EFFECT OF DIVALENT METAL IONS ON DNA STUDIED BY CAPILLARY ELECTROPHORESIS

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It is well known that certain divalent metal cations such as Zn^{2+}, Co^{2+}, or Ni^{2+} can replace the imino protons of DNA base pairs at pH conditions above 8, forming novel DNA-metal ion complexes, termed M-DNA. However, a consensus has not yet been reached on the structural effect of these transition metals binding to DNA. We utilized capillary electrophoresis (CE), circular dichroism (CD), and atomic force microscopy (AFM) to further characterize the structural properties of these metal-modified DNA molecules. Using the ABI 310 Genetic Analyzer we found that the presence of these metal cations in a typical DNA buffer can cause broad peaks with low fluorescence intensities. Furthermore, there was a change in the electrophoretic mobilities of the affected DNA molecules. Both of these effects were dependent on the length of time the DNA sample existed in the formamide before injection and also the fragment size. These results suggest that metal ion contamination might be responsible for the poor results obtained when typing some forensic DNA samples.

To metallate the molecules for CE, a DNA sample (single allele, DNA ladder, or PCR amplified product) was placed in 1.5 mM-100 mM $NiCl_2$, $ZnCl_2$, or $CoCl_2$ in a 10 mM Tris-HCl buffer, pH 8.3. The samples were allowed to interact for at least 30 minutes under ambient conditions. For controls, these same DNA fragments were also mixed in Ni, Zn, and Co buffer solutions at the same concentrations but at pH 7 and in buffers at pH 8.3 but with cations such as Mg^{2+} and Fe^{3+} that are only capable of binding to the DNA phosphate backbone. Aliquots of each of these solutions were taken, added to ROX/formamide and immediately analyzed on the ABI 310 using standard injection and run conditions.

DNA samples at pH 7 and those with cations incapable of causing an M-DNA conversion gave sharp, intense peaks. However, when the pH of this buffer was raised to a level normally used in DNA typing, pH 8.3, the DNA sample alleles and the ROX internal standard gave broad peaks. The affected DNA fragments also migrated at a different mobility than the normal, B-conformation DNA. The mobilities were typically slower although some alleles did migrate faster than their B-DNA counterparts. These CE results were readily reversible by adjusting the pH of the M-DNA sample down to a level where metal intercalation could not occur or by the addition of EDTA. Furthermore, subsequent CE runs of the same M-DNA sample in formamide showed a gradual improvement in peak shape, eventually giving the same resolution and mobility as the control sample. The increased electrophoretic mobilities for some M-DNA alleles indicates that these CE results are at least partially due to a structural change in the DNA upon metal intercalation and not just a consequence of the altered charge and mass of the molecules. In agreement, CD and AFM results reveal significant structural differences between our M-DNA and B-DNA samples. Overall, our studies reveal the negative role that metal ion contamination can have on forensic DNA typing and demonstrate solutions to reverse this effect.