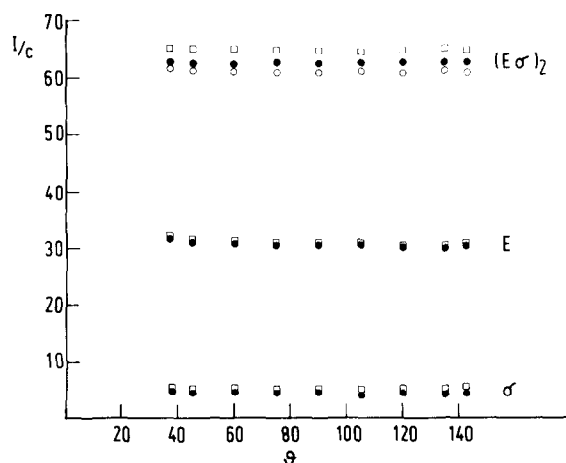

Fig.1. The scattering profile $I(\alpha)/c$ for dimeric holoenzyme $(E\sigma)_2$ at ionic strength = 0.03, core enzyme (E) and σ factor at ionic strength = 0.5: ($\square\ \square\ \square$) c = 0.25 mg/ml; ($\bullet\ \bullet\ \bullet$) c = 0.15 mg/ml; ($\circ\ \circ\ \circ$) c = 0.07 mg/ml.

2.3.1. The association constant K_σ of the system
$E + \sigma \rightleftharpoons E\sigma$

Expanding eq. (1) to the scattering intensity of the 3 component system E,σ,Eσ:

$$I = k(C_{E\sigma} \cdot M_{E\sigma}^2 + C_\sigma \cdot M_\sigma^2 + C_E \cdot M_E^2) \qquad (2)$$

The increase DI of the scattering intensity due to association of E and σ is related to K_σ according to:

$$DI = K \cdot (C_E^\circ + C_\sigma^\circ + K_\sigma^{-1} - S) \cdot k \cdot M_E \cdot M_\sigma \qquad (3)$$

where

$$S = \sqrt{(C_E^\circ + C_\sigma^\circ + K_\sigma^{-1})^2 - 4 C_E^\circ \cdot C_\sigma^\circ}$$

and

C° = total molar concentration

2.3.2. The association constant K_D of the system
$(E\sigma) + (E\sigma) \rightleftharpoons (E\sigma)_2$

The increase ΔI of the scattering intensity due to dimerisation of holozyme is related to K_D:

$$= \frac{\Delta I \cdot I_M}{2 C^\circ \cdot (I_M - I)^2} \qquad (4)$$

where

I_M = the scattering intensity of monomeric holoenzyme with the concentration C°

3. RESULTS

To decide whether the change of scattering of Eσ under various ionic strengths is caused by dimerisation of Eσ or by dissociation of Eσ into E and σ or by both, the interaction of core enzyme with σ factor was studied at different ionic strengths:

(i) At ionic strength > 0.3 core and holoenzyme are monomeric [2]. Therefore, the increase of the scattering intensity by mixing E and σ reflects the $E + \sigma \rightleftharpoons E\sigma$ equilibrium alone (fig.2b–d). From these binding curves the association constants (K_σ) were determined using eq. (3):

K(ionic strength = 0.3) = 5.8×10^7 M^{-1};

K(ionic strength = 0.4) = 2.8×10^7 M^{-1};
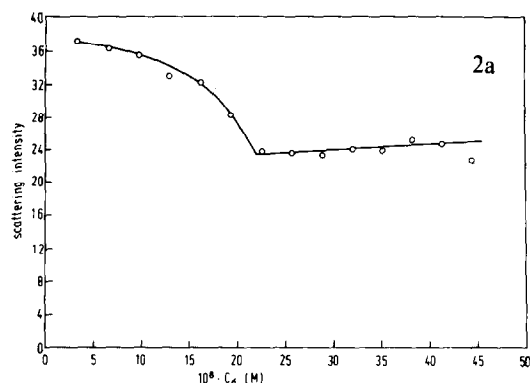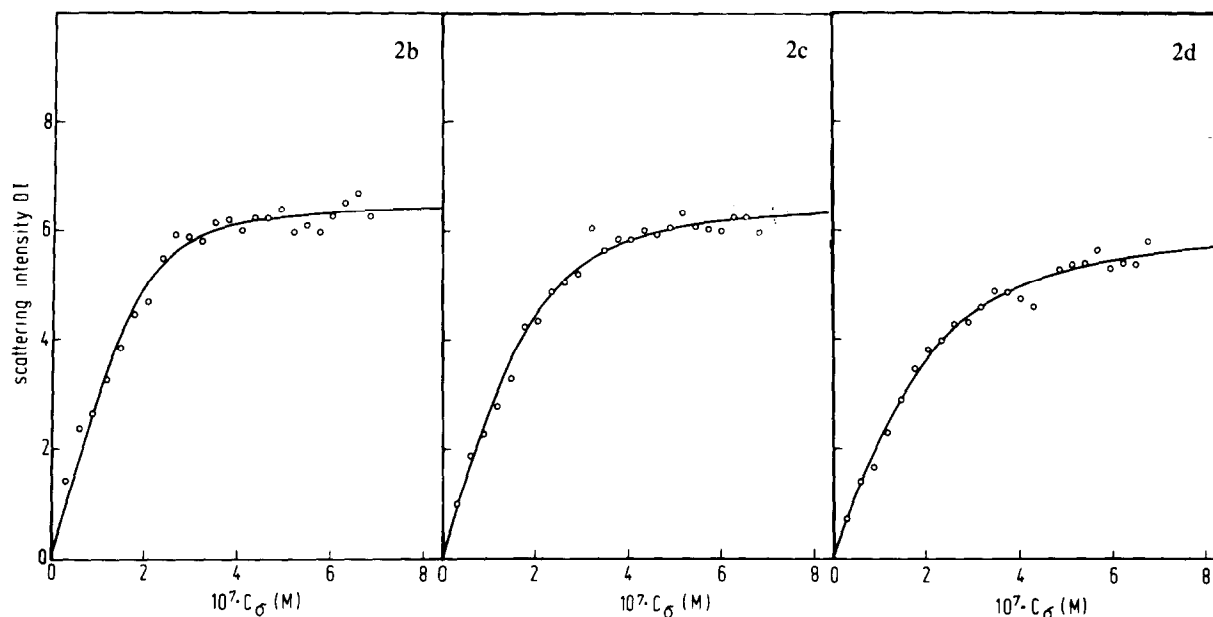
K(ionic strength = 0.5) = 1.7×10^7 M^{-1}.

Fig.2. Binding curves $I(\alpha) = 90°$ of E with σ at: (a) ionic strength = 0.03; (b) ionic strength = 0.3; (c) ionic strength = 0.4; (d) ionic strength = 0.5. Data in (b–d) obtained after subtraction of the scattering intensity of free E and free σ ($C_E = c_\sigma$ 2.0×10^{-7} M). The total increase $DI(c_\sigma = \infty)$ corresponds to the value expected from the increase of the M_r-value after binding of E and σ.

These data agree within experimental error with that in [4,5].

(ii) At low ionic strength core enzyme aggregates into oligomers (see fig.3 and 2). When σ is added to core enzyme the scattering intensity decreases until the point of molar equivalent amounts to E and σ is reached (fig.2a). From this data the association constant cannot be evaluated, since the aggregation of E additionally influences the scattering data.

The scattering intensity of monomeric holoenzyme I_M was calculated at high ionic strength taking into account the decrease of the scattering intensity due to partial dissociation of Eσ into E and σ using the data above and eq. (3). The self-association constant K_D was calculated from the scattering inten-
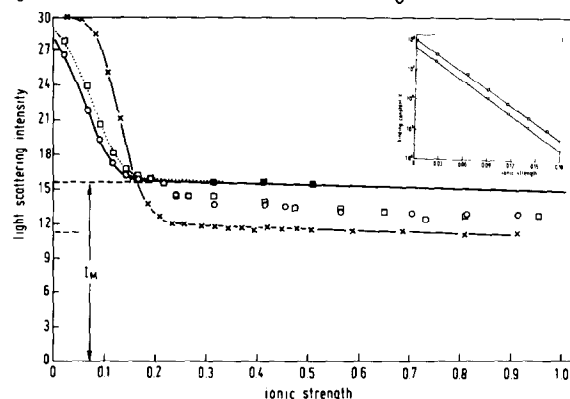

Fig.3. The scattering intensity of core enzyme (×) ($c = 1.6 \times 10^{-7}$ M) and of holoenzyme at $T = 25°C$ (□) and $T = 37°C$ (○) vs ionic strength; (×) indicates the scattering intensities of monomeric holoenzyme I_M. The curves through the points are calculated according to eq. (4). The insert shows the exponential behaviour of K_D (ionic strength).

sity difference ΔI in fig.3 between the measured intensity and the line representing I_M using eq. (4). Fig.3 shows, that the logarithm of K_D vs ionic strength can be fitted by a straight line expressed by equations:

At $T = 37°C$,

$$K_D(\text{ionic strength}) = 5 \times 10^7 \times e^{-18.7 \text{ ionic strength}}$$

(5)

At $T = 25°C$,

$$K_\sigma(\text{ionic strength}) = 10^8 \times e^{-18.3 \text{ ionic strength}}$$

4. DISCUSSION

The scattering intensities of holoenzyme at various ionic strengths measured at a fixed angle ($\alpha = 90°$) were used to study the self-association of DNA-dependent RNA polymerase. Applying eq. (1), ideal behaviour of the solution and a form-factor $P(\alpha = 90°, \lambda = 350 \text{ nm}) = 1$ (point scatterer approximation) were stated. These statements were proven for monomeric core enzyme and σ factor at the ionic strength = 0.5 (fig.1). Holo-enzyme (at ionic strength = 0.03) shows a concentration dependence of the scattering intensity (fig.1), which should be due to a change in the degree of dimerisation. Ideal behaviour of a solution of dimeric and monomeric holoenzyme was demonstrated by X-ray [6] and neutron small-angle scattering [7]. The point-scatterer behaviour observed is in agreement with the point-scatterer condition $d < \lambda/15$ [8] with $d \sim 24.5 \text{ nm}$ [9] for dimeric holoenzyme. Equation (1) describes correctly the scattering intensity in relation to the M_r-values at least for monomeric core enzyme and σ factor and dimeric holoenzyme [9,10]. It was assumed that the statements above are valid also for intermediate aggregational states of the RNA polymerase.

Although the monomer–dimer equilibrium is superimposed on the $E + \sigma \rightleftharpoons E\sigma$ equilibrium, we can contribute the scattering at various ionic strengths to each of both systems. At high ionic strength, holoenzyme is monomeric [2]. A change of the scattering intensity is caused by the $E + \sigma \rightleftharpoons E\sigma$ equilibrium. At low ionic strength the association constant for K_σ (ionic strength < 0.2) $> 10^9 \text{ M}^{-1}$, as shown by fluorescence measurements [4] and supported by the finding of a sharp inflection point in fig.2a at molar equivalent amounts of core and σ. Therefore, the change of

the scattering intensity at low ionic strength in fig.3 is caused by aggregation of holoenzyme.

The concave shape of the binding curve in fig.2a in the range of substoichiometric amounts of σ can be explained by making the assumption that in a mixture of different aggregates of core enzyme, σ binding places are partly occupied by the core pro-tomers. After addition of substoichiometric amounts of σ, the free σ binding places are occupied first. Further addition of σ induces the transition of oligomeric core to holoenzyme. This is in agreement with a model suggested by X-ray small angle scattering [9].

The self-association constant K_D(ionic strength) shows an exponential behaviour. This indicates electric-bond formation between holoenzyme molecules.

The data presented show, that under most experimental and physiological conditions the monomeric state of holoenzyme is favoured.

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