Volume 230, number 1,2, 209–211 FEB 05718 March 1988

The extraction of high-molecular-mass DNA from hair shafts

Arnd Schreiber, Eberhard Amtmann*, Volker Storch and Gerhard Sauer*

Zoologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 230, D-6900 Heidelberg and **Institut für Virusforschung, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG*

Received 27 January 1988

A simple and efficient method is presented for the extraction of DNA from hair shafts. DNA preparations obtained by this approach can be made amenable to restriction enzyme digestion, thereby allowing further molecular biological analysis.

DNA extraction; Keratinization; Hair shaft

1. INTRODUCTION

In contrast to the extensive knowledge about the protein biochemistry of keratinization and hair formation [1,2] little is known about the fate of nucleic acids during these processes. It has been suggested that both DNA and RNA completely degenerate in the initial phase of keratinization of hair follicle cells [3]. Gill et al. [4] failed to extract DNA for forensic paternity analysis from human hairs.

Here we present a technique for the extraction of high-molecular-mass DNA from clipped hair shafts. This approach is not very time-consuming and we suggest that it can be used to extract DNA for forensic purposes, as well as to obtain DNA from museum specimens of extinct species for various scientific approaches. The hitherto published extraction methods of DNA from the extinct quagga [5] or from ancient Egyptian mummies [6] were possible only on account of the fortuitous case of unkeratinized tissue having been preserved; this restricts the scope of these technical approaches.

Correspondence address: A. Schreiber, Zoologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 230, D-6900 Heidelberg, FRG

2. EXPERIMENTAL

2.1. *Extraction technique*

For DNA extraction essentially the method described by Krieg et al. [7] was used with modifications which are reported in section 3.

2.2. *DNA-filter hybridization*

After agarose-gel electrophoresis, DNA-transfer to a nitrocellulose sheet was performed according to the method of Southern [8]. Labelling of DNA for filter hybridization with ^{32}P-nucleotides occurred according to Rigby et al. [9]. After preincubation the filters were hybridized with 1×10^6 cpm overnight at 68°C. Washing was carried out at 60°C, using the following solutions: 5 × Denhardt and 0.1% SDS in 3 × SSC for 6 × 10 min, 0.1% SDS in 0.1 × SSC for 20 min and 3 × SSC for 3 × 10 min. Afterwards the filter was exposed to a diagnostic film (Kodak X-Omat) for 4 h.

2.3. *Agarose-gel purification*

After electrophoresis in 1.4% agarose gels (36 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA as gel and electrode buffer) those small pieces of the gel which contained the ethidium bromide-stained high molecular DNA were cut out and frozen at −70°C. The DNA was eluted from the gel by centrifugation through a glass fibre sheet.

3. RESULTS

The method of Krieg et al. [7] for the extraction of nucleic acids from solid tissue is based on the pulverization of the sample material with a so-called micro-dismembrator. Liquid nitrogen is used to freeze the sample and to pre-cool both a

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

metal grinding ball and the teflon chamber into which the ball and the frozen material are being transferred. Then the chamber is subjected to vibrations of 50 Hz. Hair shafts of most species turned out to be extraordinarily resistant to mechanical disruption, and complete pulverization was only possible after extended times of vibration which resulted in insignificant yields of low-molecular-mass DNA. Only lyophilization of the hair shafts and the use of grinding balls made of wolfram carbide led to a substantial shortening of the extraction time and to a distinctly increased yield. Freeze-drying (24 h in a Freezemobile 6, manufactured by Virtis Comp.) of small (5 mm) pieces of hair was accomplished after repeated (4 ×) ethanol washings.

The time required for homogenization depended on the species. Usually 60 s were found to be necessary for 200 mg of lyophilized hairs, an amount which fills about one half of the capsule. The hair powder was mixed without delay with a solution containing 10 ml of 66% phenol (in 0.3 M Na acetate, pH 5.5, equilibrated overnight with 1% hydroxychinoline) and 5 ml lysis buffer (0.3 M Na acetate, 0.5% SDS, 5 mM EDTA). After rapid shaking for 15 min, chloroform/isoamyl alcohol (25:1) was added. After shaking for another 15 min and centrifugation (10 min, 4°C, 1100 × *g*) the aqueous upper phase was transferred to a new vial and again shaken with 10 ml chloroform/isoamyl alcohol. After another centrifugation step the DNA in the aqueous upper phase was precipitated in 70% ethanol. After centrifugation (Eppendorf centrifuge, 40 min, 14000 rpm) the nucleic acid pellet was washed in 70% ethanol and dissolved in TE-buffer (20 mM Tris, pH 7.5, 1 mM EDTA).

High-molecular-mass DNA, as is exemplified in fig.1a could be extracted from hair shafts of species with different structural hair types [10,11]. After DNase digestion, no ethidium bromide signals were found. The identity of the signals in the agarose gels, which we found after extracting clipped hair tips of cattle with bovine DNA was shown using ^{32}P-labelled nick-translated calf thyroid tissue culture cell DNA under conditions of high stringency (not shown).

For further analyses it was warranted to demonstrate the cleavability of the DNA from hair with restriction enzymes. One problem that was frequently encountered probably rested in the contamination with pigments and other substances. The degree of contamination depends on hair colouration and species. This can be circumvented by agarose-gel purification, which renders the DNA accessible for restriction enzyme digestion as is documented in fig.1c and d. The cleavage patterns of satellite DNA from cattle tissue culture cells and calf hair tips correspond to each other.

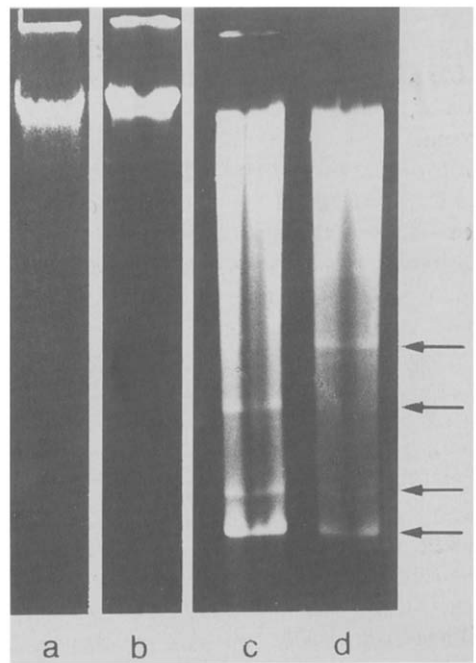

Fig.1. (a) Uncleaved DNA from clipped calf hairs after gel purification. (b) Uncleaved DNA extracted from bovine tissue culture cells. (c) Bovine cellular DNA after *Eco*RI-restriction digestion. (d) *Eco*RI-cleaved DNA obtained from calf hair shafts. The arrows mark the cleavage patterns of satellite DNA.

To our knowledge, this is the first demonstration of the persistence of high-molecular-mass DNA within cells which die in the course of their normal differentiation.

4. DISCUSSION

The above presented extraction method has been reproduced in several species, including man, vervet monkey, domestic sheep, wild boar, musk ox, horse and swamp wallaby. As up to 1 μg high-molecular-mass DNA could be obtained from

200 mg hairs, it appears certain that the approach can also be applied to extract nucleic acid from valuable specimens of extinct species for further molecular analysis.

Repeated washing of the hairs with an aqueous solution of the detergent SDS drastically reduced the yield. This could be due to leaching of the molecules out of the keratinized cells. The amount of extractable DNA was not diminished by ethanol treatment. However, in sheep wool extensive ether washings were found to remove extractable nucleic acid. Therefore, we cannot as yet exclude that at least some of the DNA resides within the lipid-rich epidermal detritus on the hair surface which contains skin flakes and dandruff. Downes et al. [3] labelled hair follicle DNA of sheep by intradermal injection of [^{3}H]thymidine. They established that all nucleic acids degrade during keratinization. As their scintillation counting measurements were only continued until 7 days after labelling, they were not able to test the possibility of nucleic acids being externally transferred to the pelage, because cell cycle rates in sebaceous glands and the germinative layer generating skin flakes are distinctly slower [12,13]. DNA or RNA are not mentioned in recent reviews on the biochemical composition of the skin lipid film or sebum [14].

Ultra-thin transverse sections of human hairs fixed in glutardialdehyde, dehydrated and embedded in an epoxyresin following the method of Spurr [15] could be stained with ethidium bromide (not shown). Although the staining patterns as discerned in the UV fluorescence microscope appeared to be confined to the keratin-poor regions of the transverse sections, unspecific staining cannot be excluded in a crystalloid dead tissue. Thus, the exact histochemical localization of the DNA within clipped hair remains to be investigated.

Acknowledgement: The financial support granted by the Hessisches Ministerium für Landwirtschaft und Forsten (grant number I A4-H110955-68571) is gratefully acknowledged.

REFERENCES

[1] Powell, B.C. and Rogers, G.E. (1986) in: Biology of the Integument (Bereiter-Hahn, J. et al. eds) vol.2, pp.695–721, Springer, Berlin.
[2] Goldsmith, L.A. (1983) Biochemistry and Physiology of the Skin, University Press, Oxford.
[3] Downes, A.M., Chapman, R.E., Till, A.R. and Wilson, P.A. (1966) Nature 212, 477–479.
[4] Gill, P., Jeffreys, A.J. and Werrett, D.J. (1985) Nature 318, 577–579.
[5] Higuchi, R., Bowman, B., Freiberger, M., Ryder, O. and Wilson, A. (1984) Nature 312, 282–284.
[6] Pääbo, S. (1985) Nature 314, 644–645.
[7] Krieg, P., Amtmann, E. and Sauer, G. (1983) Anal. Biochem. 134, 288–294.
[8] Southern, E.M. (1975) J. Mol. Biol. 98, 503–517.
[9] Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237–251.
[10] Waldeyer, W. (1884) Atlas der menschlichen und tierischen Haare sowie der ähnlichen Fasergebilde, Schauenburg Verlag, Lahr.
[11] Biedermann, W. (1928) Das Haarkleid der Säugetiere, Erg. Biol. 4, 361–680.
[12] Plewig, G. and Kligman, A. (1975) Acne – Morphogenesis and Treatment, Springer, Berlin.
[13] Weinstein, G.D. (1974) J. Invest. Dermatol. 62, 144–146.
[14] Pochi, P.E. (1985) in: Dermatology (Moschella, S.L. and Hurley, H. eds) pp.75–78, Saunders, Philadelphia.
[15] Spurr, A.R. (1969) J. Ultrastr. Res. 26, 31–43.