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Deoxyribonuclease I-facilitated electrotransfer of protein-DNA complexes from electrophoretic gels to nitrocellulose membranes

A simple and reproducible technique for efficient transfer of protein-DNA complexes from electrophoretic gels to nitrocellulose membranes is described. Transfer of DNA-protein complexes may be difficult, especially when the DNA is of high molecular mass. A considerable improvement in the efficiency of transfer can be achieved by directly digesting the DNA in the gel by DNase I. The method is illustrated in the example of the preferential binding of histone H1 to superhelical plasmid DNA.

Antibody binding is a useful method to identify and quantify proteins in DNA-protein complexes. Complexes separated by agarose or polyacrylamide gel electrophoresis must be transferred to nitrocellulose membranes in order to perform the antibody staining. This transfer may be difficult, especially when the DNA is large, as is the case with plasmid molecules. Here we report a simple procedure which facilitates the transfer, illustrated by the preferential binding of histone H1 to superhelical DNA. Earlier filter binding studies [1, 2] and more recent gel retardation assays [3, 4] have shown that histone H1 binds more efficiently to supercoiled than to relaxed circular DNA. In an attempt to extend these studies further, including linear DNA in the comparison, H1-DNA complexes were formed with an equimolar mixture of supercoiled, nicked circular, and linear DNA molecules. When these complexes were separated electrophoretically on agarose gels, the only DNA form to be retarded was the superhelical one. This apparent preference for supercoiled DNA could be real, *i.e.*, a result of

preferential binding of the histone to this DNA form; alternatively, H1 could bind all forms equally well, but affect the electrophoretic mobility of only the superhelical form by significantly altering its conformation. In order to distinguish between these two possibilities, we attempted an immunochemical quantitation of the amount of protein bound to each form. The conventional methods for electrotransfer to nitrocellulose membranes [5] were inefficient in transferring the material for the immunoreaction. Attempts to reduce the molecular weight of the DNA by irradiation of ethidium bromide-stained gels before the transfer improved the transfer efficiency only slightly. The problem was overcome by partially digesting the DNA with deoxyribonuclease I (DNase I) directly in the gel.

The gel shown in Fig. 1 was stained with ethidium bromide and photographed. One half of the gel was then gently shaken in 10 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, containing 10 units of DNase I (Sigma) per mL, for

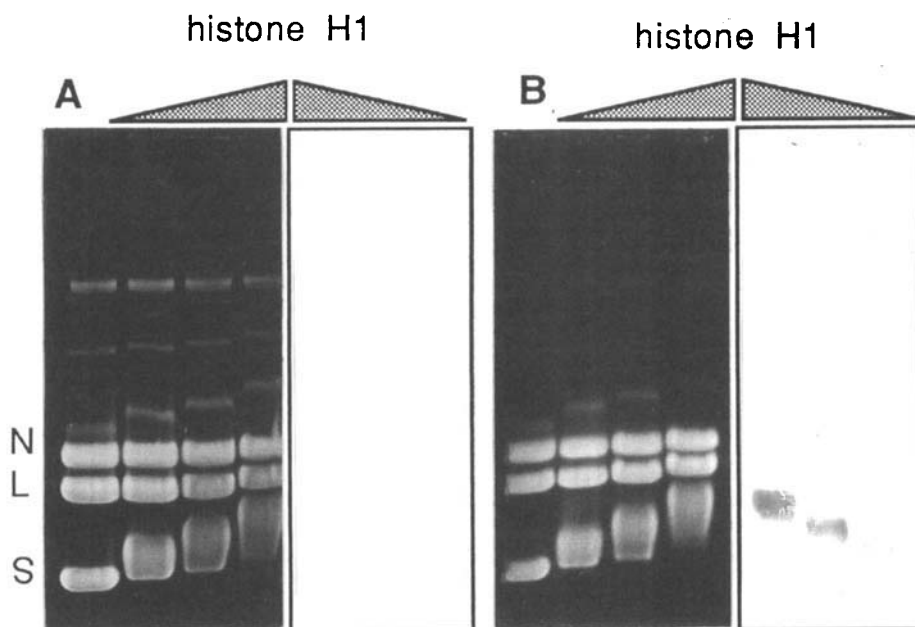


Figure 1. Selective binding of H1 to highly supercoiled DNA. A mixture of supercoiled, nicked and linear pBR322 in roughly equimolar amounts was prepared and incubated for 15 min at room temperature with chicken erythrocyte histone H1 [7] in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10 mM NaCl, and 50 µg of bovine serum albumin/mL. Supercoiled pBR322 was purified by centrifugation in the presence of CsCl [8]. Nicked DNA was prepared as in [9], and the linear form was obtained by *Pst*I cutting. The protein-DNA complexes were electrophoresed in 1% agarose gels in 40 mM Tris-acetate buffer [8]. Next, the proteins were transferred to nitrocellulose membranes without (A) and after brief DNase I digestion (B), and visualized by immunostaining. Left panels in (A) and (B) show the ethidium bromide stained gel, while right panels show the antibody-stained nitrocellulose membrane. S, L, and N denote the position of supercoiled, linear and nicked form of pBR322, respectively. For each lane 1.5 µg of DNA was used; the protein/DNA ratios were 1/400 bp, 1/200 bp, and 1/100, respectively.

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10–15 min at room temperature. The intensity of the DNA bands was slightly reduced and they appeared less sharp at the end of the incubation, indicating partial digestion of the DNA. The two halves of the gel were impregnated with transfer buffer (twofold diluted SDS-containing running buffer of Laemmli [6], containing 15% methanol) for 15 min and the transfer to nitrocellulose membranes (BA83, Schleicher and Schuell) was performed overnight in the cold room at 24 V, using the Idea Scientific Company (Minneapolis, MN) system for protein transfer. The protein-containing bands on the nitrocellulose sheet were visualized using specific rabbit anti-H1 antibody and alkaline phosphatase-conjugated anti-rabbit secondary antibody (Promega). From the intensity of immunostaining (Fig. 1B) calibrated by using known amounts of protein directly dotted onto the membrane and from the known protein content of the complexes, it was possible to estimate that the protein was transferred without any significant loss when the DNase I treatment was included in the protocol.

The results indicated that at H1/DNA ratios of up to one molecule of H1 per ~ 150 bp of DNA (which corresponds to the physiological ratio in chromatin), the histone binds highly selectively to the supercoiled DNA form. The same transfer protocol was successfully applied to study the distribution of several different proteins in fractionated chromatin (not shown), and for transfer of proteins following electrophoresis of cross-linked H1/DNA complexes in SDS-containing polyacrylamide

gels. An additional wash of the gel in the DNase I buffer was necessary in order to get rid of the SDS before the addition of the enzyme in the latter case.

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