THE SUBUNIT STRUCTURE OF THE FORM AII DNA-DEPENDENT RNA POLYMERASE FROM RAT LIVER

Barbara E. H. COUPAR and C. James CHESTERTON
Department of Biochemistry, King's College London, Strand, London WC2R 2LS

and

Peter H. W. BUTTERWORTH
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, England

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1. Introduction

Eucaryotic ribosomal RNA-coding cistrons are transcribed by one of the totally α-amanitin-resistant DNA-dependent RNA polymerases designated as form A or form I [1–3]. In 1971, Chesterton and Butterworth [4] demonstrated the existence of 2 separable species in rat liver nuclei which were subsequently termed AI and AII [3]. A similar phenomenon was observed later by Matsui et al. [5] who termed the enzymes I_A and I_B. The question as to which of these enzymes represents the transcriptionally active species in vivo has been resolved recently. Using quite different analytical approaches, Matsui et al. [6] and Kellas et al. [7] have concluded that the nucleolar transcription complex contains the I_B and the AII enzyme respectively. Work in a number of laboratories has shown that the difference between the two α-amanitin-resistant RNA polymerase species, that is AI and AII or I_A and I_B, lies in the fact that one of them lacks a subunit of about 60 000 daltons [5,8,9]. Here we report data which shows that the AII RNA polymerase possesses a full complement of subunits and is therefore the complete enzyme.

2. Methods

Form AII RNA polymerase was purified from rat liver nucleoli using 3 different methods. The procedure involving a low-salt concentration extraction of nucleoli at 37°C was carried out as described previously [10]. The high-salt concentration extraction procedure I was carried out at 4°C as follows: nucleoli, prepared as before [10], from 300 g rat liver were extracted with 7.5 ml 0.4 M $(NH_4)_2SO_4$ in buffer A (25% glycerol, 0.01 M Tris–HCl, pH 8, 0.1 mM EDTA, 5 mM $MgCl_2$ and 0.5 mM dithiothreitol) for 15 min at 10°C. After dilution to 0.1 M $(NH_4)_2SO_4$ with buffer A, the mixture was centrifuged at 39 000 × *g* for 30 min. The supernatant enzyme was precipitated with 80% saturated $(NH_4)_2SO_4$, redissolved in 2 ml buffer A, and treated with a final concentration of 12% polyethylene glycol-6000 and 0.4 M $(NH_4)_2SO_4$. The precipitate was removed by centrifugation as before and the enzyme purified by ion-exchange chromatography on columns of DEAE-cellulose and phosphocellulose as before [10] except that $(NH_4)_2SO_4$ was used as the eluting salt. The enzyme was concentrated by dialysis against saturated $(NH_4)_2SO_4$ and sedimented through a glycerol gradient [10]. The fractions containing RNA polymerase activity were analysed on disc polyacrylamide gels containing 0.1% sodium dodecyl sulphate [11].

The high-salt concentration extraction procedure II was as described for procedure I with the following modifications. Before incubation, the nucleoli were sonicated 2 × 10 s [12]. The polyethylene glycol step

was omitted. After phosphocellulose chromatography, the enzyme solution was diluted to 0.1 M $(NH_4)_2SO_4$, adjusted to 40% glycerol, and loaded onto a 5 ml column of heparin-Sepharose 4B [13]. After washing the column with 0.2 M NH_4Cl in buffer B (40% glycerol and no $MgCl_2$, otherwise as buffer A), the enzyme was eluted with 0.4 M NH_4Cl in buffer B and concentrated for sedimentation as above.

The form B RNA polymerase was purified as described previously [12].

3. Results and discussion

We have previously shown that form AII RNA polymerase can be purified from rat liver nucleoli to near homogeneity as defined by native disc polyacrylamide gel electrophoresis [10]. This procedure involves a 37°C extraction of nucleoli in buffer containing a low concentration of salt. During the final purification step, the enzyme is sedimented through a glycerol gradient at 250 000 × *g*, the gradient is cut into fractions and assayed for RNA polymerase activity. When such fractions were subjected to analysis by electrophoresis on disc polyacrylamide gels containing 0.1% sodium dodecyl sulphate [11] and stained with Coomassie Blue, it was found that the following subunits were coincident with the activity: A1, 195 000 daltons (0.07); A1′, 175 000 daltons (1.0); A2, 128 000 daltons (1.0); A3, 60 000 daltons (0.8); A4, 44 000 daltons (1.0); A5, 26 000 daltons (1.0); A6, 19 000 daltons (1.0). The relative molar ratios, determined by densitometric scanning, are shown in parentheses. Molecular weights were determined by co-electrophoresis with the subunits of *Escherichia coli* RNA polymerase (165 000, 155 000, 89 000 and 41 000 daltons [14]), bovine serum albumin (68 000 daltons), myosin (220 000 daltons) and haemoglobin (16 000 daltons). The gel from the peak fraction is shown in fig.1. It is clear that a polypeptide of 60 000 daltons is present in the AII RNA polymerase.

The original definition [4] of form A heterogeneity according to the resolution of this activity on phosphocellulose suggests that the form AII described here and the form I_B of Matsui et al. [5] are the same catalytic activity. However, the size of the subunits of the form I_B RNA polymerase were estimated as 195 000, 130 000, 65 000, 40 000 and 19 000 daltons

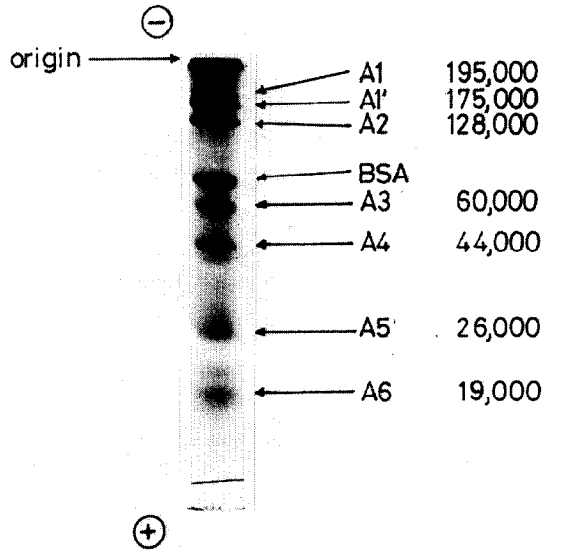

Fig.1. Separation by polyacrylamide gel electrophoresis of the subunits of form AII RNA polymerase purified from a 37°C low-salt extract of nucleoli. The enzyme (100 units) was precipitated with 5% TCA, pelleted at 20 000 × *g* for 30 min, denatured in 0.1% sodium dodecyl sulphate and 0.5 mM dithiothreitol for 30 min at 37°C, and electrophoresed on a 7.5% polyacrylamide disc-gel. Bovine serum albumin was present in the final purification step to stabilise the enzyme.

[5]. Thus 2 major differences are apparent between the structures of form AII and I_B. Firstly, the latter lacks a 26 000 dalton (A5) subunit. Secondly, the largest subunit (A1′) of the form AII enzyme (in a molar ratio of 1 with the other subunits) is of a significantly lower size than that of the heaviest subunit of I_B. It seemed possible that these differences could be associated with the method of extraction employed. An alternative technique, procedure I in Methods, was therefore tested. The new modus operandi did not subject the enzyme to a temperature of 37°C and thus minimised possible proteolysis. The structure of the purified form AII was investigated as before (fig.2a). A similar pattern was displayed to that found with the low-salt enzyme (fig.1) except that the largest subunit (A1) exhibited mol wt 195 000, clearly demonstrated by coincident electrophoresis with *E. coli* RNA polymerase subunits. As before, form AII subunits were in an approximate molar ratio of 1. A third method of purification which also avoided the

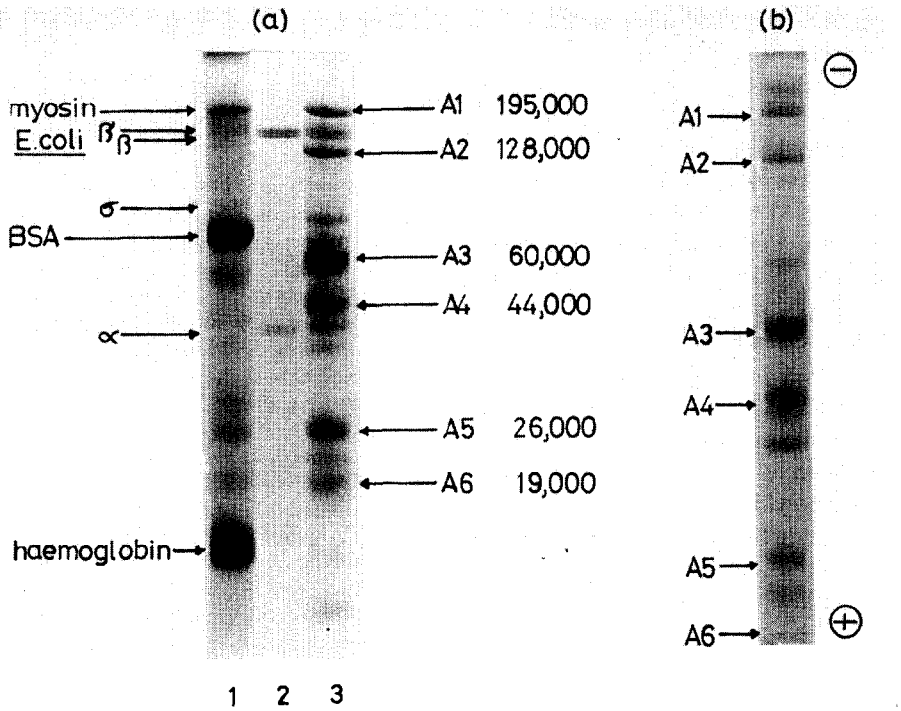

Fig.2. Separation by polyacrylamide gel electrophoresis of the subunits of form AII RNA polymerase purified from a 10°C high-salt extract of nucleoli. (a) Gel 1, 10 μg myosin, 10 μg bovine serum albumin and 10 μg of haemoglobin; gel 2, about 5 μg *E. coli* RNA polymerase; gel 3, form AII RNA polymerase (67 units) purified by procedure I. (b) Form AII RNA polymerase (20 units) purified by procedure II. Electrophoresis was carried out on 7.5% gels after the proteins were denatured as described in fig.1.

37°C-step and which incorporated a heparin–Sepharose affinity column yielded similar results (fig.2b) except that the relative levels of the smaller subunits were elevated.

It seems likely that the original difference in the size of the largest subunit was caused by limited proteolysis. It is interesting to note that a polypeptide of 195 000 daltons is detectable in the low-salt extracted enzyme (fig.1). Presumably, this is the A1 subunit from an undigested fraction of form AII molecules. Similar proteolytic alteration of structure has been observed with yeast [15] and *Drosophila* [16] form B RNA polymerases. However, the fact that the I_B enzyme lacks the 26 000 dalton subunit remains to be explained. A polypeptide of this size is present in all our preparations and in the form A enzyme from calf thymus [3] and mouse myeloma [8].

The subunit structure of form AII can be compared with that of the rat liver form B enzymes shown in fig.3. By analogy with the calf thymus system [3],

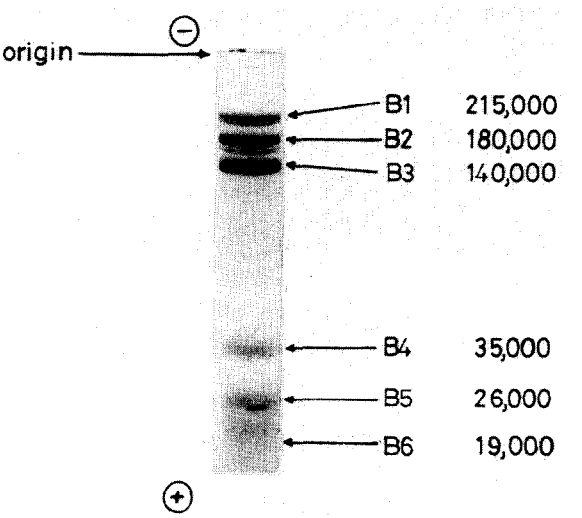

Fig.3. Separation by polyacrylamide gel electrophoresis of the subunits of form B RNA polymerase purified from rat liver nuclei. The enzyme (200 units), denatured as described in fig.1, was electrophoresed on a 5% polyacrylamide disc-gel.

this probably comprises a mixture of forms BI and BII which differ only in the size of the heaviest subunit. Enzyme BI contains subunits, B1, B3, B4, B5 and B6 whereas BII contains B2, B3, B4, B5 and B6. It is interesting to note that the size of the B5 and B6 subunits is similar to that of A5 and A6. This feature has been noted before [3] but immunological evidence suggests either that they are not in fact identical or that these subunits are not essential for transcription as assayed in vitro [17].

Form AII RNA polymerase purified by either the 37°C low-salt or the 10°C high-salt procedures contains a subunit of 60 000 daltons. It is clear, therefore, that form AII is the complete species of form A RNA polymerase. The data presented here together with that of Kellas et al. [7] concur with the findings of Matsui et al. [5,6] in implicating the complete form A or form I enzyme as the transcribing RNA polymerase in nucleoli. In all probability, forms I_B and AII are identical enzymes in vivo.

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