SUBUNIT SA3 IS NOT MANDATORY FOR THE ACTIVITY OF CALF THYMUS DNA-DEPENDENT RNA POLYMERASE AI

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1. Introduction

We have previously reported that calf thymus RNA polymerase AI, purified to apparent homogeneity, contains six subunits, [1,2]. Our data were consistent with a subunit model of $[(SA1, \text{mol wt } 197\,000)_1$ $(SA2, 126\,000)_1$ $(SA4, 44\,000)_1$ $(SA5, 25\,000)_2$ $(SA6, 16\,500)_2]$. We report here that CM-Sephadex chromatography of the highly purified enzyme AI yields two fractions, a major one which contains all six subunits and a minor one, which lacks subunit SA3. The two fractions have equal enzymatic activity using calf thymus DNA or poly[d(A-T)] as template.

2. Materials and methods

CM-Sephadex C-25 was obtained from Pharmacia. Poly[d(A-T)] was purchased from Boehringer. All
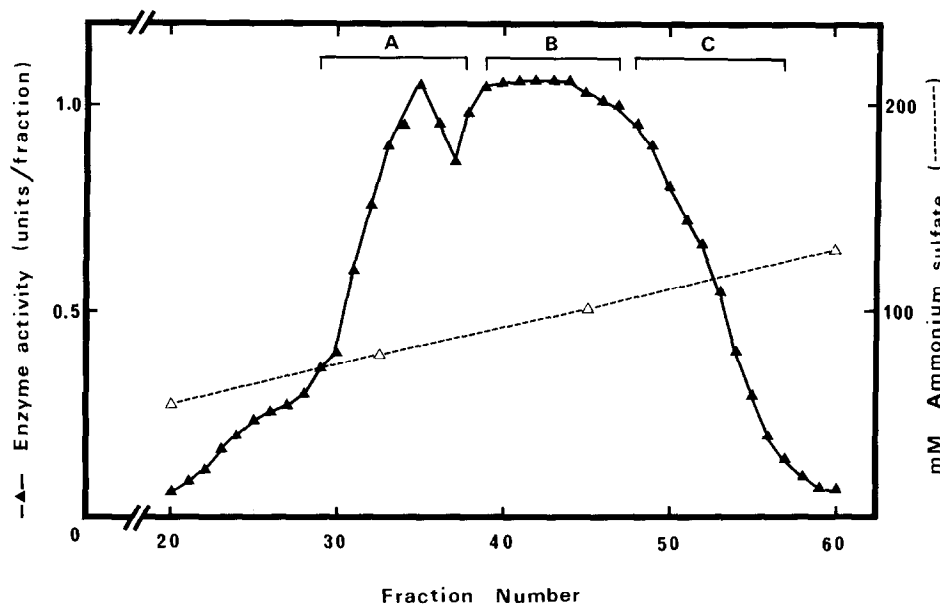

Fig.1. CM-Sephadex chromatography of glycerol gradient purified calf thymus RNA polymerase AI. 25 units of enzyme were applied (60 μl/min) to a CM-Sephadex column (1.5 × 8 cm) equilibrated with buffer E(30) containing 15 mM $(NH_4)_2SO_4$ [1]. The column was washed with 3 ml of buffer E(30) + 15 mM $(NH_4)_2SO_4$ and the polymerase activity was eluted (250 μl/min) with 70 ml of a 15 to 250 mM linear gradient of ammonium sulfate in buffer E(30). Fractions (0.5 ml each) were collected and RNA polymerase was assayed as described in Materials and methods. Fractions A, B and C were collected as indicated in the figure.
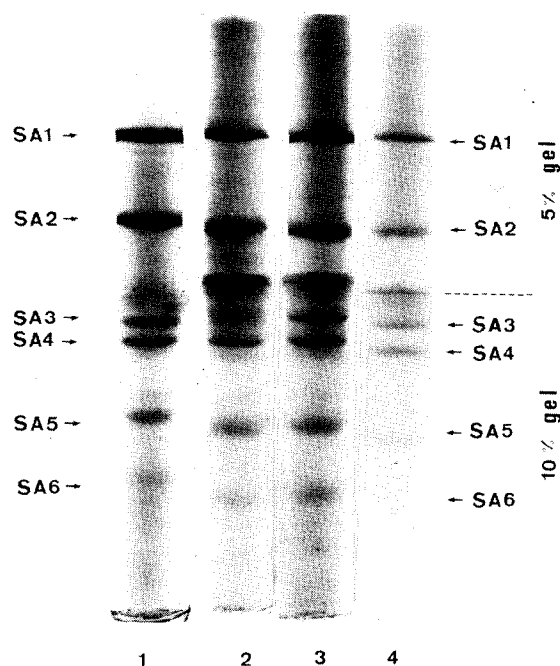
Fig.2. Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate of CM-Sephadex fractions. Aliquots of fractions A (gel 2, 2 units), B (gel 3, 3 units) and C (gel 4, 0.8 unit) (Fig.1) were electrophoresed under denaturing conditions as previously described [1,2]. 3 units of glycerol gradient purified calf thymus RNA polymerase AI were run in a similar gel (gel 1), but not in the same series.

other chemicals were as previously described [1,2]. Glycerol gradient purified calf thymus RNA polymerase AI fraction GG was prepared according to Gissinger and Chambon [1].

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out as previously described [2]. RNA polymerase activity was determined as previously reported [1]. One activity unit of enzyme incorporates 1 nmol of GMP in 10 min of incubation at $37°C$ [1]. All other methods were as previously described [1,2].

3. Results

Fig.1 shows the chromatography elution profile on CM-Sephadex of the glycerol gradient calf thymus RNA polymerase AI (fraction GG). Although the

separation was not very sharp (changing the elution conditions did not improve the resolution), two peaks were partially resolved, corresponding to about one third (first peak) and two thirds (second peak) of the total activity. Aliquots of the first peak (fraction A, fig.1) and of the second peak (fractions B and C, fig.1) were analysed by polyacrylamide gel electrophoresis under denaturing conditions in the presence of sodium dodecyl sulfate. Fig.2 shows the subunit pattern of these three fractions. The second peak (fractions B and C, gels 3 and 4) contained all of the six subunits which were present in the initial glycerol gradient purified enzyme AI (gel 1). However, subunit SA3 was barely detectable in the first peak (fraction A, gel 2), while densitometry analysis (not shown) of the five other subunits revealed that they were present in the same molar ratio as in the second peak. We termed AIa the RNA polymerase AI activity which was eluted first from CM-Sephadex and which was lacking subunit SA3, and AIb the RNA polymerase activity which contained all six subunits.

Since calf thymus DNA was used as a template to detect enzyme activity during the CM-Sephadex chromatography, subunit SA3 is clearly not required in stoichiometric amount for the transcription of this template. The comparison of the relative template efficiencies of calf thymus DNA and poly [d(A-T)] provides an additional evidence suggesting that subunit SA3 is not involved in the basic catalytic structure which is responsible for the synthesis of phosphodiester bonds by RNA polymerase AI (Table 1). Indeed, the relative template efficiencies of the two templates were identical, within the experimental error, for the unfractionated purified calf thymus enzyme AI and for the two enzyme forms, AIa and AIb, fractionated by CM-Sephadex chromatography.

4. Discussion

Two explanations can be invoked in order to account for the appearance of an enzyme AI form which is lacking subunit SA3 when a highly purified preparation of calf thymus RNA polymerase AI, which otherwise is apparently homogeneous [1,2] is chromatographed on CM-Sephadex. First, CM-Sephadex chromatography could reveal an heterogeneity which is otherwise difficult to detect because enzyme form

Table 1

Relative template efficiency of calf thymus DNA and poly[d(A-T)] for calf thymus RNA polymerase form AIa and AIb

Enzyme fraction	Calf thymus DNA (pmol)	Poly[d(A-T)] (pmol)	CT-DNA/ poly[d(A-T)] ratio
Glycerol gradient (fraction GG)	16	22	0.73
CM-Sephadex			
A	11	14	0.78
B	11	16	0.69
C	9	11	0.82

0.05 μg of either glycerol gradient purified calf thymus RNA polymerase AI (fraction GG) or CM-Sephadex fractions A, B and C (Fig.1) were incubated as described in Materials and methods for 10 min at 37°C in the presence of [³H] UTP and native calf thymus DNA or poly[d(A-T)]. The values (average of 3 assays not differing by more than 15%) correspond to pmole of [³H] UMP incorporated in acid-insoluble material.

AIa represents only a small fraction of calf thymus RNA polymerase AI and because the overall charge and molecular weight of enzyme forms AIa and AIb are too close. According to this hypothesis, enzyme form AIa would not be generated during CM-Sephadex chromatography, but its presence in the purified enzyme AI preparation might reflect the presence of subunit SA3 in a limiting amount in vivo. Alternatively, the five-subunit form AIa would be generated during the CM-Sephadex chromatography and would result from a partial dissociation of subunit SA3 from the six-subunit enzyme form. This second interpretation is favored by our previous observations [1,2] showing that subunit SA3 was present in stoichiometric amounts in the highly purified glycerol gradient fraction which was subjected to the CM-Sephadex chromatography. However, the first possibility is not excluded, because enzyme form AIa represents only a small fraction of calf thymus RNA polymerase AI and because the exact stoichiometry of the various subunits is imprecise relying only on densitometry measurements [2]. Additional studies are currently in progress to better understand the significance of the appearance of enzyme form AIa during CM-Sephadex chromatography.

Calf thymus subunit SA3 is clearly not the enzyme AI component required for initiation on intact double-stranded DNA, since even the six-subunit enzyme does not initiate RNA synthesis on an intact double-stranded DNA [3,4]. From the present studies, using as template either calf thymus DNA or poly[d(A-T)] where initiation is thought to occur mainly on single-stranded regions and single-stranded nicks, it appears that subunit SA3 is also not involved in the basic catalytic structure which is responsible for the synthesis of phosphodiester bonds. It is likely that calf thymus subunit SA3 plays some regulatory function which will be elucidated only when more physiological transcriptional systems will be available.

Enzyme AI forms, corresponding to calf thymus AIa and AIb forms, have recently been separated by phosphocellulose chromatography of purified rat liver nucleolar RNA polymerase AI [5] and by polyacrylamide gel electrophoresis of purified mouse myeloma nucleolar RNA polymerase AI [6]. In the case of rat liver, the five-subunit form lacking subunit SA3 was eluted first from phosphocellulose, like calf thymus enzyme form AIa from CM-Sephadex, while in the second case a faster migrating component lacking subunit SA3 was resolved from a slower enzyme form containing all six subunits. The two rat liver enzyme forms are most likely the counterparts of the two calf thymus enzyme forms, since both appear to be enzymatically active. In contrast the five-subunit component of mouse myeloma RNA polymerase AI was apparently inactive. However, as pointed out by

the authors, no definitive conclusion can be drawn concerning the possible role of subunit SA3 in the activity of mouse myeloma RNA polymerase AI, since the yield of activity eluted from polyacrylamide gel was very low even for the six-subunit component.

Multiple peaks of partially purified class A RNA polymerase has been previously reported in rat liver [7], ascites tumor [8], *Xenopus laevis* [9], *Drosophila* [10] and HeLa cells [11] after either phosphocellulose, CM-Sephadex or DEAE-Sephadex column chromatography. It is unlikely that the basis for these heterogeneities could lie in the absence or the presence of subunit SA3, because the separation of the enzyme peaks was much more clearcut than that presently observed for calf thymus enzyme forms AIa and AIb. Since in no case were the various enzyme A peaks pure enough to allow meaningful structural studies, additional purification work is required before it will be possible to decide whether the multiple peaks of class A RNA polymerase correspond to multiple enzymes with distinct subunits or to multiple forms of the same enzyme lacking anyone of its subunits.

Ackowledgements

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