PROTEINASE K-RESISTANT AND ALKALI-STABLY BOUND PROTEINS IN HIGHER PLANT DNA

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1. Introduction

In [1] we demonstrated that distinct proteins which are, according to their electrophoretical mobility, between 54 000–68 000 mol. wt cannot be removed from animal cell DNA (Ehrlich ascites tumor cells, rat liver, calf thymus, sea urchin sperm) by phenol, proteases, alkali nor by any combination of these methods [1]. The presence of these proteins can be demonstrated by radioiodination of purified DNA samples. After degradation of DNA the iodinated material can be analysed by SDS–polyacrylamide gel electrophoresis and autoradiography of the gels. The pattern of the bands observed is very characteristic and reveals the presence of proteins which are 54 000–68 000 mol. wt. Since these proteins cannot be removed from DNA except by degradation of DNA [1] we assume that these proteins are covalently bound to DNA. Here we show that proteins of identical size and characteristics are also found in DNA of dicotyledonous and monocotyledonous plants. These most tightly bound proteins can also be labelled by treatment of purified DNA from plant cells with ^{125}I. Following degradation of the DNA the labelled proteins are released and can be analysed by SDS–polyacrylamide gel electrophoresis and autoradiography. According to their size and characteristics these proteins from plant cell DNA are identical with the most tightly bound proteins found in animal cell DNA. This is of importance because it indicates that the presence of these proteins is a general characteristic of eukaryotic cell DNA. Furthermore, our results indicate that DNA from plant cells generally considered to be free of protein still contains distinct protein components.

2. Experimental

2.1. *Isolation of DNA from plant cells*

DNA was isolated from *Sinapis alba* hypocotyls, *Pisum sativum* epicotyls, *Cymbidium pumilum* protocorms and *Rhoeo discolor* leaves. The material was frozen in 0.05 M Tris, 1 M NaCl, 0.1 M EDTA (pH 8) and ground while frozen. After thawing SDS was added to 1% final conc. DNA was purified by shaking with chloroform–isoamylalcohol (24:1) repeatedly until the protein interphase disappeared. Ethanol precipitation, ribonuclease and proteinase K digestions were followed by further chloroform–isoamylalcohol treatment and precipitation with ethanol [2–4]. Pigments and RNA fragments were removed from the DNA by passing it over a Sepharose 4B (Pharmacia) column equilibrated in 0.12 M phosphate buffer [5,6].

2.2. *Further purification of DNA*

For Ag^+/Cs_2SO_4 gradient centrifugation the ratio of Ag^+ to DNA phosphate in 0.01 M Na_2SO_4 and 5 mM sodium tetraborate buffer (pH 9) was 0.4 M and the initial density of Cs_2SO_4 was 1.5 g/cm^3 [7,8]. Centrifugation was made in a fixed-angle Ti 75 rotor on a Beckman preparative ultracentrifuge for 68 h at 32 000 rev./min and 20°C. DNA containing fractions were extensively dialyzed before iodination or further purification.

Finally the DNA samples were alkali-denatured and rebanded in alkaline (pH > 12) Cs_2SO_4 gradients prior to iodination [1]. This was done in a fixed angle Ti 50 rotor for 48 h at 35 000 rev./min and 20°C. Fractions containing the DNA were collected, dialysed and precipitated by ethanol. The DNA was redissolved

in a solution which was prepared by mixing of 3.5 ml 0.5 M Tris–HCl (pH 7.6), 3 g urea and 100 mg SDS.

2.3. *Radio-iodination of DNA*

Fractions containing 1–2 A_{260} units of DNA were treated with ^{125}I (Radiochemical Center, Amersham) as in [1]. After removal of the unreacted iodine, aliquots were mixed with equal vol. 60 mM Tris–HCl, 2% SDS, 10% glycerol, 10% mercaptoethanol at pH 6.8, heated to 95° for 30 min and subsequently submitted to SDS–polyacrylamide (12%) gel electrophoresis [9]. Electrophoresis was performed at 150 V for 4 h. The gels were exposed to X-ray film for 1–12 h and the films were traced by a Vernon-densitometer.

3. Results

Figure 1 shows densitometer tracings demonstrating the progress of purification of one DNA sample

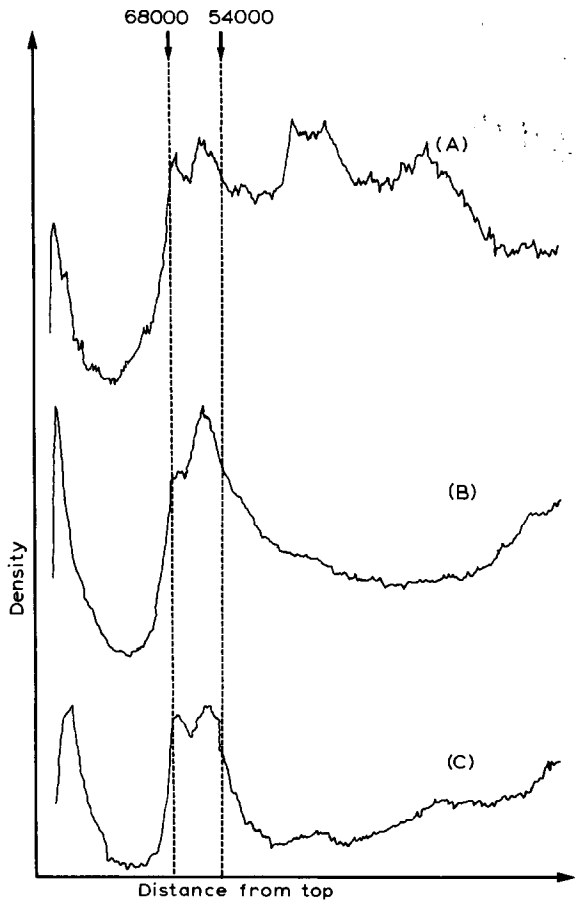

Fig.1. Demonstration of proteins which are tightly bound to DNA from *Cymbidium pumilum* after sequential purification steps: (A) after Sepharose 4B; (B) after complexing by Ag^+/Cs_2SO_4; (C) after rebanding in alkaline Cs_2SO_4-gradient. The DNA samples were purified as described under (A–C) treated with ^{125}I and heat-degraded. The ^{125}I-labelled material was analyzed by SDS–polyacrylamide gel electrophoresis, autoradiography and densitometer-tracing of the films.

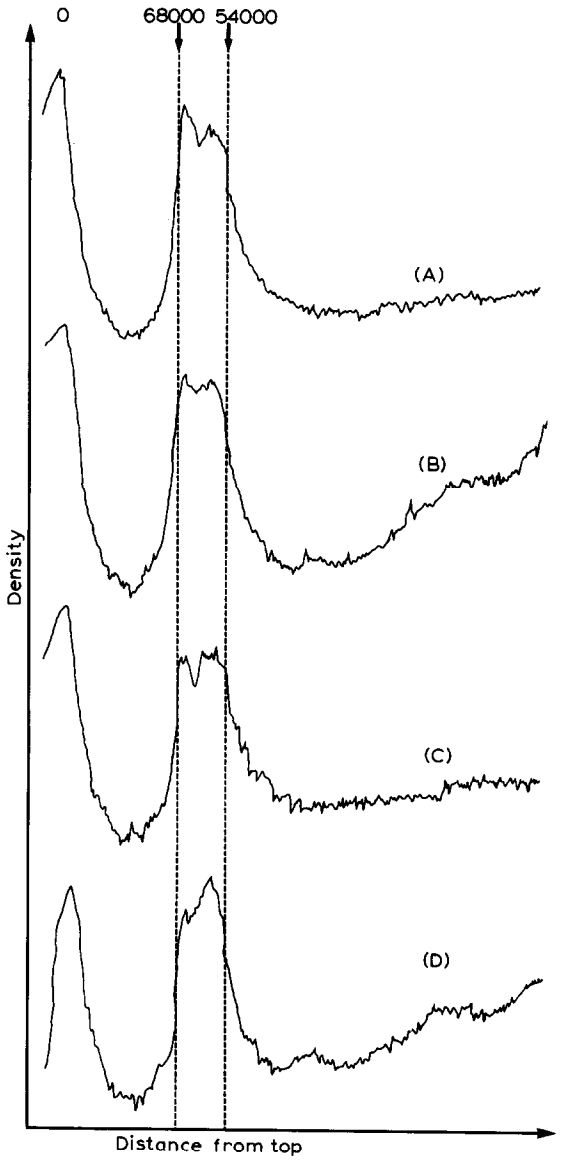

Fig.2. Demonstration of proteins alkali-stably bound to plant and animal cell DNA. DNA was isolated from (A) *Pisum sativum*, (B) *Rhoeo discolor* leaves, (C) *Sinapis alba* and (D) Ehrlich ascites tumor cells. Prior to iodination all DNA samples were rebanded in alkaline Cs_2SO_4-gradients. The iodination procedure was performed as in fig.1.

(Cymbidium pumilum) by the following sequential purification steps: Isolated DNA from Sepharose 4B, DNA after Ag^+/Cs_2SO_4 complexing, and DNA after banding in alkaline Cs_2SO_4-gradients. The results show that the original DNA preparation contains still a small quantity of residual proteins which are 54 000–68 000 mol. wt and in addition to this material other labelled material is detected which is smaller in size. However, the smaller material is removed after centrifugation of DNA in a Ag^+/Cs_2SO_4 gradient whereas the 54 000–68 000 mol. wt material is not removed by this treatment. The 54 000–68 000 mol. wt material is also not removed by rebanding in alkaline Cs_2SO_4, indicating that it is alkali-stably bound to DNA. Figure 2 shows densitometer tracings demonstrating that the alkali-stably bound proteins are also present in alkali-treated DNA of other higher plant species. Furthermore, the densitometer tracings show that the size and the pattern of these alkali-stably bound proteins in plant cell DNA are identical with that of animal cell DNA.

4. Discussion

The data presented here indicate that the alkali-stably bound protein component found in DNA from animal cells is also found in highly purified and alkali-treated DNA from plant cells. It was suggested in [1] that these most tightly bound proteins found in DNA from animal cells could play a role in maintaining the stability of superhelical loops and 3-dimensional structure of chromatin [10–12]. Therefore, the presence of identical proteins in DNA from plant cells is consistent with the former interpretation. Furthermore, since the DNA is isolated from plant cells by different procedures than that from animal cells and since the copurifying proteins are identical this is taken as a further indication for the specificity of this component.

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