INTERACTION OF CYTOSOL DNA-BINDING PROTEINS WITH ADENINE-RICH POLYNUCLEOTIDES

Donald B. Carter and Chi-Bom Chae

Department of Biochemistry, University of North Carolina
Chapel Hill, N.C. 27514

Received April 30,1974

SUMMARY. In liver cytoplasm there are two small molecular weight DNA-binding proteins (20,000-30,000 daltons) which show high affinity for adenine-rich polynucleotides.

A special class of cytoplasmic proteins which bind to DNA *in vitro* can be isolated by either DNA-cellulose chromotography (1,2) or DNA-polyacrylamide chromotography (3). It is possible that the affinity of this class of proteins for DNA-cellulose or DNA-polyacrylamide is related to its *in vivo* function (4,5).

We report here a cytoplasmic DNA-binding protein which has a high affinity for adenine-rich nucleic acid in the presence of Mg^{+2}.

MATERIALS AND METHODS

Rat, bovine and porcine liver were excised from animals no more than 10 minutes after sacrifice and kept at 0°C for approximately one hour before homogenization in 0.15 M NaCl, 1 mM β-mercaptoethanol, 1 mM EDTA, 20 mM TRIS-HCl, pH 7.4 at 4°C in a Waring blender (3:1 v/w) for 1 1/2 min. All further steps were carried out at 4°C. In order to remove nuclei, mitochondria and plasma membranes the homogenate was centrifuged at 15,000 g for 30 min. and the lipid layer on top of the supernatant removed by aspiration. The supernatant was then centrifuged for 1 1/2 hours at 65,000 xg in a Beckman No. 30 rotor and the lipid layer again removed by aspiration from the supernatant. The supernatant was then centrifuged at 30,000 xg for 30 min to remove a light precipitate. The resulting clarified supernatant was made 10% in glycerol and applied either to a DNA-cellulose column or a phosphocellulose column equilibrated in 0.15 M NaCl-Buffer A (1 mM β-mercaptoethanol, 1 mM EDTA, 10% glycerol, 20 mM TRIS-HCl pH 7.4).

The DNA-cellulose columns were prepared by the method of Litman (6) using either rat or calf thymus double-stranded DNA prepared by the method of Brown and Weber (7). Whatman P-1 phosphocellulose washed 2 x with 1 N NaOH, 2 x with 0.1 N HCl, and deionized water to neutrality was titrated to pH 7.4 with 0.1 5 M NaCl-Buffer A.

The chromotography on DNA-cellulose columns was carried out by washing the columns with at least 3 column bed volumes of 0.15 M NaCl-Buffer A before and after protein sample was applied to the column, and DNA-binding protein were eluted with 0.6 M NaCl-Buffer A and 2 M NaCl-Buffer A. The phosphocellulose chromotography was carried out by washing the phosphocellulose extensively with 3 to 4 column bed volumes of 0.6 M NaCl-Buffer A, and the phosphocellulose-binding proteins were eluted with 2 M NaCl-Buffer A.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was carried out as described (8).

Poly dA-dT (MW > 100,000), Poly A (MW > 100,000) and Poly G (MW > 100,000) were obtained from P-L Biochemicals. Poly d(A,T) (MW > 100,000) was the kind gift of Dr. Henry Berger.

RESULTS AND DISCUSSION

The cytosol extract fractionated from a DNA-cellulose column by stepwise elutions with 0.15 M, 0.6 M and 2 M NaCl containing Buffer A showed no DNA unwinding activity with either native calf thymus DNA or poly dA-dT as substrates. This is in agreement with the report of Herrick and Alberts that the DNA-unwinding proteins do not bind to double-stranded DNA-cellulose (9). However, considerable precipitation occurred on the introduction of poly dA-dT into the 0.6 M NaCl fraction dialyzed into Buffer B (0.12 M NaCl, 1 mM β-mercaptoethanol, 0.1 mM EDTA, 2% glycerol, 10 mM $MgSO_4$ and 2 mM TRIS-HCl pH 7.4). The 0.6 M NaCl DNA-cellulose fraction completely binds to phosphocellulose and the poly dA-dT precipitating activity was found to be eluted by 2 M NaCl suggesting that the poly dA-dT precipitating protein has a strong affinity for phosphates. As shown in Fig. 1, the electro-

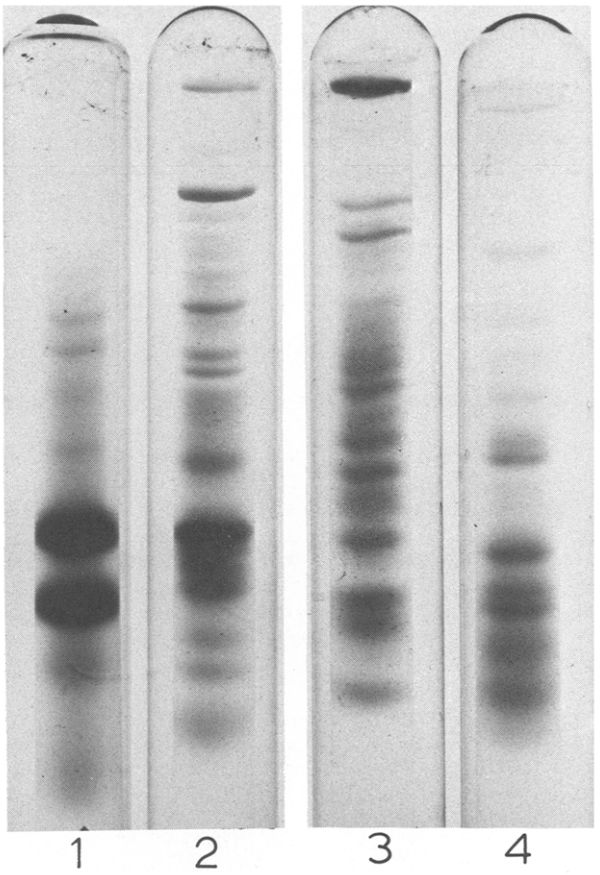

Figure 1 SDS gel electrophoretic patterns of DNA-cellulose and phosphocellulose binding proteins. (1) Rat liver DNA-binding proteins precipitated with poly dA-dT. The 0.6 M NaCl fraction was dialyzed against Buffer B before reaction. (2) Total rat liver DNA-binding proteins (0.6 M NaCl fraction). Phosphocellulose-binding proteins (2 M NaCl fraction) of bovine liver (3) and porcine liver (4).

phoretic patterns of the DNA-cellulose 0.6 M NaCl fraction and the phosphocellulose 2 M NaCl fraction obtained from cytosol extract reveal quite heterogeneous protein compositions for the respective fractions. The amount of protein eluted from DNA-cellulose by the 0.6 M NaCl-Buffer A is 0.3% of the total protein applied. Similarly the 2 M NaCl-Buffer A fraction of phosphocellulose contains 0.05% of the total protein applied.

The 0.6 M NaCl fraction from DNA-cellulose and 2 M NaCl fraction from phosphocellulose apparently precipitate preferentially adenine-rich poly-

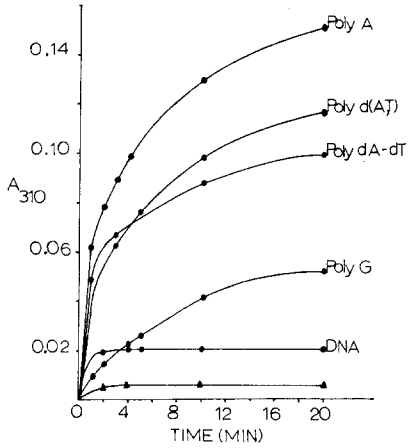

Figure 2 Turbidity formation of phosphocellulose-binding proteins and various polynucleotides as a function of time. The 2 M NaCl fraction from phosphocellulose (porcine liver) was dialyzed into Buffer B. 0.8 ml (126 µg total protein) of the dialyzed fraction was added to the cuvette in a Beckman DB spectrophotometer and allowed to sit for 10 to 20 min. The polynucleotides designated by the labels in the figure were added in volumes no greater than 100 µl (10 µg for poly A, poly d(A,T) poly dA-dT and native rat thymus DNA, 20 µg for poly G). The turbidity increases were followed by monitoring the absorbance at 310 nm. Curves with dots ●—● designate presence of 10 mM Mg^{+2} except curve for poly G which was 5 mM Mg^{+2}. The curve labeled ▲—▲ refers to turbidity formation by poly A, poly dA-dT and poly d(A,T) in the stated fraction in the absence of Mg^{+2}.

nucleotides. An experiment with the phosphocellulose fraction is shown in Fig. 2. It is clear that when an equal amount of various polynucleotides including double-stranded DNA is mixed with the phosphocellulose fraction the rate of formation of turbidity is much greater with poly A, poly dA-dT and poly d(A,T) than poly G and DNA. The formation of precipitates was also dependent on the presence of Mg^{++}. The order of turbidity formation seems to be poly A > poly dA-dT = poly d(A,T) >> poly G > DNA (double- and single-stranded).

The liver protein which is precipitated with adenine-rich polynucleotides seems to be mainly composed of two polypeptides as shown in Fig. 1 and 3. The same proteins seem to be involved in the precipitation of poly A, poly dA-dT and poly d(A,T). The same results were obtained with rat and porcine liver (not shown). The molecular weight of the two proteins is in the range of 20,000 and 30,000 daltons, and the two proteins have mobilities different from histones (not shown here).

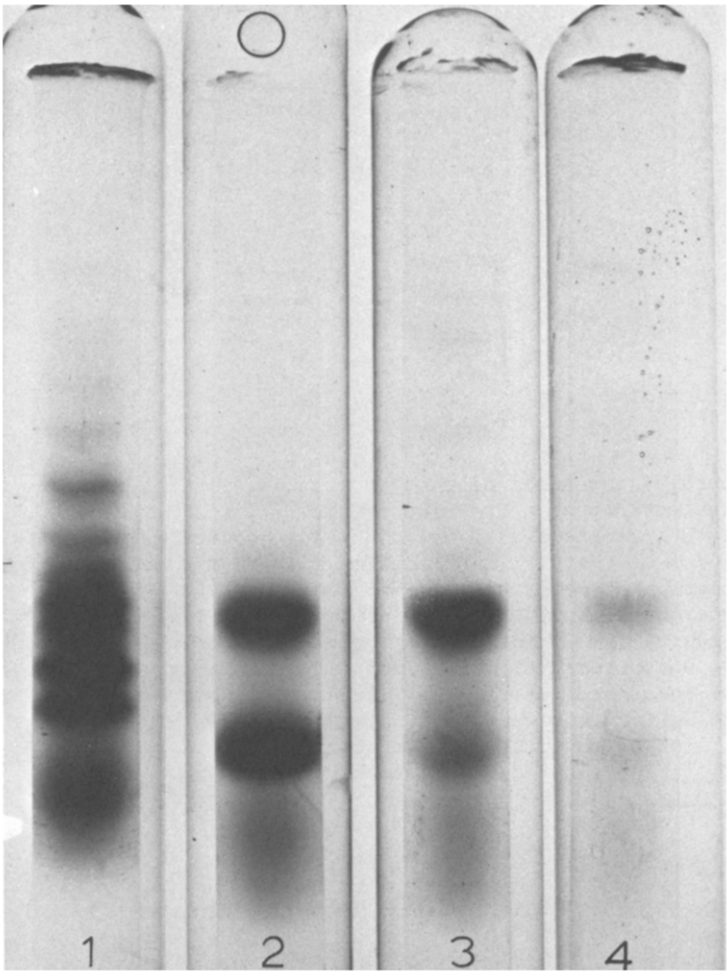

Figure 3 Phosphocellulose-binding bovine liver proteins precipitated with adenine-rich polynucleotides. (1) Total phosphocellulose-binding proteins (2 M NaCl fraction); proteins precipitated with poly A (2), poly dA-dT (3), and poly d(A,T) (4). SDS gel electrophoresis was from top to bottom.

The fact that the poly A-precipitating proteins bind more tightly to phosphocellulose than to DNA-cellulose is an indication of their rather high affinity for phosphate groups. Nevertheless there is some degree of specificity in the precipitation reaction occurring. The inability of the poly A-precipitating proteins to precipitate either poly G, and native or single-stranded DNA to the extent observed for the adenine-rich polynucleotides is evidence that the poly A-precipitating proteins prefer to bind to adenine-rich sequences. However, the relative inability of the proteins

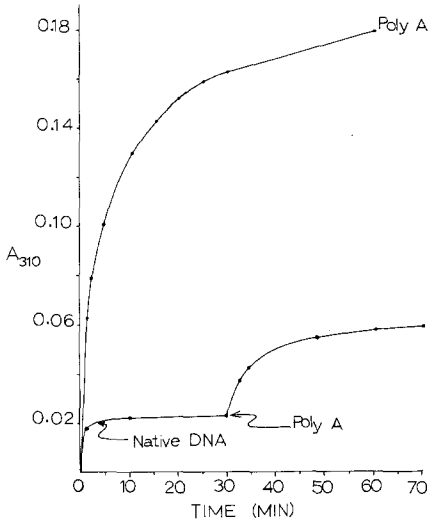

Figure 4 Competition of DNA and poly A for the poly A precipitating proteins. The 2 M NaCl fraction from phosphocellulose (porcine liver) was dialyzed into Buffer B, and 10 µg of native rat thymus DNA was added initially (lower curve) to 0.8 ml of 2 M NaCl fraction containing 126 µg total protein. At 30 min 10 µg of poly A was added and the resulting turbidity was followed by absorbance change at 310 nm. In the upper curve 10 µg of poly A was added to 0.8 ml of the 2 M NaCl fraction (126 µg protein).

to precipitate DNA may be due to the binding selectivity on DNA, perhaps binding to adenine-rich regions in DNA, leaving some phosphates open to water space. In the case with poly A, poly dA-dT or poly d(A,T) the binding proteins may saturate the phosphate groups due to recognition of the adenine-rich sequences present and thus cause the complex to lose its overall charge and solubility. The precipitation of histones and DNA at a mass ratio of 1:1 is explained on a similar basis (10). This contention is supported by several experiments one of which is shown in Fig. 4. A 2 M NaCl fraction (porcine liver) from phosphocellulose was dialyzed into Buffer B and mixed with native rat thymus DNA. The precipitation was minimal. After an incubation period poly A was added which caused some precipitation but the degree of precipitation was not nearly as great as when the poly A was added to another aliquot of the same fraction without the presence of DNA. One explanation for this kind of behavior is that the DNA decreased the avail-

ability of a portion of the precipitating proteins to bind to poly A. Work is in progress for isolation and characterization of this protein.

ACKNOWLEDGEMENTS

This work was supported by grants from the University of North Carolina (VC 427 and VC 333) and the American Cancer Society (IN15-0).

REFERENCES

1. Crippa, M. (1970) Nature 227, 1138-1140.
2. Tsai, R.L. and Green, H. (1973) J. Mol. Biol., 307-316.
3. Vaughan, S.T. and Comings, D.E. (1973) Exptl. Cell Research 80, 265-274.
4. Alberts, B. and Frey, L. (1970) Nature 227, 1313-1318.
5. Sigal, N., Delius, H., Kornberg, T., Gefter, M. and Alberts, B. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3537-3541.
6. Litman, R. (1968) J. Biol. Chem. 243, 6222-6233.
7. Brown, D. and Weber, C. (1968) J. Mol. Biol. 34, 681-697.
8. Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412.
9. Herrick, G. and Alberts, B. (1973) Fed. Proc. 32, 497 Abs.
10. Shih, T.Y. and Bonner, J. (1970) J. Mol. Biol. 48, 469-487.