APPLICATION OF DNA POLYMORPHISMS TO THE FORENSIC EXAMINATION OF DRIED BLOOD STAINS

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Abstract

We have examined the possibility of using DNA purified from dried blood stains for the analysis of restriction fragment length polymorphisms. High molecular weight DNA was recovered from blood dried on cotton cloth, aged from one day to three years. These DNA samples were digested with restriction endonucleases, separated by agarose gel electrophoresis and hybridized to recombinant DNA probes that recognize two different highly polymorphic DNA sequences. A set of controlled samples was aged at room temperature for up to 28 days. The polymorphic DNA pattern observed with each probe remained the same at all time points. The patterns of DNA polymorphisms observed in the two and three year old dried blood stains was consistent with patterns observed in the general population. These results indicate that DNA recovered from dried blood stains can be used for identification purposes.

Introduction

The analysis of dried blood stains to help determine identity presently relies on the examination of polymorphic proteins and cellular antigens (1,2). Methods to detect nucleotide sequence polymorphisms within DNA are presently available. Using specific restriction endonucleases and defined probes, many heritable restriction fragment length polymorphisms (RFLPs) have been described (3,4). This report investigates the recovery and RFLP analysis of DNA from dried blood stains.

Materials and Methods

Sources of Dried Blood Samples

Dried bloodstains were prepared by applying 6 one cc aliquots of freshly drawn blood to sections of cotton cloth or to the bottoms of glass beakers. These stains were allowed to age at room temperature for up to 28 days before DNA was isolated. Three forensic blood stains taken post-mortem and stored 2 or 3 years at 4° C were also analyzed. All blood stains on cloth were about 45 mm in diameter.

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Preparation of DNA

Peripheral blood samples were mixed with 4 volumes of blood lysis buffer (0.32 M sucrose; 10 mM Tris-HCl, pH 7.6; 5 mM MgCl; 1.0% Triton X-100). The white cells were pelleted by centrifugation at 2000 x g for 10 minutes at 4° C and resuspended in DNA lysis buffer (10 mM Tris-HCl, pH 7.4; 10 mM NaCl; 10 mM EDTA). Proteinase K and SDS were added to final concentrations of 100 µg/ml and 1.0% respectively. Samples were incubated at 37° C overnight with gentle mixing. Sodium perchlorate was added to 1.0 M and either stored at 4° C for processing later or extracted twice with phenol-chloroform (1:1), twice with chloroform, and dialysed against 3 changes of a 1000 fold excess of TE (10 mM Tris-HCl, pH 7.4; 1 mM EDTA). Ammonium acetate was added to a final concentration of 0.3 M and DNA precipitated overnight at -20° C with 2.5 volumes of 95% ethanol. DNA was pelleted by centrifugation at 10,000 x g for 20 minutes at 4° C, dried, and resuspended in 1.0 ml of TE. Blood stained cotton cloth cut into small strips or dried blood samples from glass beakers was suspended directly in DNA lysis buffer and treated as above. DNA concentrations were determined by absorbancy at 260 nm in a spectrophotometer.

Restriction Endonuclease Digestion and Electrophoresis

One to ten micrograms of DNA was digested with a six fold excess of the restriction endonucleases Eco Rl or Taq 1 (Bethesda Research Laboratories) according to the conditions recommended by the manufacturer. Digested DNAs were size fractionated with appropriate size markers by agarose gel electrophoresis in TAN buffer (40 mM Tris-HCl, pH 7,9; 20 mM sodium acetate; 2 mM EDTA). Eco Rl digested DNA was electrophoresed in 0.4% agarose gels at 0.6 volts/cm for 3 days while Taq 1 digested DNA was electrophoresed in 1.2% agarose gels overnight at 1.8 volts/cm. The total length of each gel varied from 15 cm to 22 cm. DNAs were visualized post-electrophoresis by ethidium bromide staining and photographed under ultraviolet light.

DNA Transfer and Hybridization

DNA was denatured in gel and transferred to nylon membranes (Zetabind, AMF Cuno) using standard blotting techniques (5). DNA probes were radioactively labelled by nick translation (6) to specific activities $>10^8$ cpm/µg using all four alpha-^{32}P deoxyribonucleotide triphosphates (Amersham). Membranes were hybridized with labelled probes and washed to remove non-specifically bound radioactivity as described by the manufacturer.

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Bands were visualized by autoradiography at -70° C using x-ray film (X-Omat, Kodak) and Dupont Lightning-Plus intensifying screens.

DNA Probes

Two DNA probes were prepared from cloned inserts in the plasmid pBR322. pAW101 (kindly provided by R. White) contains a 6.5 kilobase (kb) insert that hybridizes to the D14S1 region on chromosome 14 and is polymorphic with Eco R1 (7). pLM0.8 contains a 879 base pair Cla 1 - Sph 1 insert derived from the 3'-flanking region of the HRAS-1 oncogene on chromosome 11 (8) and is polymorphic with Taq 1. Both inserts were purified from their pBR322 vectors and used as probes.

Results and Discussion

The amount of DNA recovered from 1 ml dried blood stains (45 mm in diameter) varied from 27 µg to 73 µg with the average recovery of 40 µg, similar to the amount obtained from one ml of peripheral blood. Titration experiments indicate that 1 to 4 µg of DNA are sufficient for RFLP analysis using unique sequence probes. Thus, a 200 microliter (9 mm) bloodstain could yield enough DNA for analysis using two probes.

High molecular weight DNA is essential for RFLP analysis. A band on an autoradiograph is obtained only if enough intact copies of the desired fragment are present. Broken fragments will migrate faster and will not be concentrated in a single band after electrophoresis. To measure the size of DNA recovered from blood stains, we used agarose gel electrophoresis with appropriate size markers. Undigested DNA which ran slower than the largest size marker was considered to be high molecular weight (ie. >23 kb). High molecular weight DNA was isolated from all stain samples including the 2 and 3 year old specimens.

The RFLP patterns obtained from each probe rely on the binding (hybridization) of the probe only to complementary DNA sequences bound to the membrane. Autoradiography of a membrane after hybridization with a labelled probe reveals one (homozygote) or two (heterozygote) allelic bands for each digested DNA sample. Because the RFLPs display Mendelian inheritance patterns, they can be used to establish identity. More than 30 alleles can be distinguished for pAW101 with fragment sizes ranging from 14 to 32 kb. For pLM0.8, 18 distinct alleles are observed with fragment sizes ranging from 1.8 to 4.5 kb.

High molecular weight DNA isolated from bloodstains on cotton cloth and glass at time points of 0, 1, 3, 7, 14, and 28 days were examined for RFLPs using the pAW101 and pLM0.8 probes. Clearly defined bands of equal intensity were seen on autoradiographs for all samples.

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Allele sizes remained unchanged as the samples aged. High molecular weight DNA isolated from two and three year old bloodstains produced bands with both probes within the size range of alleles observed in the general population.

Non-allelic bands can not arise by DNA degradation. Rather, breakage of DNA produces a dispersion of DNA fragments resulting in the loss of any specific autoradiographic signal. Incorrect bands larger than expected are obtained only if high molecular weight DNA is not digested to completion by the restriction endonuclease. This possibility is avoided by digestion with at least a five fold excess of enzyme.

The ultimate power of an RFLP for identification is dependent on the frequency of the alleles. Hundreds of non-related individuals from the New York City area have been analyzed to generate a database of allele frequency. Presently the average power of identification using the two probes is about 1 in 1,000. Other probes which detect multiple allele RFLPs are under investigation and will increase the power of the DNA test.

Conclusions

Enough high molecular weight DNA was recovered from blood stains as old as three years for restriction fragment length polymorphism (RFLP) analysis using two probes which recognize highly polymorphic regions. For identification purposes, analysis of DNA RFLPs should prove to be a powerful addition to the current protein techniques.

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