Transfer of Small Plasmid DNA Fragments from Polyacrylamide Gels onto Nitrocellulose Paper

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The incorporation into a 7% polyacrylamide gel of a nucleic acid-specific photochemical reagent 4,5′,8-trimethylpsoralen at a concentration of 1 mg/dl of acrylamide solution improves both the sensitivity and the efficiency of the transfer of plasmid DNA fragments from the gel onto nitrocellulose filters. This improvement allows detection by blot hybridization of DNA fragments as small as 57 bp long.

KEY WORDS: Southern transfer; blot hybridization; psoralen; plasmids; DNA; restriction enzymes.

The blot-hybridization method (1) is among the more extensively used techniques in nucleic acid research. This technique is limited in detecting DNA fragments smaller than 400 base pairs (bp), however. We show in this report that the incorporation of a psoralen derivative, 4,5′,8-trimethylpsoralen (TMP),[1] in the resolutive gel improves the transfer of 50- to 500-bp-long plasmid-DNA fragments onto nitrocellulose filters.

Psoralen and its derivatives are furocoumarins which intercalate with DNA in the dark in a manner similar to other polycyclic aromatic hydrocarbons such as acridine orange, ethidium bromide, or actinomycin. When intercalation is followed by near-uv light irradiation (UV-A; $\lambda \sim 360$ nm), the excited psoralen molecules then bind covalently to the pyrimidine bases of the DNA (2,3). Psoralens can form complexes not only with double-stranded DNA, but also with RNA or single-stranded DNA (3).

The psoralens have been used clinically for treatment of patients with photosensitive or pigmentation skin disorders (4), or for diseases characterized by proliferative cell growth, i.e.,

in psoriasis. In DNA research they constitute useful photochemical probes for studying chromatin structure (5,6) or examining the dynamics of secondary structure in DNA and RNA (7–9).

MATERIALS AND METHODS

Samples preparation. pBR 322 plasmid (BRL Laboratories) was cut with *Hae*III restriction endonuclease (Lyphozyme from BRL) according to the manufacturer's recommendations; 10-ng samples of *Hae*III-digested pBR 322 were submitted to electrophoresis, together with 20 μg of sonicated carrier DNA (calf thymus or salmon sperm DNA, Sigma Chemical Co.).

Polyacrylamide gel electrophoresis. Electrophoresis was performed on 20 × 15 × 0.3-cm vertical polyacrylamide gels (T = 7%; c = 5%) in a 50 mM Tris–HCl, 50 mM borate buffer, pH 8.3, containing 2 mM EDTA, at 750 to 950 V-h. The procedure was performed in ambient light since the glass plates filtered out activating light. TMP (Trioxsalen, Sigma Chemical Co.), in alcoholic solution (120 μg ml⁻¹) for amounts smaller than 1 mg or as powder for amounts greater than 1 mg, was

[1] Abbreviation used: TMP, 4,5′,8-trimethylpsoralen.

dissolved in the acrylamide solution just before polymerization at concentrations ranging from 200 µg to 12 mg TMP/dl of acrylamide solution. Extreme caution should be used in handling TMP due to its potent mutagenicity and carcinogenicity.

Gel treatment and transfer. After electrophoresis the DNA fragments were denatured by soaking the gel in a 0.5 M NaOH, 1.5 M NaCl solution for 30 min, followed by neutralization in 1 M Tris–HCl, 3 M NaCl, pH 7.4, for 30 min. These two steps (alkaline denaturation and neutralization of the DNA fragments) were carried out in the dark. The gels were then exposed to ambient laboratory light for various periods of time before the blotting procedure, which was performed by electroelution of the DNA fragments onto nitrocellulose filters (Schleicher and Schuell; µ = 0.1 µm) in a Trans-Blot cell (Bio-Rad Laboratories) or comparable apparatus at 30 V for 3 h; the electrotransfer buffer was 50 mM Tris–HCl, 50 mM borate, pH 8.3, containing 2 mM EDTA. Ethidium bromide staining must be done with a guide strip or on a second electrophoresis. After denaturation and neutralization in the dark, the gels were exposed to ambient light for varying periods of time while being loaded into the holder in which they are pressed against the nitrocellulose membrane. Electroelution is then done in ambient light.

Hybridization and autoradiography. Baking, prehybridization, hybridization, and washing of the filters was done according to Jeffreys and Flavell (10). As a probe, 100 ng of nick-translated pBR 322 plasmid with a specific activity of 1.0 to 2.0 × 10^8 cpm µg^{-1} were used per filter. The position of the bands was revealed by autoradiography using X-Omat AR-Xray films (Kodak) and intensifying screens (Cronex X-ray screens, Dupont).

RESULTS

In Figure 1 are presented the autoradiographs of *Hae*III-digested pBR 322 showing a comparison of the results obtained without TMP (Fig. 1A) and with increasing amounts of TMP incorporated in the polyacrylamide gels (Figs. 1B–D). Incorporating TMP into the polyacrylamide gels before electrophoresis clearly has an effect on the pattern obtained upon autoradiography; the optimal results (i.e., more intense as well as smaller detectable bands) are obtained when TMP is used at a concentration of 1 mg/dl of acrylamide solution (Fig. 1D). Further increasing the TMP concentration, from 2 to 12 mg TMP/dl of acrylamide solution (the last value corresponding to the solubility limit of TMP in aqueous medium) leads to patterns less intense than Fig. 1D to barely visible under the same conditions of exposure (data not shown).

In Figure 2, the DNA fragments were submitted to 7% polyacrylamide gel electrophoresis in the presence of 1 mg TMP/dl of acrylamide solution, with varying times of exposure of the gels to ambient light between the end of the denaturation–neutralization steps (carried out in the dark) and the electroblotting of the fragments. The optimal exposure is 30 s to 1 min (Figs. 2C and D). The shortest observable fragments are 57, and occasionally 51, bp long. Although near-uv irradiation facilitates covalent cross-linkage of DNA fragments, psoralen in the complete absence of light mediates the reaction slightly, giving more intense and more complete patterns than in the case of experiments carried out without the presence of TMP.

In an attempt to detect smaller size fragments (for example, the 21-bp fragment present in the *Hae*III-digest of pBR 322), we used 10 to 12% polyacrylamide gels, but the smallest detectable fragments remained in the 51 to 57-bp range. Under these conditions, the observed patterns were always suboptimal.

Adding carrier DNA (20 µg of sheared calf thymus or salmon sperm DNA) to the digested plasmid when loading the samples for electrophoresis enhanced the intensity of the patterns obtained upon autoradiography, which appeared to be true whether TMP was used or not.
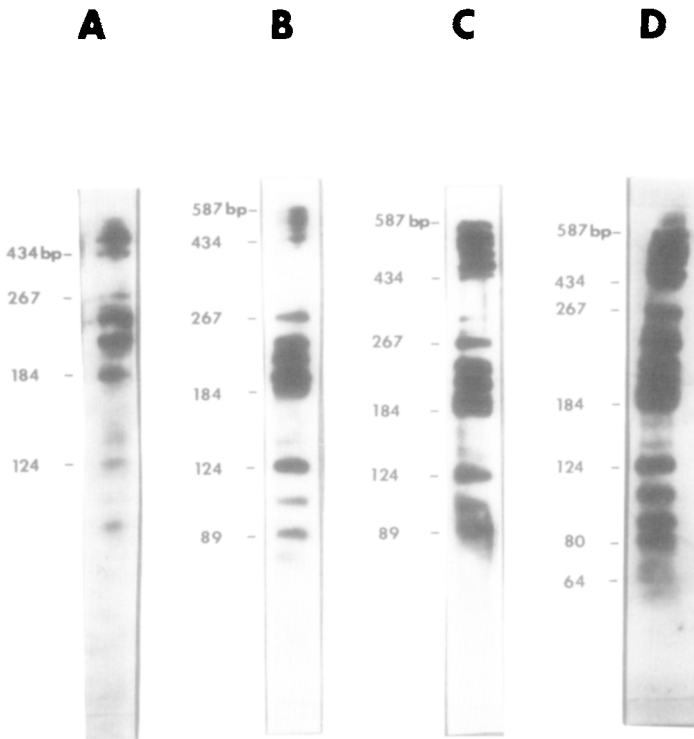
A   **B**   **C**   **D**

FIG. 1. Autoradiographs of *Hae*III digests of pBR322 submitted, together with 20 μg of sheared calf thymus DNA, to electrophoresis in a 7% polyacrylamide gel. (A) without TMP; (B), (C) and (D) with 250 μg, 400 μg, and 1 mg TMP/dl acrylamide solution, respectively. Electrotransfer was performed as described in the text after exposure of the gels to light for 30 s. Autoradiographs were exposed for 6 h.

DISCUSSION

We have established that the use of TMP in a 7% polyacrylamide gel improves the electroblotting of plasmid DNA fragments, leading to more intense patterns upon autoradiography and allowing visualization of fragments as small as 57 bp long. Optimal results are obtained when DNA fragments are submitted to electrophoresis in a gel containing 1 mg TMP/dl of acrylamide solution (this step is carried out in ambient light), denatured and neutralized in the dark, and then submitted to ambient light for 30 s to 1 min before being electrotransferred. The last step can be performed at ambient light. Photography after electrophoresis is avoided; a parallel electrophoresis is required if this is desired.

These results do not allow us to explain the mechanism of action of TMP in these experiments and we can only propose some hypotheses. TMP, for example, does not seem to act during the course of electrophoresis since discrete bands are obtained and the patterns are similar whether TMP is used or not. One possible mechanism is that small fragments are cross-linked into larger networks that transfer efficiently and bind to the nitrocellulose membrane. This mechanism is supported by the fact that excess psoralen, thereby increasing the size of the network, appears to diminish the efficiency of the procedure. Similarly, excessive exposure to light may produce networks too large to transfer. We cannot exclude the possibility that 3,4-monocycloadducts are formed between TMP and partially denatured DNA fragments in the polyacrylamide gel upon light irradiation; these monocycloadducts might then transfer and the other reactive group (4′,5′-C = C bonds) of
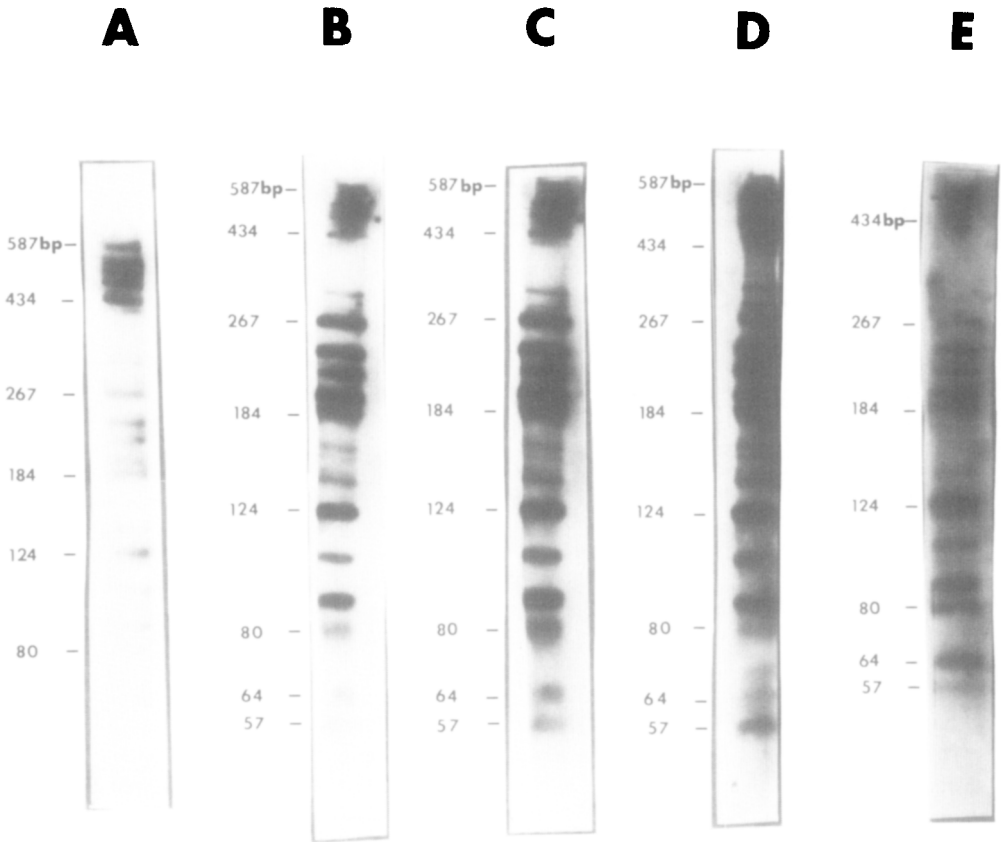
FIG. 2. Six-hour exposed autoradiographs of HaeIII-digested pBR322 submitted, together with 20 μg of sheared calf thymus DNA, to electrophoresis in a 7% polyacrylamide gel containing 1 mg TMP/dl acrylamide solution. Electrotransfer was performed after exposure of the gels to light for 0 s (A), 10 s (B), 30 s (C), 1 min (D), and 10 min (E).

TMP bind with the nitrocellulose matrix. Darkness appeared to be essential only during the denaturation–neutralization steps. Most likely that is because the glass plates and plastic apparatus in which the electrophoreses and electrotransfer are performed filtered out the near-uv light.

All hypotheses remain open and require further investigation. The aim of this report is to present a simple method allowing the visualization of 50- to 500-bp-long plasmid-DNA fragments.

REFERENCES

1. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
2. Cole, R. S. (1970) *Biochim. Biophys. Acta* **217**, 30–39.
3. Song, P. S., and Ou, C. N. (1980) *Ann. N. Y. Acad. Sci.* **346**, 355–367.
4. Fitzpatrick, T. B., Arndt, K. A., El Mofty, A. M., and Pathak, M. A. (1966) *Arch. Dermatol.* **93**, 589–600.
5. Wiesehahn, G. P., Hyde, J. E., and Hearst, J. E. (1977) *Biochemistry* **16**, 925–932.
6. Cech, T., and Pardue, M. L. (1977) *Cell* **11**, 631–640.
7. Cassuto, E., Gross, N., Bardwell, E., and Howard-Flanders, P. (1977) *Biochim. Biophys. Acta* **475**, 589–600.
8. Schen, C. K., Hsieh, T. S., Wang, J. C., and Hearst, J. E. (1977) *J. Mol. Biol.* **116**, 661–679.
9. Cantor, C. R. (1980) *Ann. N. Y. Acad. Sci.* **346**, 349–356.
10. Jeffreys, A. J., and Flavell, R. A. (1977) *Cell* **12**, 429–439.