EFFECT OF DEGRADATION ON PCR BASED DNA TYPING

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INTRODUCTION

In almost 50% of all cases, DNA extracted from forensic evidence turns out to be either not enough to be tested for molecular weight or severely degraded (Perlee and Balasz 1993). Using DNA polymorphisms with short alleles and the PCR approach it is possible to achieve results from minimal amounts of DNA and degraded samples. But, as has been described e.g. by Reynolds et al. (1993) for D1S80, the degradation can lead to dubious or even wrong typing results through causing preferential or sole amplification of the smaller allele.

In cases where the degree of degradation cannot be tested beforehand, degradation artefacts will not always be expected and recognized. In order to provide a basis for accurate interpretation of these artefacts using artificially degraded DNA, we have systematically studied the effects of different degrees of degradation on PCR typing results. To get information for different size allele ranges, the results for one AmpFLP system (Apo B, standard and nested) were compared to results for the STR system VWA.

Furthermore, we investigated the effect of DNA degradation on quantification results achieved by the dot blot approach and chemiluminescent probe D17Z1, and tested the molecular weight of DNA from bloodstains after Chelex extraction.

MATERIAL & METHODS

DNA degradation:
DNA of three individuals was diluted to 40µg/ml. The concentration was reexamined, and if necessary, adjusted using the "Human DNA Quantitation System" (Gibco/BRL) according to the manufacturers instructions. Aliquots of the DNA solutions were subjected to ultrasonic waves (Rinco Ultrasonics 9320 Arbon) for different time spans, which resulted in increasing degrees of degradation as is shown in fig. 1. After degradation the aliquots were diluted further and employed for dot blot quantitation and gene amplification.

Amplification:
The two loci chosen for amplification were:
Apo B - VNTR (Boerwinkle et al. 1989)
allele range: 570-900 bp, length of repeat unit 14-16 bp
VWA - STR (Kimpton et al. 1992)
allele range: 135-167 bp, length of repeat unit 4 bp
Apo B was amplified as described elsewhere (Prinz et al. 1993) using the original primers by Boerwinkle et al. (1989). For nested PCR the DNA was first amplified for 20 cycles using external primers by Schmitt et al. (subm.). A second round was performed with 1µl of the first round, the original primers, and 25 cycles. VWA primers were synthesized according to Kimpton et al. (1992), the PCR conditions were as given in Schmitt et al. (subm). Electrophoresis and silver-staining have been modified after Allen et al. (1989).

Chelex extraction:
Bloodstains on cotton cloth (2 days and 10 weeks old) that were equivalent to 20µl of fresh blood were shredded and subjected to Chelex extraction following Singer-Sam et al. (1989),

with 400µl of 5% Chelex (Biorad) in aqua bidest. and 5µl (20mg/ml) proteinase K, incubation for 2 h at 56°C, 30 sec vortex, 8 min boiling water bath, 30 sec vortex, and centrifugation at 14.000 xg for 5 min. Parallel samples were extracted using DNA lysis buffer (Gill et al. 1985), proteinase K incubation, phenol/chloroform extraction, and Centricon 100 (Amicon) concentration.

RESULTS AND DISCUSSION

The exposure to ultrasonic waves resulted in severe degradation with no high molecular DNA left after only 30 seconds (fig. 1). As is shown in fig. 2 using the dot blot quantification, the degraded DNA produced only faint signals which completely disappeared for increasing degrees of degradation. The DNA probe contained in the kit (D17Z1) is based on probe p17H8, that has been developed by Waye and Willard (1986) and hybridizes with an α-satellite repeat on chromosome 17 consisting of 500-1000 copies of a 2,7kb EcoRI fragment, that is composed of 171bp repeat units. Considering the short length of the repeat unit and the fact that D17Z1 is an oligonucleotide probe, the system shouldn't be as sensitive to DNA degradation as, according to our results, it is. The effect of the reduced signal intensity is an underestimation of the true DNA concentration. For the PCR this could cause background smear or inhibition if overly high amounts of DNA are used. Definitely, depending on the PCR system, samples that don't show hybridization signals can still be amplified. For all degradation steps, aliquots containing 500pg or 1ng of DNA could not be detected on the dot blot, but showed correct VWA genotypes.

This phenomenon and preferential amplification have also been noted for samples that had been extracted using the Chelex method. Since Chelex extraction yields single stranded DNA, it is not possible to test the molecular weight by yield gel electrophoresis and ethidium-bromid staining. Therefore a yield gel has been blotted and hybridized with probe D17Z1 (see fig. 3). The DNA extracted with phenol/chloroform was of high molecular weight and showed only slight degradation for the 10 week old stains. For both storage times the DNA extracted with Chelex was severely degraded to a fragment size of smaller than 5kb. Singer-Sam et al. (1989) postulated that Chelex as a chelating agent would prevent DNA degradation that could be catalyzed by metal ions during boiling the sample in a low ionic strength solution. Maybe due to the presence of, e.g., high amounts of Fe^{++} ions stemming from the heme components, this could be especially important for the extraction of bloodstains. Chelex extraction of saliva stains yielded high molecular weight DNA (data not shown). Therefore the degradation shown here for 20µl bloodstains may be less for other materials and smaller bloodstains. This will be further investigated.

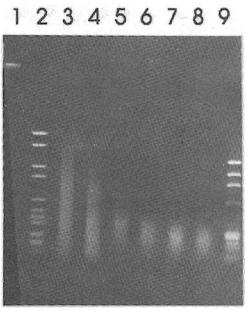

Fig. 1 Degradation test gel
lane 1: high molecular weight control
3 - 9: 30'',1',4',60', 120',150' exposure to ultrasonic waves
lane 2: Boehringer VI
lane 9: PhiX174/HaeIII

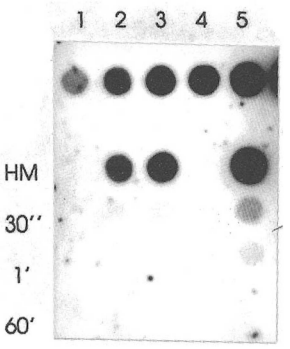

Fig. 2 Dot blot results
lane 1-5: 0.2,0.4,1,2,4ng control, 4,1 and 0.4ng of degraded DNA were spotted

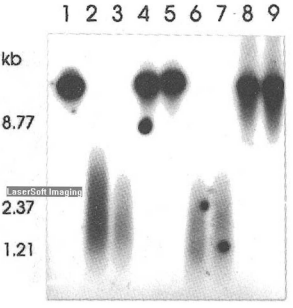

Fig. 3 Yield gel hybridization
lane 1 high molecular reference
lane 2,3,6,7 Chelex extraction
lane 4,5,8,9 phenol/chloroform ex.

As expected the comparison of the amplification results on degraded DNA for Apo B and VWA, showed the lower susceptibility of VWA to degradation. All three individuals were heterozygote for both loci. Their genotypes were VWA (14/16), (14/18), (17/19)
Apo B (31/33), (37/39), (39/51).
There was no influence of the genotype on the VWA typing results. The highest degree of degradation that has been tested here (after 150 minutes of exposure to ultrasonic waves) still contained DNA fragments larger than the longest VWA allele of 167bp. By employing 1ng and 500pg of DNA, reliable results could be obtained. Only with smaller amounts of DNA (for instance 50pg), artefacts as allelic drop out (of the longer and the shorter allele) and additional bands appeared with increasing degradation.

For Apo B, preferential amplification and therefore the genotype of the DNA had a great influence on the PCR results. Both alleles of genotype (31/33) could be detected in DNA fragmented to less than 500bp (60 min degradation time), while the DNA of genotype (39/51) (allele 51 = approx. 900bp) showed strong preferential amplification of the shorter allele (see fig. 4), and, depending on the DNA amount, a single band pattern after 30 seconds to 60 minutes.

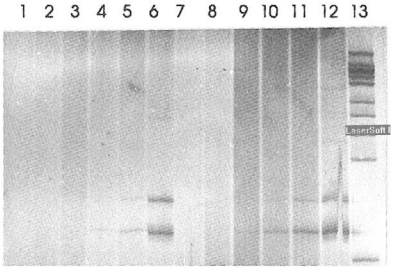

Fig. 4
Amplification test gel for Apo B genotype (39/51)
lane 1-6: 10 ng of DNA
lane 7-12: 100 ng of DNA
150',120',60',4',1',30'' and highmolecular control each
lane 13: 123 bp ladder

Caused by the higher sensitivity, it was possible to somewhat improve the Apo B typing results by employing nested PCR. Probably because the second round of amplification enhances unequal amounts of short and long allele products from the first round (Schmitt et al. subm.), nested PCR is even more susceptible to preferential amplification, so that there was a stronger influence of the allele length. Even closely spaced alleles as (37/39) showed an allele 37 single band pattern for some degradation steps. Increasing the amount of DNA also resulted in an improved allele detection but this was connected to a considerable background smear. Akane et al. (1993) report the separation of high molecular weight DNA from low molecular fragments, which is advisable to solve the background problem.

Our results show that the ability to type degraded DNA is a function of the degree of degradation and the DNA concentration. Since, as we have shown here, the DNA content of a degraded sample cannot reliably be determined, it is therefore, even for a specific polymorphism, not possible to relate a degradation effect, like faint bands or preferential amplification, to the degree of degradation. A more promising approach that has been suggested by Reynolds et al. (1993), is a test amplification of two DNA sequences of different size, which would simultaneously determine the presence of DNA sequences of a specific size range and the amplifiability of the DNA sample.

Because of their short allele lengths the STR polymorphisms will become important tools for analysing degraded DNA. For minute amounts of stain material the requirement of species determination will have to be reconsidered. Even samples that, according to the dot blot results, don't contain any human DNA should be employed for gene amplification, since this result could be caused by degradation.

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