Separation of carcinogen-damaged DNA fragments from undamaged DNA

Paul T. Strickland and Cristl Gentile

Department of Environmental Health Sciences, The Johns Hopkins Medical Institutions, 615 North Wolfe Street, Baltimore, MD 21205, USA

Submitted October 21, 1991

Immunological methods employing antibodies specific for carcinogen-damaged DNA have been used to detect DNA damage in human and animal cells (1, 2). These antibodies also provide a potential approach to examining the distribution of carcinogen damage within the genome. We have developed a separation procedure based on immunoprecipitation to separate carcinogen-damaged DNA fragments from undamaged DNA. The procedure involves (i) digestion of carcinogen-damaged DNA with restriction enzyme, (ii) precipitation of complexes between damage-specific monoclonal antibodies and carcinogen-damaged DNA fragments using anti-mouse secondary antibody under conditions where undamaged DNA fragments do not precipitate, and (iii) electrophoretic fractionation of damaged-DNA fragments on agarose gels. Fragments can subsequently be identified by Southern transfer to nitrocellulose and gene-specific DNA hybridization. The procedure has been demonstrated using a monoclonal antibody specific for UV-induced DNA photoproducts to separate damaged and undamaged fragments of UV-irradiated DNA (Figure 1) in a dose-dependent manner. This approach should also be useful in separating DNA damaged by chemical carcinogens when antibodies specific for chemical carcinogen-modified DNA are used.

Figure 1. DNA samples (25 μ g) irradiated with UVC (0, 1, 3, 10 KJ/m^2) were digested with EcoRI under standard conditions, heat-denatured, and incubated with mouse monoclonal antibody (0.05, 0.5, or 5 μ g) specific for UV-induced DNA photoproducts (3). Antibody-UV-DNA complexes were precipitated by incubation with anti-mouse IgG secondary antibody (Sigma). After 18 hrs at 4°C, precipitate was pelleted (25 min at 3000 g) and resuspended in Tris-EDTA. Supernatant and resuspended pellet samples were heat-denatured (5 min at 90°C) and clarified by centrifugation (5 min at 3000 g). Gel sample buffer (4 μ l) was added to clarified supernatant (25 μ l) and pellet samples, which were fractionated by agarose gel (1%) electrophoresis in the presence of ethidium bromide (0.5 μ g/ml). Lanes 1–12; immunoprecipitated pellets. Lanes 13–24; supernatants. Lanes 1–3, 13–15; unirradiated. Lanes 4–6, 16–18; irradiated with 1 KJ/m^2 UVC. Lanes 7–9, 19–21; 3 KJ/m^2 . Lanes 10–12, 22–24; 10 KJ/m^2 . Lanes 1–3, 4–6, 7–9, 10–12, etc; samples precipitated with 5, 0.5, or 0.05 μ g antibody.

ACKNOWLEDGEMENT

Supported in part by NIH award AR38884.

REFERENCES

1. Strickland, P.T. and Boyle, J.M. (1984) *Prog. Nucleic Acids Res. Mol. Biol.* **31**, 1–58.
2. Bartsch, H., Hemminki, K. and O'Neill, I.K. (1988) *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*. IARC Sci. Publ. No. 89, Lyon.
3. Strickland, P.T. and Boyle, J.M. (1981) *Photochem. Photobiol.* **34**, 595–601.