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Molecular analysis of in vitro damaged DNA samples

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Abstract. A single DNA sample underwent controlled damaging treatments represented by UV radiations, free radicals, formaldehyde, reactive oxygen species (ROS), metal ions radical starters such as Fe^{3+} and Cu^{2+}, HCl, and NaOH. Capillary electrophoresis showed that most of the treatments produced chemical alteration of the primary structure of the DNAs. In addition, both Alu probing and real-time PCR provided data lower than the controls. Both STR typing and Y-specific SNPs analysis were influenced by the altered composition of the templates. The results of this study indicate that more information on the molecular composition of the template can be extremely useful for the evaluation of the PCR-based evidences. © 2006 Published by Elsevier B.V.

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1. Introduction

DNA is a macromolecule whose integrity can be altered both by enzymatic and physical/chemical processes [1]. DNA typing is performed, in any field of Science, by PCR, an error-prone method of DNA amplification [2]. PCR fidelity is affected by several factors, with the integrity of the primary structure of the template playing the major role. As far as regards the biological samples usually tested in the Forensic Laboratory, these have been shown to exhibit a wide range of DNA decay which degree, however, is not

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related to the aging of the specimen [3,4]. In order to investigate how common environmental compounds (reactive oxygen species, metal ions, UV radiation, acidic and basic pH) can interfere with the reliability of genetic analyses, controlled damaging treatments were carried out on a single DNA sample. The treated sample was subsequently analysed by conventional methods. The results show that even trivial treatments can make the DNA molecule an unsuitable source of genetic information.

2. Materials and methods

A single DNA preparation was carried out by phenol/chloroform method from the buffy coat obtained from 500 ml of fresh blood of a male donor. After quantification by spectrophotometry at OD_{260}/OD_{280} nm, this sample was aliquoted in Eppendorf tubes each containing 26 μg of DNA in a volume of 100 μl. The samples were then treated, in duplicate, with the agents listed in Table 1. After ethanol precipitation each sample was redissolved in 100 μl of H_2O. Suitable dilutions of the same sample were then quantified by the following methods: EtBr staining [4], Alu probing [4] and real time PCR [5]. In addition, CE (capillary electrophoresis) was performed in 60 μl of each sample employing 50 nM of ^{5}BrU as internal standard following the method describe elsewhere [6]. At least, STR typing was performed from comparable amounts of the samples following a "single-shot" approach (see Table 1 for more details). The Y-specific SNPs analysis was performed according to Briòn et al. [7].

Table 1

Sample	Treatment	EtBr	Alu	CE	RT–PCR	1° STR	2° STR	Y-SNPs
4	UV	+++	++	0.65	0.91	13/15; 1d.o.	15/15	+
8	–	+++	+++	0.66	1.08–1.36	15/15	15/15	+
14	Formal.	+	+	0.22	0.19–0.27	0/15	5/15	–
20	HCl	+++	++	0.49	0.71	13/15; 1d.o.	14/15 1d.i.	+
26	H_2O_2	+++	++	0.43	0.26	14/15	15/15	+
32	Fe	+++	++	0.58	1.06	14/15	15/15	+
39	Cu	+++	++	0.59	0.78	14/15; 1d.o.	15/15	+
44	H_2O_2+Cu	+++	++	0.48	0,39	14/15; 1d.o.	15/15	+
48	–	+++	+++	0.66	0.94–1.04	15/15	15/15	+
49	–	+++	+++	0.67	0.63–1.17	15/15	15/15	+
54	H_2O_2+Fe	n.d.	n.d.	0.55	0.01–0.02	0/15	0/15	–
60	NaOH	+++	++	0.66	0.77	14/15 1d.i.	15/15	+

Treatments: UV=UV radiation at 254 nm for 5 min at the distance of 5 cm (in ice); –=control sample; Formal.=3% formaldehyde for 5 min; HCl=1% HCl for 5 min; H_2O_2=3% H_2O_2 for 5 min; Fe=$FeCl_3$ (10 nM) for 5 min; Cu=$CuSO_4$ (10 nM) for 10 min; H_2O_2+Cu=3% H_2O_2+$CuSO_4$ (10 nM) for 5 min; H_2O_2+Fe=3% H_2O_2+$FeCl_3$ (10 nM) for 5 min; NaOH=NaOH (10 nN) for 5 min. **EtBr:** DNA quantification performed by spot agar; +++=1.00–0.75 of the expected value (e.v.); ++=0.75–0.50 of e.v.; +=<0.50 of e.v.; n.d.=no detectable. **Alu:** DNA quantification performed by slot blot hybridisation: +++=1.00–0.75 of the expected value (e.v.); ++=0.75–0.50 of e.v.; +=<0.50 of e.v.; n.d. =not detectable. **CE:** the data of this column show the ratios obtained by the following formula: area of the four DNA bases/total area of the effluents. Analysis performed by quantitative Capillary Electrophoresis. **RT–PCR:** DNA quantification performed by real time PCR using the Quantifiler™ Human Quantification Kit (Applied Biosystem). The data are given in ng/μl. Samples 8, 14, 48, 49 and 54 were analysed by replicate tests (the higher and lower values are provided). **1° STR:** The ratio indicates the number of loci successfully typed by employing the AmpF*l*STR Profiler Plus™ (Applied Biosystem) for 32 cycles of PCR. As template, for each sample, a volume containing, before the treatments, 500 pg of DNA was employed. **2° STR:** Results of the STR typing of 500 pg of DNA as assessed by real-time PCR. **Y-SNPs:** Results obtained by the employment of the 29 Y-chromosome SNPs multiplex system. 35 cycles of PCR were performed from a volume containing, before the treatments, 8 ng of DNA. +=indicates that >15 out of 29 loci were successfully typed so allowing the definition of the haplogroup; –=indicates that <5 out of 29 loci were typed.

3. Results

The first remarkable effect of the treatments employed is a reduced susceptibility/ sensitivity both to the EtBr staining and Alu probing (Table 1). In addition, real-time PCR analysis provided data lower than those obtained from the untreated control samples. Again, the traces obtained by CE analysis were always anomalous with reduced relative amounts of the four canonical DNA bases.

As far as regards the STR typing, the amplification of comparable amounts of these damaged samples showed the following phenomena: no PCR products, drop out and drop in. PCR amplification of SNP loci showed no or poor quality DNA profiles for samples 14 and 54. In addition, the reproducibility of the minisequencing reaction was lower in the treated samples (data not shown).

4. Discussion

In this study, a single DNA sample, which underwent controlled treatments with ROS, metal ions, UV radiation, acidic and basic pH, was analysed by several conventional methods.

CE analysis showed that most of the experimental conditions employed were able to modify the chemical composition of the DNAs, mainly inducing the hydrolysis of the *N*-glycosilic bond. Such finding provides an explanation for the others results. Actually, it is well known that DNA damage interferes with DNA probing [4], the step that plays the major role in PCR fidelity [2].

In this study, the reliability of all the PCR-based methods employed (real-time PCR, STR typing and SNPs typing) was strongly influenced by the chemical integrity of the template. In addition, full DNA profiles could not be achieved even increasing the amount of template. However, the data here presented further confirm that DNA quantification (performed either by DNA probing or real-time PCR) is a fundamental step of the genetic analysis.

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