The Dissociation of σ -Factor from Ribonucleic Acid Polymerase

By AILSA M. CAMPBELL and PETER A. LOWE

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

(Received 7 January 1977)

The σ -factor of *Escherichia coli* RNA polymerase was shown to dissociate from the core enzyme as a function of absolute concentration. The association constant is in the range 10^6 – 10^8 litre/mol. This implies that the amount of holoenzyme, core enzyme and σ -factor in RNA polymerase assays may vary according to the absolute concentration of the enzyme.

RNA polymerase from *Escherichia coli* has five subunits: two α -subunits with mol.wt. 39000, one β -subunit with mol.wt. 155000 and one β' -subunit with mol.wt. 165000. The combination of these four is known as the 'core' enzyme. When the fifth subunit, σ (mol.wt. 95000), is present the complex is known as 'holoenzyme' (Chamberlin, 1974). Both core enzyme and holoenzyme are known to be present in their monomeric forms in solutions of high ionic strength and to form dimers in solutions of lower ionic strength.

Many factors affect the binding of the σ -subunit to core enzyme. Thus phosphocellulose, single-stranded polynucleotides and initiation on DNA all cause the release of σ -factor from the holoenzyme complex. The relative ease with which such dissociations occur, and the difficulty of isolation of enzyme with a full complement of σ -factor, suggest that the degree of association of σ -factor with core enzyme may be dependent on other physiological factors. One of the most obvious of these is absolute concentration. In the case of a loosely associated complex such dissociation may occur within a measurable concentration range which is within the range of most experimental assay systems. In such a circumstance the molecular weight of the holoenzyme would be expected to vary from an average of the molecular weights of the dissociated components at low concentration, to the molecular weight of the fully associated holoenzyme at high concentration.

Light-scattering is an exceptionally suitable technique for experimental measurements in this size range. It requires relatively small amounts of material when a laser-light source is used, and it lacks the complications found in sedimentation experiments at low ionic strength when the dimerization of the enzyme complicates its sedimentation properties. By using this technique we have shown that the σ -factor dissociates from the core enzyme under conditions of low ionic strength at protein concentrations above that normally used for assays and that the amount of holoenzyme present is a function not only of the

amount of σ -factor apparently present but on the absolute concentration of the components. We also estimate the association constant to within an order of magnitude at the ionic strengths used for assay and preparation, and suggest that this value should be borne in mind when experiments involving initiation are undertaken.

Materials and Methods

Laser-light scattering

The apparatus used has been described before (Campbell, 1976) and calibrated with Ludox Colloidal Silica. The refractive increment for RNA polymerase over the concentration range 0.1–0.6 mg/ml was 0.168 ± 0.03 ml/g in high-ionic-strength buffer [0.05 M-Tris/HCl (pH 8)/0.1 mM-dithiothreitol/0.1 mM-EDTA/0.4 M-KCl] and 0.171 ± 0.03 ml/g in the lower-ionic-strength buffer, which was identical with the high-ionic-strength buffer but contained only 0.18 M-KCl. The dissymmetry of the scattering from the enzyme solutions (which is the measurement of the ratio of scattering at 45° to that at 135°) was always less than 1.03, indicating the absence of any interference from high-molecular-weight aggregates or DNA contaminants. Clarification of the solutions was through Schleicher and Schuell filters of pore size $0.45 \mu\text{m}$. RNA polymerase is retained on such filters, but not extensively, especially in the high-salt buffer, and the filter readily became saturated by the first $100 \mu\text{g}$ of polymerase. The molecular weight measured is a weight average, $\Sigma M_i^2 N_i / \Sigma M_i N_i$ (where M_i is the molecular weight and N_i the molar concentration of the i th species), and as such is biased towards the heavier molecules in solution. Dilution of the enzyme was performed gravimetrically with the addition of filtered buffer and reweighing of the cell with each sample. This is particularly important in analyses of associating systems as one or other component of the mixture may be preferentially retained on the

filter if refiltration occurs before each measurement. The total error in the molecular weight was $\pm 5\%$.

RNA polymerase was isolated from *E. coli* M.R.E. 600 by a modification of the method of Burgess (1969) as described previously (Lowe & Malcolm, 1976). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis indicated a purity in excess of 95% (Shapiro *et al.*, 1967). The σ -factor stoichiometry, as judged by scanning densitometry, was 0.35 mol ($\pm 10\%$) per mol of core enzyme (Lowe & Malcolm, 1976). Core enzyme was isolated by the Polymin P precipitation technique of Burgess & Jendrisak (1975) with a purity in excess of 98%, as judged by polyacrylamide-gel electrophoresis.

Results and Discussion

Fig. 1 shows the relationship between apparent weight-average molecular weight and concentration in high-salt buffer for the preparation of RNA polymerase used. Above 200 $\mu\text{g/ml}$ there is a linear relationship between molecular weight and concentration, and linear extrapolation to zero concen-

Fig. 1. Relationship between apparent molecular weight and total protein concentration for RNA polymerase in high-salt buffer in the presence and absence of σ -factor

Empirical linear extrapolation of the eight high-concentration points leads to the correct value for a preparation with $40 \pm 5\%$ σ -factor. Empirical linear extrapolation of the five low-concentration points leads to a weight-average molecular weight of the dissociated components. Theoretical curves (----) for the association constants of 10^6 , 10^7 and 10^8 litre/mol, corrected for the observed virial effect shown by the core enzyme, are also shown. ●, Core enzyme with σ -factor; ○, core enzyme alone.

tration gives the expected mol.wt. of 440000, indicating that the holoenzyme is undissociated in solution at these higher concentrations. However, below 200 $\mu\text{g/ml}$ the graph becomes biphasic, and linear extrapolation of the last five points on the curve leads to a mol.wt. of 370000, which corresponds to an apparent weight-average molecular weight of dissociated σ -factor and core enzyme. The slight molecular repulsion apparent at the higher concentration is a common virial effect, particularly in solutions of high ionic strength, and reflects an affinity of the molecules for the solvent rather than for each other (Noren *et al.*, 1971). The preparation of core enzyme gives no such biphasic curve and extrapolates to the expected mol.wt. of 400000, indicating that dissociation of σ -factor is responsible for the observed fall in molecular weight.

Fig. 2 shows the relationship between apparent weight-average molecular weight and concentration for RNA polymerase in low-salt buffer. Here again it is apparent that extrapolation occurs to a mol.wt. of 370000, which corresponds to free core enzyme and dissociated σ -factor being again present in solution. At high protein concentration the well-known tendency of the enzyme to form dimers becomes apparent, although to a small extent. However, below 150 $\mu\text{g/ml}$ dissociation of σ -factor is occurring as a function of absolute concentration.

The dissociation of RNA polymerase observed under these two sets of solution conditions indicates that in both the preparation under conditions of high and low ionic strength and the assay of the enzyme (at low ionic strength) the relative proportions of core enzyme, σ -factor and holoenzyme present in solution may be far removed from those apparent in sodium dodecyl sulphate/polyacrylamide-gel electro-

Fig. 2. Relationship between apparent molecular weight and total protein concentration for RNA polymerase in low-salt buffer in the presence of σ -factor

The theoretical curves shown in Fig. 1 are not included because of the unknown virial contribution, but the data can be seen to extrapolate to a similar apparent weight-average molecular weight.

phoresis. This may be a major factor in studies involving initiation, and so we have attempted to estimate the order of magnitude of the association constant. Fig. 1 shows that this is between 10^6 and 10^8 litre/mol and close to 10^7 litre/mol at the high ionic strength. The values are similar in solutions of low ionic strength at low concentrations at which the dimerization of the polymerase is presumed to be minimal. The effect of extensive dimerization would be to lower the apparent degree of dissociation. It is not possible to quantify this with core enzyme alone as its polymerization properties differ from those of holoenzyme (Berg & Chamberlin, 1970). It is not possible to estimate the value very much closer than one order of magnitude, and indeed the association of the polymerase to DNA is customarily described only within one or two orders of magnitude.

The dissociation of σ -subunit confirms the observation by Travers (1975) that σ -factor and core enzyme are in dynamic equilibrium under high-salt conditions. In addition it allows measurement of the amount of free σ -factor present in solution and extends the observation to lower ionic-strength values used for assay of the enzyme.

All multisubunit enzymes must dissociate at infinite dilution according to Le Chatelier's principle. Doubtless RNA polymerase does also, but within the measuring range of our instrument we find no evidence

of further dissociation of the polymerase core enzyme, which shows the correct and well-established molecular weight obtained by addition of its four component subunits. The fall in apparent molecular weight of holoenzyme at low concentrations must reflect the dissociation of the σ -factor. Absolute concentration must therefore be a parameter to be considered in preparations and assays of RNA polymerase.

This work was supported by a grant from the Science Research Council.

References

- Berg, P. & Chamberlin, M. J. (1970) *Biochemistry* **9**, 5055–5064
- Burgess, R. R. (1969) *J. Biol. Chem.* **244**, 6160–6167
- Burgess, R. R. & Jendrisak, J. J. (1975) *Biochemistry* **14**, 4634–4638
- Campbell, A. M. (1976) *Biochem. J.* **155**, 101–105
- Chamberlin, M. J. (1974) *Enzymes* **10**, 333–374
- Lowe, P. A. & Malcolm, A. D. B. (1976) *Eur. J. Biochem.* **64**, 177–188
- Noren, I. B. E., Chien, H. & Cassassa, E. F. (1971) *Biochemistry* **10**, 3222–3229
- Shapiro, A. L., Vinuela, E. & Maizel, J. V. (1967) *Biochem. Biophys. Res. Commun.* **28**, 815–820
- Travers, A. (1975) *FEBS Lett.* **53**, 76–79