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Anomalous Migration of PCR Products Using Nondenaturing Polyacrylamide Gel Electrophoresis: The Amelogenin Sex-Typing System

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ABSTRACT: Sex-typing of biological samples can be accomplished using the polymerase chain reaction (PCR) to amplify DNA sequences that are specific for the Y-chromosome. One such system is based on PCR amplification of the X-chromosome amelogenin gene and the amelogenin-like sequences located near the centromere of the Y-chromosome. The X and Y PCR products can be distinguished from each other on the basis of a 177 basepair (bp) insertion in the X relative to the Y. In this report, we demonstrate that the amelogenin PCR products migrate anomalously using non-denaturing polyacrylamide gel electrophoresis (ND-PAGE) as opposed to agarose gel electrophoresis or denaturing PAGE. These results may be relevant to the choice of electrophoretic system used to analyze highly polymorphic loci for individual identification.

KEYWORDS: pathology and biology, sex-typing, polymerase chain reaction, amelogenin gene, electrophoretic anomalies, nondenaturing PAGE

The polymerase chain reaction (PCR) [*1*] provides rapid and sensitive approaches for the analysis of highly polymorphic loci in human DNA [*2,3*]. PCR protocols have also been developed for gender determination or *sex-typing* [*4–7*]. This involves amplification of sequences which are specific for the Y-chromosome, often in combination with autosomal or X-chromosome sequences as a control for the amplification reaction. One such system is based on amplification of the X-chromosome amelogenin gene (AMG) and the amelogenin-like sequences (AMGL) located near the centromere of the Y-chromosome [*6,7*]. The principal difference between the X and Y loci is a 177 basepair (bp) insertion in the X relative to the Y [*8*]. Using a single set of primers, the X and Y sequences can be amplified and distinguished from each other on the basis of size (X = 977 bp; Y = 788 bp) [*6,7*].

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The presence of the X and Y fragments indicates that the DNA originated from a male, whereas amplification of X fragment alone is indicative of female DNA.

In this report, we describe the effects of using different electrophoretic detection systems to distinguish between the X and Y amelogenin PCR products. Electrophoresis of the PCR products on agarose gels or denaturing polyacrylamide gels gave fragment lengths that were consistent with their published sequences (Y = 788 bp; X = 977 bp). In contrast, the PCR products exhibited retarded migration when analyzed by non-denaturing polyacrylamide gel electrophoresis (ND-PAGE). The 977 bp X fragment migrated approximately 18% slower (1150 bp), and the 788 bp Y fragment migrated more than 70% slower (1350 bp). These electrophoretic anomalies may be due to sequences which alter the curvature of the double helix, a phenomenon which has been reported for many types of DNA sequences [*9,10*]. Electrophoretic anomalies of this type may be undesirable for applications which require accurate sizing of DNA fragments. This consideration may be relevant to the choice of electrophoretic system used to analyze highly polymorphic loci for individual identification.

Materials and Methods

PCR Amplification of the Amelogenin Locus

The primers used to amplify the X and Y amelogenin sequences were previously described by Nakahori and colleagues [*6*]. The primer sequences are as follows:

primer AMXY-1F (5′-CTGATGGTTGGCCTCAAGCCTGTG-3′)
primer AMXY-2R (5′-TAAAGAGATTCATTAACTTGACTG-3′)

PCR was conducted using the Perkin-Elmer GeneAmp® PCR System 9600 Thermal Cycler. Genomic DNA samples were amplified in a 100 μL reaction volume containing 0.2 mM of each dNTP, 50 mM KCl, 10 mM Tris.HCl pH 8.3, 4.0 mM $MgCl_2$, 120 pmoles of each primer, and 2.5 U *Taq* polymerase. PCR was run for 30 cycles of 94°C for 1 min (denature), 65°C for 2 min (anneal), and 72°C for 3 min (extend). Following amplification, the PCR products were analyzed by electrophoresis on 1.2% agarose gels run with 1 × TBE (89 mM Tris borate, 89 mM boric acid, 2 mM EDTA) or 6% nondenaturing polyacrylamide gels run with 1 × TBE. The gels were stained with ethidium bromide and the fragments were visualized by fluorescence under ultraviolet light.

Results and Discussion

The amelogenin PCR products from two females and two males were split and analyzed by agarose gel electrophoresis or ND-PAGE (Fig. 1). Using agarose gel electrophoresis, the Y fragment migrates faster than the X fragment, and their sizes are in close agreement with the predicted sizes derived from the published sequences of the X and Y amelogenin loci [*8*]. Similarly, the expected fragment sizes were obtained when the PCR products were analyzed by denaturing PAGE (6% polyacrylamide/8 M urea gels) (data not shown). In contrast, ND-PAGE resulted in the Y fragment actually migrating slower than the X fragment (Fig. 1). Relative to the marker fragments, the Y fragment appeared to be approximately 1350 bp with ND-PAGE. This is more than 550 bp (70%) larger than its predicted size. Migration of the X fragment was also retarded with ND-PAGE. Relative to the marker fragments, the X fragment appeared to be approximately 1150 bp. This is more than 170 bp (18%) larger than its predicted size. Although both the X and Y fragments exhibited retarded migration with ND-PAGE, the effect was much greater for the Y fragment. It is possible that the 177 bp insertion of the X fragment somehow interrupts or displaces the sequences that are responsible for the dramatically altered mobility of the Y fragment.

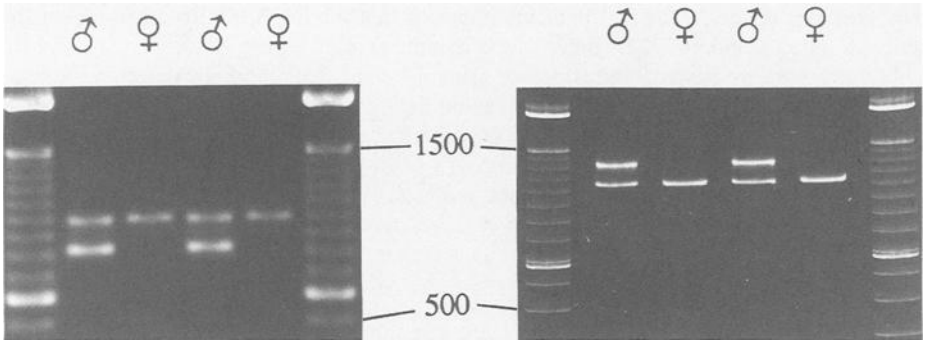

FIG. 1—*Sex-typing based on PCR amplification of the X and Y amelogenin sequences. PCR amplified DNA from two females (♀) and two males (♂) were analyzed by agarose gel electrophoresis* (left panel) *or ND-PAGE* (right panel). *The sizes of the molecular weight markers are given in basepairs (bp). The predicted sizes of the X and Y fragments are 977 bp and 788 bp, respectively.*

Anomalous migration of DNA fragments with ND-PAGE is not limited to the amelogenin sex-typing system. In fact, one of the marker fragments also exhibited retarded migration with ND-PAGE. The 600 bp "rung" of the sizing ladder should appear several times more intense relative to the other fragments, as was the case for the marker fragments separated on agarose gels (Fig. 1). With ND-PAGE, the intensely staining "rung" of the sizing ladder migrated closer to 700 bp. This artifact of ND-PAGE is noted in the product information which accompanies the 100 bp sizing ladder (GIBCO BRL, Burlington ON).

It is well established that certain sequence arrangements can alter the curvature of double-stranded DNA molecules and retard their migration through sieving matrices such as polyacrylamide gel [*9,10*]. The amelogenin results presented here provide an extreme example of the inherent problems associated with using ND-PAGE to estimate the sizes of PCR fragments. It should be noted that anomalous migration of the amelogenin fragments with ND-PAGE does not preclude correct sex-typing. However, this type of artifact may be important with respect to the choice of electrophoretic system used to analyze highly polymorphic loci such as short tandem repeats (STRs) [*11*]. In light of the potential artifacts associated with ND-PAGE, it may be prudent to use agarose gel electrophoresis or denaturing PAGE for applications that require accurate sizing of PCR fragments.

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