

Binding of Chromosomal Acidic Proteins to DNA and Chromatin¹ (36364)

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(Introduced by S. Weinhouse)

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Recently a considerable amount of attention has been directed towards chromosomal acidic proteins as possible regulators of gene expression in general (1-4) and cell proliferation in particular (5-7). Their localization in chromatin and ability to counteract gene repression by histones (1), first suggested their probable role in gene regulation. Species specificity of chromosomal acidic proteins in binding to DNA has also been reported (8, 9).

Chromatin has nearly equal numbers of basic amino acids and negatively charged nucleotide residues (10). In order to have a specific regulatory function, chromosomal acidic proteins must interact either with open sites on the DNA strands, or with the basic proteins, and thus cause structural modifications in the chromatin. Thus, the binding ability of chromosomal acidic proteins to chromatin, compared to DNA, may provide some insight into the specificity of their interaction with chromatin and the mechanism of gene expression.

We report here that (a) chromosomal acidic proteins bind to chromatin as efficiently as they bind to DNA; (b) total cytoplasmic proteins or cytoplasmic acidic proteins compete with chromosomal acidic proteins in binding to chromatin; and (c) about 12-15% of chromosomal acidic proteins cannot be competed out by any fraction of cytoplasmic proteins in binding to chromatin.

Materials and Methods. Exponentially growing HeLa S₃ cells were used in this

study. Chromatin was prepared by the method of Paul and Gilmour (3) from a purified nuclear fraction (11). Chromosomal proteins were extracted with 3 M NaCl-5 M urea-0.01 M Tris, pH 8.3, and after removing DNA by centrifugation (12) the supernatant was extensively dialyzed against 5 M urea-0.01 M Tris, pH 8.3. Chromosomal acidic proteins, free of histones, were isolated from the dialysate by fractionating through QAE Sephadex A-50 (a strongly basic anion exchanger) according to Gilmour and Paul (13). To isolate total cytoplasmic proteins and cytoplasmic acidic proteins, the cells were suspended in a solution containing 0.02 M NaCl-0.02 M Tris, pH 8.3-0.005 M MgCl₂. They were then homogenized in a Potter-Elvehjem homogenizer and centrifuged at 12,000g for 20 min to discard nuclei and intact cells. The supernatant was made 1 mg/ml protein and treated with 10 μg/ml RNAase A (Protease free, Schwarz) for 60 min. Solid NaCl and urea were added, stirred until dissolved, and the final concentration of NaCl and urea made 1 M and 5 M, respectively. The mixture was dialyzed against 5 M urea-0.01 M Tris, pH 8.3; a portion of the dialysate was used as the total cytoplasmic proteins while another portion was fractionated through QAE Sephadex A-50 as above. Acidic cytoplasmic proteins were eluted with 3 M NaCl-5 M urea-0.01 M Tris, pH 8.3, and then dialyzed against 5 M urea-0.01 M NaCl-0.01 M Tris, pH 8.3.

To isolate radioactively labeled chromosomal acidic proteins, HeLa cells were labeled for 60 min with L-leucine-³H (sp act 58 Ci/mmol, New England Nuclear Corp.) in leucine-free Eagle's minimum essential medium and the chromosomal proteins isolated as

¹ This investigation was supported by a research grant from the Damon Runyon Memorial Fund (1019B), and by a training grant (CA-05222) from the National Cancer Institute, U.S. Public Health Service.

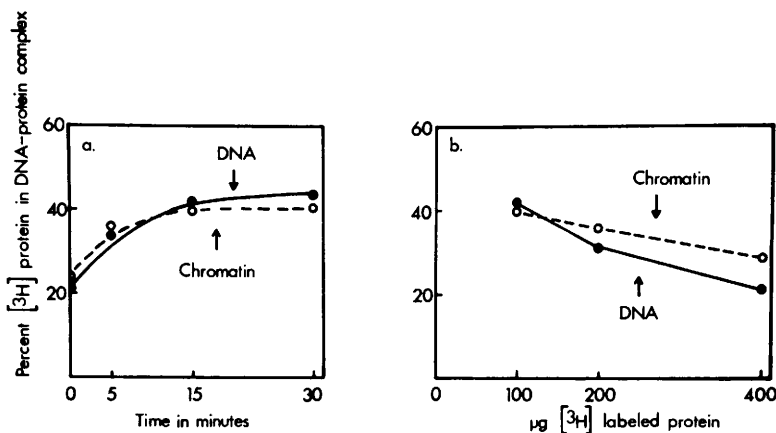


FIG. 1. Binding of chromosomal acidic proteins to DNA and chromatin.

a. Time curve of binding. Pure DNA or DNA as chromatin (100 µg) was mixed with labeled chromosomal acidic proteins in a DNA:protein ratio 1:1 (w/w) and incubated for the time periods indicated on the abscissa.

b. Binding studies with increasing amounts of chromosomal acidic proteins and a constant amount of DNA or chromatin. DNA or chromatin (100 µg) was mixed in a DNA:protein ratio 1:1, 1:2, 1:4 and incubated for 15 min. In both cases, after incubation, the DNA-protein complexes were collected by centrifugation through 1.7 M sucrose. The DNA content and the radioactivity of the nucleoprotein complexes were determined. The results are represented as the percent of total labeled chromosomal acidic proteins bound to DNA.

above. DNA was purified from exponentially growing HeLa S₃ cells according to Marmur (14). Proteins were estimated by the method of Lowry *et al.* (15).

In binding experiments, either pure DNA or DNA as chromatin, both in 5 M urea-0.01 M NaCl-0.01 M Tris, pH 8.3, were mixed with labeled chromosomal acidic proteins in the same buffer and incubated at 4° with stirring for a specified period of time. After incubation the samples were layered on top of 25 ml 1.7 M sucrose, the upper two-thirds were gently mixed and nucleoproteins were collected by centrifugation in a SW 25.1 rotor at 55,000g for 4 hr. The pelleted nucleoprotein fraction was solubilized in 1 N NaOH, neutralized and an aliquot used for radioactivity measurement in Triton-toluene liquid scintillation cocktail (16). DNA content was measured from the absorbancy at 260 mµ.

Results and Discussion. Figure 1a shows that under the present experimental conditions maximum binding of chromosomal acidic proteins to DNA occurred after 15-min incubation. About 40 µg of proteins were bound to DNA when 100 µg of proteins were

incubated with 100 µg of DNA. Further incubation did not cause any significant increase in binding. A considerable amount of binding also occurred by just mixing DNA and proteins followed by immediate centrifugation.

Chromosomal acidic proteins were also found to bind extensively to chromatin. When 100 µg of chromatin were incubated with 100 µg of proteins, the amount of protein bound to chromatin was similar to that of DNA. But when the amount of protein in the incubation mixture was increased (DNA:protein ratio 1:2, 1:4, w/w), a 20-40% more binding of acidic proteins to chromatin was observed. The results are shown in Fig. 1b. It should be noted that when 100 µg of DNA were incubated with 100 µg of acidic proteins in the presence of an equal amount of histones, about 48 µg of acidic proteins were bound to DNA (results not shown).

To compare the extent of binding obtained with the present procedure to other methods reported in the literature (9, 13), the binding of chromosomal acidic proteins to DNA was also studied according to the method employed by Teng *et al.* (9), except that the

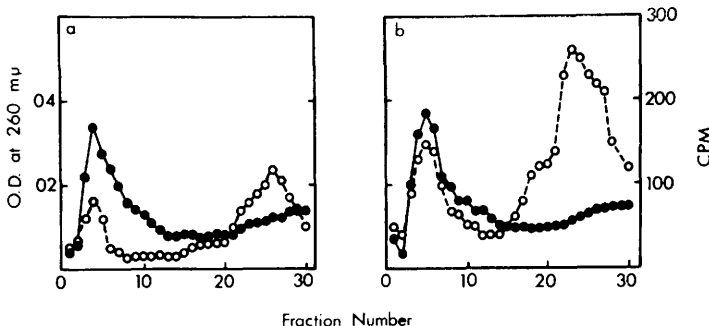


FIG. 2. Binding of chromosomal acidic proteins to DNA by a conventional technique employed for the study of the reconstitution of chromatin (9). Pure DNA and chromosomal acidic proteins in 3 M NaCl-5 M urea-0.01 M Tris-HCl, pH 8.3 were mixed and dialyzed against decreasing concentrations of salt. Finally the urea was removed by dialysis against 0.01 M Tris-HCl, pH 8.3-0.01 M NaCl and the DNA-protein complex was banded by centrifugation in a 5-25% linear sucrose gradient. The percent of total proteins bound to DNA in a DNA-protein ratio 1:1 (a) and 1:2 (b) is 46 and 38, respectively. ●—● od at 260 mμ; ○—○ radioactivity.

sucrose gradient centrifugation was carried out in a SW 25.1 rotor. The results are shown in Fig. 2. The percentage of proteins bound to DNA at a DNA:protein ratio of 1:1 and 1:2 (w/w) is similar to that obtained with the first procedure.

The extensive binding of chromosomal acidic proteins to chromatin suggested the possibility that the binding was random and possibly due to nonspecific interaction with chromatin. Thus the possibility was considered that cytoplasmic proteins might similarly interact with chromatin and effectively compete with chromosomal acidic proteins in binding to chromatin. The binding ability of cytoplasmic proteins to chromatin is shown in Fig. 3. When incubated with chromatin the amount of total cytoplasmic proteins and cytoplasmic acidic proteins sedimentable with the nucleoprotein complex, were found to be 12 and 22 μg, respectively, per 100 μg of DNA. When labeled chromosomal acidic proteins were incubated with chromatin in the presence of unlabeled cytoplasmic proteins, total cytoplasmic proteins competed, to some extent, with chromosomal acidic proteins in binding to chromatin, but the competition was much more effective with cytoplasmic acidic proteins. However, even in the presence of an 8-fold concentration of cytoplasmic acidic proteins about 12-15% of chromosomal acidic proteins were still able to

bind to chromatin. The results are shown in Fig. 4.

The results suggest that any protein that is acidic in nature may interact nonspecifically with chromatin, and that studies on protein binding to DNA (17, 18) may involve some nonspecifically bound proteins which are like-

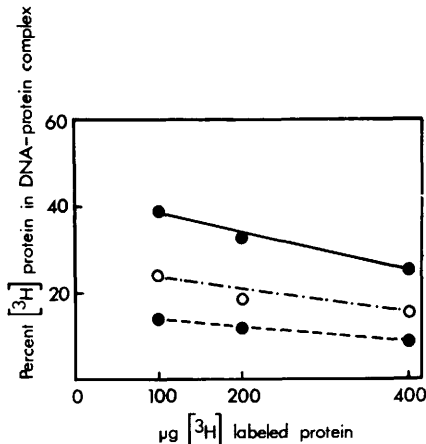


FIG. 3. Binding of total cytoplasmic proteins, cytoplasmic acidic proteins and chromosomal acidic proteins to chromatin. DNA as chromatin was mixed at a DNA (100 μg):protein ratio 1:1, 1:2 and 1:4 and incubated for 15 min. The nucleoprotein complexes were isolated as in Fig. 1. The results are represented as the percent of total labeled proteins bound to DNA. ●—● chromosomal acidic proteins; ○---○ total cytoplasmic proteins; ⊗—⊗ cytoplasmic acidic proteins.

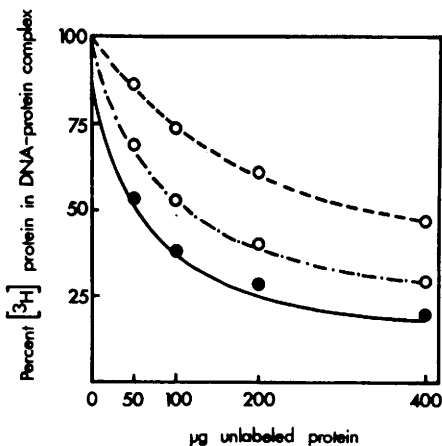


FIG. 4. Binding of labeled chromosomal acidic proteins (50 μg) to chromatin (50 μg DNA) in presence of varying amounts of unlabeled chromosomal acidic proteins, total cytoplasmic proteins, and cytoplasmic acidic proteins as shown on the abscissa. Labeled chromosomal acidic proteins were mixed with unlabeled proteins before incubating with chromatin. Incubation period was 15 min. The nucleoprotein complexes were isolated as in Fig. 1. ●—● chromosomal acidic proteins; ○---○ total cytoplasmic proteins; ⊗—⊗ cytoplasmic acidic proteins.

ly to interfere with identification of the specific regulatory molecules.

While a variety of molecules must interact with DNA or chromatin in order to initiate the basic genetic processes, to identify the specific groups of molecules competitive binding studies may prove to be useful. The competition experiments in the present studies show that while cytoplasmic proteins effectively compete with chromosomal acidic proteins in binding to chromatin, there is a fraction (about 15%) of chromosomal acidic proteins which are not competed out by cytoplasmic protein. The specificity may arise from specific binding sites for these proteins in DNA, which cannot be saturated by cytoplasmic proteins. Recently Chytil and Spelsberg (19) also have reported by immun-

ochemical method that 20% of the chromosomal non-histone proteins possess tissue specific antigenic activity.

Summary. Binding studies of chromosomal acidic proteins to DNA and chromatin show that these proteins bind to chromatin as effectively as they bind to DNA. While cytoplasmic proteins compete with these proteins, about 15% of chromosomal acidic proteins cannot be competed out by cytoplasmic proteins in their ability to bind with chromatin.

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Received Dec. 20, 1971. P.S.E.B.M., 1972, Vol. 139.