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CONFORMATIONAL CHANGE OF DNA BINDING SUBUNIT OF RNA POLYMERASE II ON BINDING TO DNA

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SUMMARY: The spatial arrangement of the subunits of RNA polymerase II from Ehrlich ascites tumor cells was investigated by measuring the sensitivity of each subunit in the native enzyme to various proteinases. The results showed that the largest two subunits (a and b) were sensitive to all the proteinases tested, whereas two smaller subunits (e and h) were resistant to these enzymes. These results suggest that in the native enzyme subunits e and h are located in the inside of RNA polymerase II, forming a core. It was also found that the conformation of the DNA binding subunit a changes when the enzyme binds to DNA, and it becomes much more susceptible to chymotryptic digestion.

Eukaryotic cells contain three distinct RNA polymerases termed RNA polymerase I, II and III (1,2). These enzymes consist of multiple subunits forming a huge assembled structure with a molecular mass of about 500,000 (1-4). Since RNA polymerase II is responsible for the synthesis of heterogeneous nuclear RNA (2) and thus messenger RNA, elucidation of the function of each subunit is important in understanding the regulation of eukaryotic gene expression.

In the formation of active enzyme subunits are assembled systematically to construct the multimeric structure. Therefore, the spatial arrangements of these subunits are supposed to be important in understanding the function of RNA polymerase II. However, little is known about the spatial structure of RNA polymerase II, mainly because the pure enzyme is difficult to obtain in quantity.

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Recently, we established a method for large scale purification of RNA polymerase II from Ehrlich ascites tumor cells, separated ten subunits of the enzyme, and identified a DNA-binding subunit (5,6). In this work we investigated the proteinase sensitivity of each subunit of RNA polymerase II to obtain information about the conformation of this enzyme.

MATERIALS AND METHODS

Chemicals

Chymotrypsin, plasmin and elastase were purchased from Sigma. TPCK-treated trypsin was from Worthington. Ficin and V-8 proteinase were from Boehringer and Miles Lab., respectively. All other chemicals used were of reagent grade.

Preparation of RNA polymerase II

RNA polymerase II was purified to homogeneity from Ehrlich ascites tumor cells as described before (5). About 2 mg of pure enzyme was routinely obtained from 0.8 kg of wet cells. It was separated into 10 subunits named in order a to j by SDS-polyacrylamide gel electrophoresis.

Proteinase digestion of RNA polymerase II

RNA polymerase II (3 µg) was dissolved in 56 µl of 15 mM Tris-HCl buffer, pH 7.9 (25°C), containing 2 mM $MnCl_2$, 5 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 0.1 mM EDTA, 2 mM 2-mercaptoethanol, 200 µM each of GTP and ATP, 20 µM UTP, 0.04%(v/v) Triton X-100 and 11%(v/v) glycerol. This is a standard reaction mixture for RNA synthesis *in vitro* except that it does not contain template DNA or radioactive nucleosidetriphosphate (7,8). This mixture was incubated at 22°C for 20 min with 4 µl of an appropriate concentration of proteinase in 25 mM Tris-HCl buffer, pH 7.9 (25°C), containing 0.05 mM EDTA, 0.15 mM dithiothreitol, and 12.5%(v/v) glycerol. Then the reaction was terminated by adding 0.5 ml of 10%(w/v) trichloroacetic acid solution and the resulting precipitate was collected by centrifugation at 9,000 x *g* for 5 min, washed successively with 1 ml volumes of cold ether and deionized water, and subjected to SDS-polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis under denaturing conditions

Electrophoresis on polyacrylamide SDS slab gel (1 mm x 14 cm x 13 cm) was carried out by the method of Laemmli (9). After electrophoresis the gels were stained with Coomassie brilliant blue R-250 by the method of Fairbanks et al. (10).

For quantitative determination of the extent of digestion of each subunit of RNA polymerase II with proteinase, the gel was scanned at 600 nm, and the relative amount of each subunit was calculated from the weight of chart paper corresponding to each peak.

RESULTS

Digestion of RNA polymerase II with proteinases

RNA polymerase II purified from Ehrlich ascites tumor cells consisted of ten subunits. The molecular masses of two of them

are larger, being 165,000 and 140,000, respectively, whereas those of the other eight subunits are less than 40,000 (5). Since the enzyme is stable in the reaction mixture for RNA synthesis reported before (8), the three-dimensional structure of the enzyme is supposed to remain intact under these conditions. Therefore, to obtain information about the spatial arrangements of the subunits, we investigated the susceptibility of RNA polymerase II to trypsin, chymotrypsin V-8 proteinase, plasmin, ficin and elastase.

A fixed amount of RNA polymerase II was digested with increasing amounts of these enzymes and the digestion products were subjected to SDS-polyacrylamide gel electrophoresis. The resulting bands of subunits were then scanned in a densitometer and the relative amount of each subunit remaining was calculated as described in the Materials and Methods, and plotted against the concentration of enzyme. As shown in Fig. 1, the largest two subunits, a and b, were digested appreciably by relatively low concentrations of all six enzymes. In contrast, subunits e and h were insensitive to all these enzymes, and remained almost intact even after incubation with the highest concentrations of enzymes used.

Change in susceptibility to chymotrypsin of the largest subunit in the presence of DNA

As reported before, the largest subunit a of RNA polymerase II is a DNA binding subunit (6). Therefore, we examined whether its susceptibility to proteinase changes when RNA polymerase II binds to DNA. For this, a fixed amount of RNA polymerase II was digested with various concentrations of chymotrypsin in the presence or absence of Ehrlich ascites tumor cell DNA under conditions in which RNA polymerase II forms a complex with DNA (8), and the resulting digestion products were analyzed by

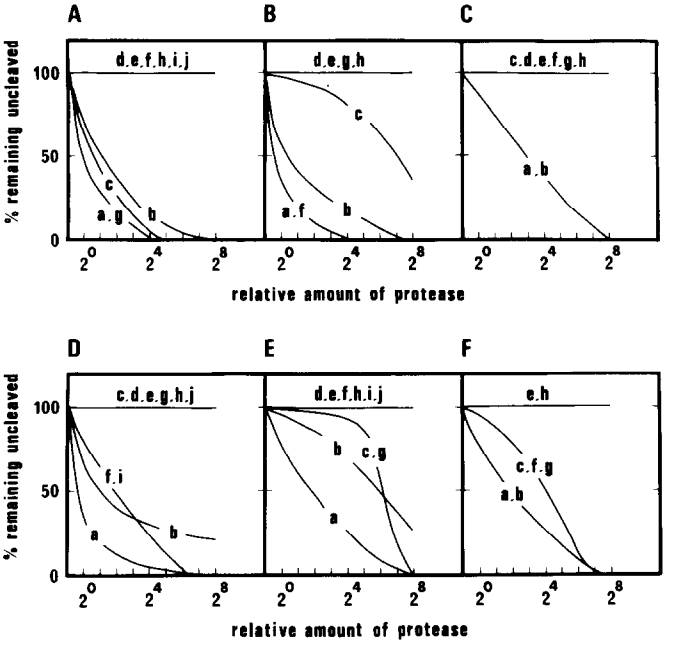

Fig. 1. Comparison of the susceptibilities of RNA polymerase II to various proteinases. A fixed amount of RNA polymerase II (3 μg) was digested with increasing amounts of various proteinases. After electrophoresis of the digestion products, the gels were scanned at 600 nm to quantify the amount of subunits remaining, as described in the Materials and Methods, and the relative amount of each subunit (a to j) was plotted against the relative amount of proteinase used. A, trypsin; B, chymotrypsin; C, V-8 proteinase; D, ficin; E, plasmin; F, elastase. Initial doses of enzymes corresponding to 2^0 are 0.10 (A), 0.83 (B), 0.83 (C), 1.7 (D), 1.7 (E), and 1.7 (F) μg/ml, respectively.

SDS-polyacrylamide gel electrophoresis. As evident from Fig. 2, the band of the largest subunit was visible when the enzyme was treated with 1.7 and 3.3 μg/ml of chymotrypsin, respectively, in the absence of DNA (B, lanes 3 and 4). However, it became fainter and disappeared under the same conditions when RNA polymerase II was digested in the presence of DNA (A, lanes 3 and 4). Thus the largest subunit became more susceptible to chymotrypsin in the presence of DNA, suggesting that when RNA polymerase II binds to DNA its conformation changes in such a way that more cleavage sites become available for chymotrypsin. The susceptibilities of other subunits to chymotrypsin did not seem to change in the presence of DNA.

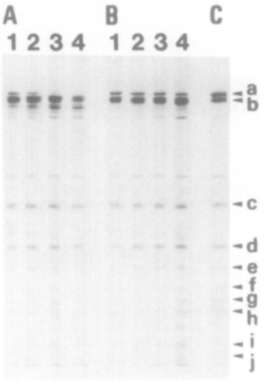

Fig. 2. Digestion of RNA polymerase II with chymotrypsin in the presence or absence of DNA. RNA polymerase II (3 µg) was mixed with 0.5 µg of Ehrlich ascites tumor DNA in the reaction mixture described in the Materials and Methods, and incubated at 37°C for 5 min to allow the enzyme to bind to DNA. Then increasing amounts of chymotrypsin were added and incubation was continued at 22°C for 20 min. Then the reaction was terminated by adding trichloroacetic acid solution, and the resulting precipitate was analyzed by SDS polyacrylamide gel electrophoresis. A, digestion in the presence of DNA; B, digestion in the absence of DNA. The enzyme concentrations used for lanes 1 - 4 were 0.42, 0.83, 1.7 and 3.3 µg/ml, respectively. C, control RNA polymerase II. Subunits of RNA polymerase II (a to j) are indicated by arrows.

DISCUSSION

The present results indicate the following new aspects on the spatial structure of RNA polymerase II. Subunits e and h may form a core with other subunits surrounding them. Subunits a and b, the two largest subunits, seem to be exposed to outside in this assembly, protecting the core part of the enzyme from attack by proteinases. Since these two subunits represent more than 60% of the total molecular mass of RNA polymerase II, it is conceivable that these two subunits are exposed to outside of the multimeric enzyme structure, and thus proteinases are easily accessible to these subunits. Previously, by stepwise dissociation of subunits of RNA polymerase II in urea, we demonstrated that subunits b and c have affinity to subunit a (6). As shown in Fig. 1, B, E and F, subunit c was more resistant to chymotrypsin, plasmin and elastase than subunits a and b, although it was digested completely with higher doses of

these enzymes. Thus it may be spatially protected by subunits a and b, but during digestion of the latter it may become exposed and then gradually digested by the enzyme. With the procedure used in this work, it was difficult to determine the amounts of the digestion of some subunits, especially d, i and j, because they had the same mobilities as the degradation products of other subunits on SDS-polyacrylamide gel. Thus, although it is impossible to deduce the arrangement of all ten subunits of this multimeric enzyme, a rough idea about that of some subunits such as a, b, c, e and h could be obtained from the patterns of its proteinase digests.

In this work we also demonstrated that when RNA polymerase II binds to template DNA, the conformation of its DNA binding subunit changes. This possibility was deduced for the finding that the susceptibility of this subunit to chymotrypsin changed when RNA polymerase II bound to DNA. The molecule may unfold when it binds to DNA, thus exposing more cleavage sites to chymotrypsin. This is the first evidence suggesting that the DNA binding subunit of RNA polymerase II changes in conformation depending upon the state of the enzyme. Although RNA polymerase II purified from Ehrlich ascites tumor cells is type IIB (2), and thus the molecular mass of a is 165,000 in stead of 220,000, this finding may be important in understanding the structure and function of subunit a.

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