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Optimization of intercalation dye concentration for short tandem repeat allele genotyping using capillary electrophoresis with laser-induced fluorescence detection

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Abstract

DNA analysis using capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection requires that polymerase chain reaction products either be prepared using primers with fluorescent molecules covalently bonded to them, or stained with a fluorescent intercalation dye following amplification. The intercalation technique has the advantage of allowing fluorescence detection of any double-stranded DNA (dsDNA) product regardless of the amplification primers used. The increased sensitivity of LIF detection is sometimes compromised by the intercalation dye changing the mass to charge ratio of the DNA. The purpose of this study was to evaluate the changes of migration rate, resolution and fluorescent intensity of dye–DNA complexes during electrophoretic separations, and to establish the optimal parameters for short tandem repeats alleles profiling. The alleles of three STR loci THO1, F13A01 and vWFA31 were intercalated with the monomeric dyes TOPRO-1 and YOPRO-1, and their corresponding dimers, TOTO-1 and YOYO-1 (Molecular Probes, Eugene, OR, USA). Alleles intercalated before injection onto the CE column resulted in loss of resolution and sensitivity when compared to the on-column labeling technique. The results of this experimentation were then applied to a STR typing assay using a commercially available polymer and buffer matrix. This assay included development of a unique internal standard used for migration time normalization assignment of alleles. Consequently, the 9 allele and the 9.3 microvariant of the THO1 locus were separated and typed correctly with a resolution of 0.49 in less than 20 min, and the only sample preparation necessary after amplification was a dilution step.

Keywords: Intercalation dyes; Short tandem repeat alleles; DNA

1. Introduction

In recent years, capillary electrophoresis (CE) has generated considerable interest as an analytical technique with great potential in the analysis of biomolecules [1]. Low sample volume, greater applied voltages, resulting in shorter analysis time, real time detection, and many detection modes contribute to CE's popularity. Originally, UV absorbance was the most common means of monitoring CE separations [2,3]. DNA can be analyzed at 260 nm with limited

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detection. Laser-induced fluorescence (LIF) detection offers a considerable increase in sensitivity over the traditional UV analysis [4–8]. Since DNA has little native fluorescence, analysis using LIF detection requires the fragments either be labeled using a primer with a fluorescent moiety attached or stained using fluorescent (intercalating) dye [6,9].

With the development of polymerase chain reaction (PCR) protocols for DNA fingerprinting techniques, successful amplification is possible even when the sample is limited or subject to environmental degradation. Now available for forensic and paternity testing are short tandem repeat (STR) kits such as AmpFISTR Profiler PCR Amplification Kit (Perkin-Elmer, Foster City, CA, USA), which use primers with a fluorescent molecule covalently bond to them allowing LIF detection [10]. After denaturing, the single-stranded alleles are separated and detected by LIF with increased resolution when compared to double-stranded DNA (dsDNA) analysis [11]. The increase in resolution of single-stranded DNA (ssDNA) is due to the elimination of secondary structures and conformational changes of the fragment when it is denatured. Williams et al. [12] reported an increase of resolution of 0.58 between denaturing and nondenaturing conditions when analyzing PCR fragments and restriction digest samples. They reported the ssDNA fragments migrate more uniformly within the sieving matrix monitored by UV detection at 260 nm. Using a fluorescent-labeled primer, which requires less sample to be injected on to the CE system, increases the detection of single strand of DNA. This eliminates problems of resolution caused by sample overloading of the capillary. This fluorescent primer technique was reported by Isenberg et al. [13] to allow a resolution of 1.4 bases. Mansfield et al. found this technique amenable to capillary array electrophoresis [14]. To save time and expense during development of DNA assays, intercalation offers the advantage of using any primers (without fluorescent labels) and fluorescent emission detection. Kim and Morris [15] and Zhu et al. [16] reported that intercalation dyes alter the DNA helix and total mass-to-charge ratio, which alters migration rate and resolution of restriction digest DNA. Other evaluations of intercalation dyes for fluorescence detection of dsDNA include the work of Figeys et al. [17], Clark and Sepaniak [18], and Skeidsvoll and Ueland [19]. The purpose of this study was to determine the changes and optimize the resolution and fluorescent detection of intercalated STR alleles dsDNA during electrophoresis using a commercially available separation polymer.

STR alleles are ideal targets for determination of intercalation effects due to their polymorphic nature. In a previous study of intercalation of STR alleles, the maximum base pair-to-dye ratio was determined to be 5:1 in solution (non-electrophoresis experimentation) [20]. In the discussion that follows, alleles of the STR loci, THO1, F13A01 and vWFA31 were separated by CE and detected by LIF after being intercalated with fluorescent dyes. The alleles were intercalated by two methods. In the first method, the DNA fragments were mixed with intercalation dyes before electrophoretic separation and for the second method, the fragments were intercalated on-column during separation. This study monitored the changes in detection and resolution of the STR alleles as a function of intercalation dye concentration, and then applied the most efficient dye parameter to a model STR typing assay.

2. Experimental

2.1. Samples

DNA was extracted from whole blood using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) following the manufacturer's protocol.

The samples of PCR-amplified STR loci were amplified according to manufacturer's instruction [ABI Prism STR Primer Set Protocol 903223, Perkin-Elmer-Applied Biosystems Division (PE-ABD), Foster City, CA, USA]. The samples were amplified using the Perkin-Elmer 9600 thermal cycler (Perkin-Elmer, Norwalk, CT, USA) [10 min at 95°C, 28× (45 s at 94°C, 1 min at 54°C – 2 min ramp, 1 min at 72°C), 10 min at 72°C, 4°C soak]. For a complete listing of STR loci and alleles see http:\\ibm4.carb.nist.gov:8800\DNA\home.htm.

2.2. Allele purification and isolation

The ion-pair reversed-phase (IPRP) DNA purification was described previously [21]. In brief, high-performance liquid chromatographic isolation was

performed using a Dionex DX-500 system with UV detection at 260 nm. The DNASep column 50×4.6 mm (Transgenomic, San Jose, CA, USA) was heated to 55°C using an Eppendorf Model CH-30 oven (Eppendorf, Madison, WI, USA). The mobile phase consisted of high-performance liquid chromatography (HPLC)-grade acetonitrile (ACN) (EM Science, Gibbstown, NJ, USA) and triethylammonium acetate (TEAA) (Applied Biosystems, Foster City, CA, USA). The mobile phase pH was adjusted to 7.0 using glacial acetic acid (Mallinkrodt, Paris, KY, USA) [22].

2.3. Instrumentation

All electropherograms were generated using the Thermo Capillary Electrophoresis Crystal CE 310 (Franklin, MA, USA) with an laboratory LIF detector and Dionex data acquisition software AI450 (ver. 3.32). The separation matrix was the DNA Fragment Analysis Buffer also called gene scan polymer (GSP) (PE-ABD, Foster City, CA, USA). GSP is a hydrophilic polymer that provides molecular sieving and noncovalent wall coating when used in uncoated fused-silica capillaries The specific composition is being withheld by PE-ABD on the material safety data sheet, as a trade secret in accordance with OSHA 29CRF 1910.1200. GSP is provided in a 7% stock solution in water. The 3.5% polymer solution used in the analysis was prepared from the stock solution by dilution with water. The CE system was programmed to fill the capillary with the 3% polymer solution using a pressure of 2000 mbar for 6 min before each analysis. The separation parameters included electrokinetic injection of −90 V/cm for 6 s, run field of −300 V/cm, excitation using multi-wavelength argon-ion laser, and fluorescence emission monitored at 540 nm. For additional information on design of the detector systems see Yeung et al. [5]. The fused-silica capillary (60 cm×50 μm I.D.) (PolymicroTechnologies, Phoenix, AZ, USA) had an effective length of 49 cm. The capillary was flushed with 0.3 *M* NaOH, water and 1.0 *M* HCl prior to each set of samples.

2.4. Intercalation dyes

The intercalator dyes were purchased from Molecular Probes. All intercalator dye solutions were prepared fresh daily.

3. Results and discussion

3.1. Pre-electrophoresis intercalation

The THO1 (6,6) allele, containing one 158 base pair (bp) fragment, was intercalated with TOTO-1. The base pair-to-dye ratios for this sample set were infinity (~) (no dye), 50/1, 25/1, 10/1, 5/1 and 2/1. The DNA concentration was 40 pg/μl and the sample volume was 20 μl. All electrophoresis parameters remained constant for each sample (see Experimental). The electrophoresis buffer was the 3.5% ABI fragment analysis buffer, with no intercalator added. The blank contained DNA only, bp/dye ratio equal to infinity (~), showed no fluorescence. As the intercalator concentration increased, the peak intensity also increased from 60 mV (bp/dye 50/1) to 130 mV (bp/dye 2/1), and the migration time lengthened from 12.2 min to 15.4 min, a decrease in velocity of 0.7 cm/min. These observations indicated an increase in the number of dye molecules bound to the DNA and the corresponding neutralization of the DNA charge. Similar experimental results were obtained in 1994 when researchers Kim and Morris [15], and Zhu et al. [16] evaluated the effect of intercalation dyes on restriction digest fragments.

The electropherograms did not contain a single peak as expected for the single allele (6) analysis. The bisintercalators have two aromatic systems linked together by a flexible chain of linear atoms. The structures of TOTO-1 or YOYO-1 permit these dyes to intercalate into the DNA helix at two different places. According to the findings reported by Carlsson et al. [23], the smear of bands occurring in slab gel electrophoresis resulted from inhomogeneous number of dye molecules distributed through the DNA fragment. Carlsson et al. indicate that the DNA and intercalation dye, after reaching equilibrium, migrates as a single band. To ensure the DNA–dye equilibrium, the THO1 samples were heated and stored for one week, at which time a homogenous distribution of dye molecules should be attained. The samples were then re-analyzed by CE–LIF. Fig. 1 illustrates the electropherograms of the THO1 (6,6) locus at all the dye/bp ratios. The smear of product in the electropherograms did not diminish with time as reported by Carlsson et al. There were two differences between this research and that of

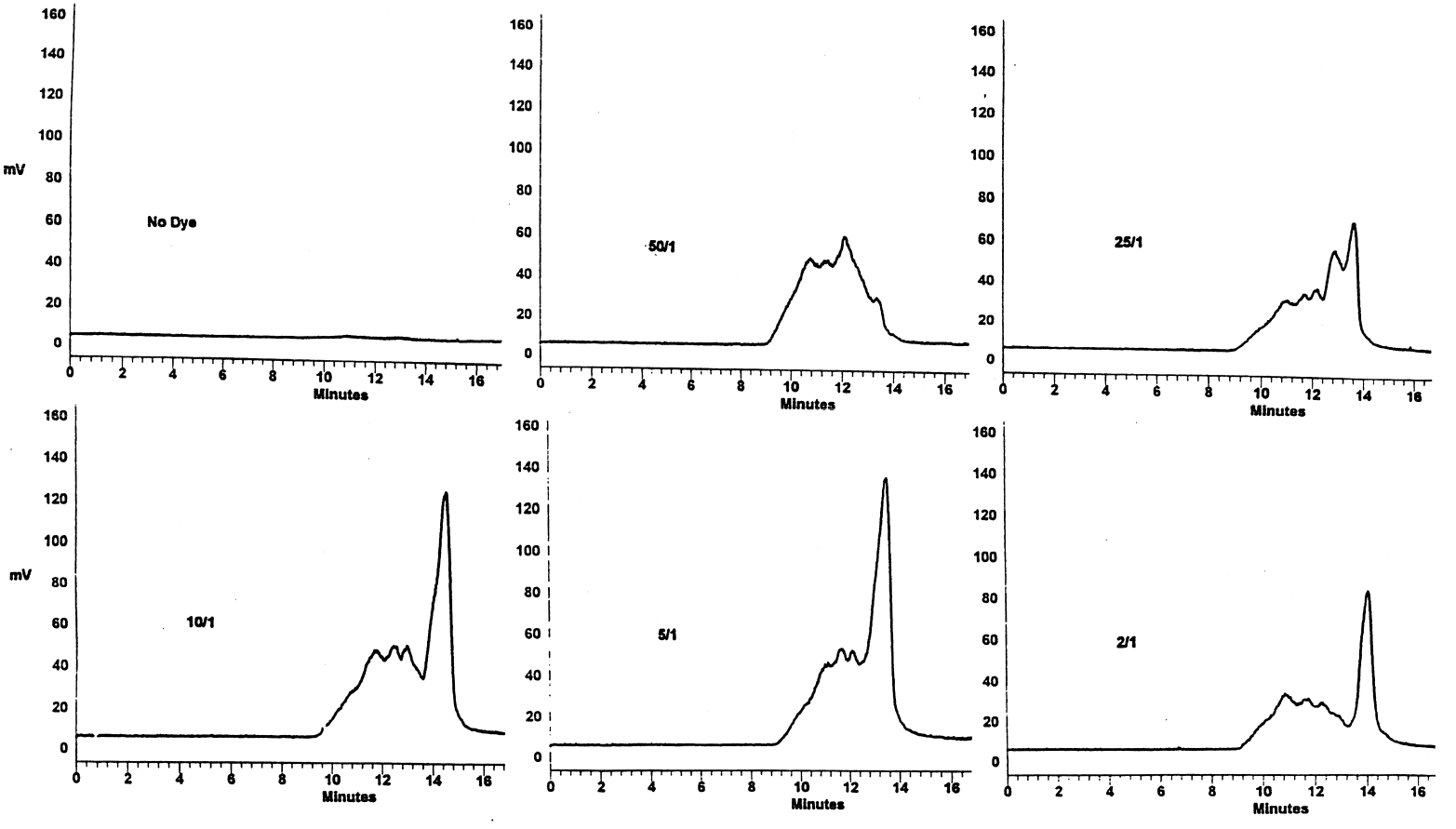

Fig. 1. STR locus THO1 (6,6) intercalated with TOTO-1 before electrophoresis. The electromigration of the THO1 (6) allele (158 bp), using the Thermo Capillary Electrophoresis Crystal CE 310 (Franklin, MA, USA), was monitored at 540 nm, and the separation media was 3.5% ABI fragment analysis buffer (Foster City, CA, USA). The allele was electrokinetic injection at −90 V/cm for 6 s and electrophoresed at reverse polarity voltage of 300 V/cm. TOTO-1 was the intercalation dye used for this sample set. The numbers above the electropherograms are of the base pair-to-dye ratio. All samples were stored for five days before being analyzed. All data were gathered with Dionex AI-450 data acquisition software (Dionex, Sunnyvale, CA, USA) version 3.32.

Carlsson et al. First, the DNA fragments analyzed by Carlsson et al. were much larger (kb fragments) than the STR alleles studied (less than 300 bp). And second, slab gel electrophoresis, not CE, was used to analyze the products. CE has greater resolving potential than the slab method [24].

Another mode of intercalation has been suggested by Glazer and Rye [25] where the bisintercalator molecules cross-link more than one DNA fragment. When the DNA–bisintercalator mixture was allowed to reach equilibrium, two different DNA fragments could possibly be joined by one intercalation molecule. The short size of the fragments and the concentration of base pair and dye molecules favor the additional binding (cross-linking) mode.

The results of the monomeric intercalation dyes TOPRO-1 and YOPRO-1 were totally inconsistent and non-reproducible for the loci THO1 and vWFA31, with limited success for the locus F13 (data not shown). To confirm the CE system integrity, an external standard a 220 bp fragment labeled with the fluorescent dye phosphoramidites, 6-FAM amidite (PE-ABD) was analyzed every six samples.

The heterozygote individuals with the alleles THO1 (6,9), F13A01 (5,8), and vWFA31 (17,19) were also amplified, purified and intercalated using the monomeric dyes. The samples were mixed with the intercalators and stored in the dark for a minimum of two days before being analyzed. The results were the same as the homozygote alleles: irreproducible. Sample prestaining with monomeric dyes results in a loss of sensitivity and resolution from the dyes not being permanently bound to the DNA as noted by Benson et al. [26]. When the DNA strand was completely saturated with intercalation dye, at bp/dye ratio 2/1 [20,27], the mass has increased and the charge decreased. These conditions restrict the electrokinetic migration of DNA onto the column during injection. Also, as the intercalated fragment electromigrates through the separation media, the positively charged dye molecules migrate in the opposite direction to that of the negatively charged DNA. Once the dye leaves the DNA helix, the fluorescent detection (sensitivity) diminishes. The dye molecules not covalently bonded to the DNA can be released by cation-exchange with the ions in the separation buffer [28,29]. A solution to this problem was described by Zhu et al. [16], where 9-aminoacridine (9AA) was added to the run buffer and polymer during analysis of ≠X174/HaeIII samples.

3.2. Internal standard

Determining the changes of intercalated DNA fragments, an internal standard was needed that possesses the following characteristics: a standard that would minimally bind with the intercalation dyes, a standard that would not bind to any of the other DNA fragments and finally one that would be detectable by LIF. A short oligonucleotide with the sequence 5′-FAM-GCGAAAGAATGAGAC-TACAT-3′ fit the criteria. This is a single strand of DNA with a fluorescent label, 6-FAM (PE-ABD) attached to the 5′ end and a non-complementary sequence of the STR alleles. When the standard was analyzed using the ABI polymer with and without intercalation, there was no change in the electrophoretic velocity (migration time). The FAM-labeled standard was added to each sample prior to being analyzed.

3.3. DNA intercalated on-column

To optimize the CE separation parameters, the applied (run) voltage was varied to balance run time and resolution. At negative reverse polarity 200 and 250 V/cm, a slight increase (0.1) in resolution was obtained when compared to the analysis at −300 V/cm. The increase was accompanied by an increase in analysis time. Diffusion (band broadening) resulted at fields below −200 V/cm. At applied voltages greater than −310 V/cm, faster migration times resulted in significant loss of resolution. Optimizing for both analysis time and resolution, the best electrophoresis voltage for the STR alleles was −300 V/cm. The polymer concentration of 3.5% ABI Fragment Analysis Buffer was determined optimal for the analysis based on the sample time and resolution of a 12 bp size difference (data not shown). Using the run field of negative 300 V/cm and 3.5% polymer solution, the intercalated alleles differing by 12 bp were separated and detected in less than 20 min.

The low DNA mass ladder (LDML) standard (GibcoBRL, Gaithersburg, MD, USA) was used to

determine the low detection limit of the CE–LIF system using electrokinetic injection parameters of −90 V/cm for 6 s and the Argon laser intensity of 4 mW. The detection limit was determined to be less than 1 pg/μl (sample size of 20 μl) with excess TOTO-1 added to the separation polymer (data not shown). The minimal amount of intercalator was experimentally determined to be $50 \cdot 10^{-6}$ m*M* intercalation dye added to the matrix.

DNA from heterozygote individuals of the STR loci THO1, F13A01 and vWFA31 with the alleles of (6,9), (5,8) and (17,19), respectively were amplified and purified for use in the on-column intercalation study. The alleles of THO1 and F13A01 differ in size by 12 bp and the vWFA31 alleles by 8 bp. The alleles ranged in size from 151 to 199 bp vWA (17) and F13 (8), respectively. The electrophoresis parameters were as previously stated except the polymer contained the intercalation dye.

Samples were then prepared with a concentration of 40 pg/μl. The alleles fluorescent intensity varied slightly for the increased TOPRO-1 concentration (100 to $500 \cdot 10^{-6}$ m*M*). Also, the migration time for the DNA fragments did not increase with the increase of dye concentration in the polymer (data not shown).

Another monomeric intercalator YOPRO-1 was also evaluated. The locus F13A01 was used for a series of experiments. Two different concentrations of the alleles were prepared to check concentration dependence on resolution. There was little difference in resolution as a function of monomeric dye concentration (data not shown).

The fluorescent response for the TOPRO-1 was 30.0 mV versus 6.0 mV for YOPRO-1. This decrease in fluorescence intensity was due to TOPRO-1 excitation maximum of 515 nm which corresponds closely to the 514.5 nm line of the argon laser, where as YOPRO-1 maximum is 491 nm and the corresponding laser line is 488 nm. Also the decreased fluorescent response occurred because the intercalation dye YOPRO-1 has a maximum emission wavelength at 509 nm [30], and the filter system on the LIF detector contains a 540 nm (±5 nm) band-pass filter. Higher background fluorescence also contributed to reduced sensitivity for both monomeric intercalators. Their backgrounds were higher than the corresponding dimeric dyes. This can be seen as the

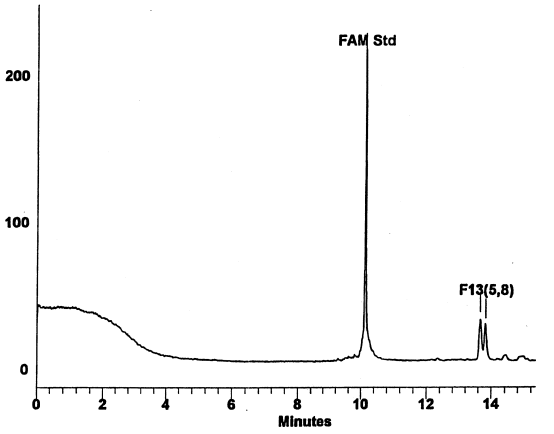

Fig. 2. Analysis of locus F13 alleles (5,8) intercalated on-column with YOPRO. The sample of F13 (5,8) concentration was 80 pg/μl with 1 μl of a 10 n*M* solution of FAM-labeled oligonucleotide added. The alleles were intercalated on-column with YOPRO-1. The analysis parameters and data acquisition were the same as in Fig. 1. The drop in the baseline at 3 min was due to the intercalation dye moving past the LIF detector window.

intercalator moving opposite to the migration of the DNA. Fig. 2 is an example of the F13 alleles (5,8) analysis where the intercalation dye YOPRO-1 is electromigrating from the destination electrode toward the source electrode. The front at time 3 min is the YOPRO-1 passing the detector window a distance of 11 cm from the destination electrode.

The dimeric intercalator TOTO-1 was mixed with the polymer in the concentrations of 50, 75, 100, 175 and $250 \cdot 10^{-6}$ m*M*. The DNA samples contained 10 pg/μl DNA. All sample volumes were 20 μl, with 1 μl of the 10 n*M* FAM standard added. The LDML was also analyzed as an external standard. Table 1 contains the resolution calculations for the three STR loci. Each locus at the lowest concentration of intercalation dye gave no resolution of the alleles. All the allele pairs co-migrated as a single peak at the lower dye concentration. Fig. 3 represents the

Table 1
Resolution of STR alleles intercalated on-column with TOTO-1

Loci (alleles)	Dye concentration ($\cdot 10^{-6}$ m*M*)				
	50	75	100	175	250
THO1 (6,9)	0.00	0.62	1.04	1.80	1.45
F13 (5,8)	0.00	0.60	1.08	1.26	1.19
vWFA (17,19)	0.00	0.61	0.88	1.08	1.06

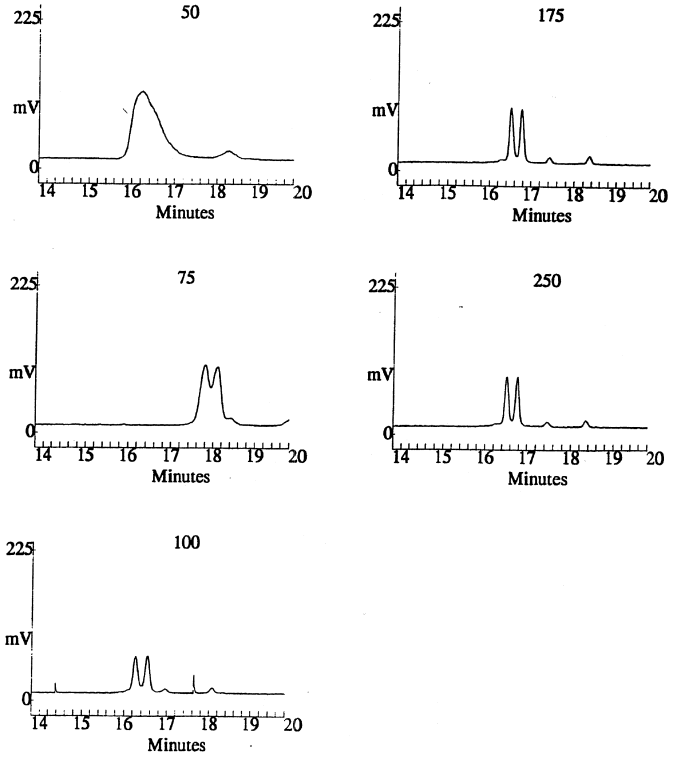

Fig. 3. F13 alleles separated by CE using different dye concentration. The F13 locus with alleles (5,8) were separated with the same electrophoresis conditions as in Fig. 1. The 3.5% ABI buffer contained different concentrations of the intercalation dye TOTO-1. The number above the electropherograms was the dye concentration ($\cdot 10^{-6}$ m*M*).

electrophoretic separation of locus F13 alleles at each dye concentration. The last two electropherogram dye concentrations 175 and $250 \cdot 10^{-6}$ m*M* have similar migration times and fluorescent intensities. At those intercalator concentrations, the DNA has been intercalated to the same degree. The migration time appears to change for $100 \cdot 10^{-6}$ m*M* TOTO-1. When the migration times are normalized using the internal FAM standard, there was little difference in migration time (Table 2). But there was a dramatic change in resolution as a function of bisintercalator concentration. Therefore, as the dye concentration increases the DNA is intercalated homogeneously.

The same TOTO-1 polymer solutions were used to analyze the same alleles at a lower DNA concentration (1.0 pg/μl). For this sample set, there was little change in the resolution, fluorescent intensity or migration time at any dye concentration (data not shown). Therefore, the fewer base pairs in each sample were uniformly intercalated even at the lowest dye concentration ($50 \cdot 10^{-6}$ m*M*).

All the samples used for this experiment were only

Table 2
Migration time of STR alleles intercalated on-column with TOTO-1

Loci (alleles)	Dye concentration ($\cdot 10^{-6}$ m*M*)				
	50	75	100	175	250
THO1 (6,9)	1.24	1.64/1.66	1.64/1.66	1.63/1.66	1.52/1.55
F13 (5,8)	1.56	1.67/1.70	1.68/1.71	1.70/1.72	1.70/1.72
vWFA (17,19)	1.50	1.34/1.36	1.34/1.36	1.37/1.39	1.60/1.62

injected once. If samples are repeatedly injected, the intercalator will migrate into the sample vial and pre-intercalate the sample resulting in a loss of resolution and sensitivity. An example of a sample that has been intercalated while injected is illustrated in Fig. 4. To prevent any additional contamination, a step was programmed on the CE system to immerse the capillary into deionized water, between the capillary filling and sample injection steps.

Another series of THO1 and F13A01 alleles (20 pg/μl) were analyzed using the bisintercalator YOYO-1. Table 3 contains the calculated resolution data. The fluorescent intensity was consistent (approximately 220 mV) for the YOYO-1 samples. The

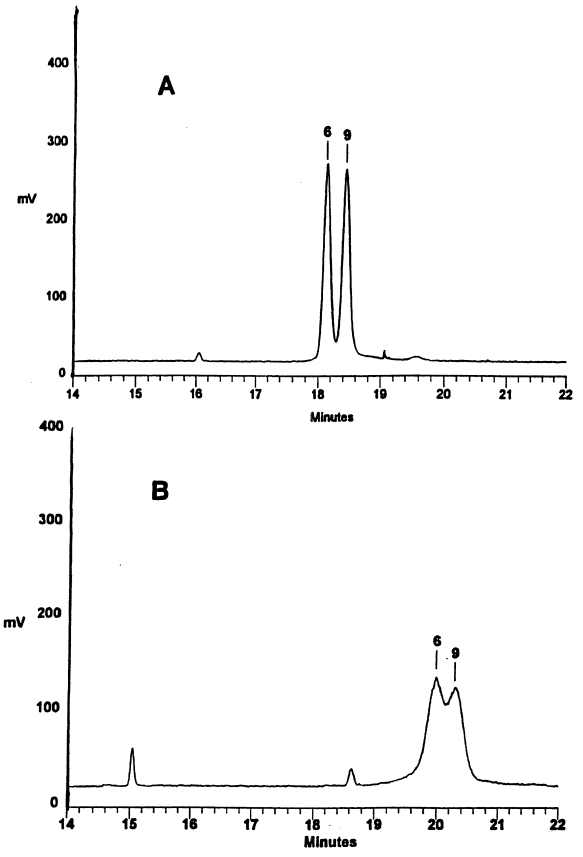

Fig. 4. Sample intercalated during injection. The THO1 (6,9) sample was injected at −90 V/cm onto the CE column containing $250 \cdot 10^{-6}$ m*M* TOTO-1. Same conditions as in Fig. 1. Panel A was the first injection of the standard. Panel B is the fifth injection. The loss of resolution was the result of the TOTO-1 migrating into the sample vial during injection of the sample.

Table 3
Resolution of STR alleles intercalated on-column with YOYO-1

Loci (alleles)	Dye concentration ($\cdot 10^{-6}$ m*M*)				
	50	75	100	175	250
THO1 (6,9)	0.8	1.40	1.71	1.79	2.00
F13 (5,8)	0.6	1.28	1.33	1.59	1.88

YOYO-1 fluorescent dye intensity was greater than the TOTO-1 analysis, as seen in previous study [20].

3.4. THO1 typing study

Using the information obtained from this experimentation the following allele typing procedure was developed. This procedure uses the intercalation dye TOTO-1 and normalized migration time (NMT) calculations. HPLC-purified alleles (dsDNA) were analyzed by CE–LIF with the FAM standard to create a normalized allelic ladder. The following individuals alleles of THO1 were chosen: homozygote 6, heterozygote 6,9; 7,9; and 8,10. The HPLC-purified alleles (10 pg/μl) were analyzed using $250 \cdot 10^{-6}$ m*M* TOTO-1 in the ABI 3.5% polymer. Since the alleles differed in size by 4 bp, the voltage was reduced to negative 230 V/cm to increase resolution. The NMT was calculated and the values for each allele averaged between the closest alleles. This average was used as a range (bin) of NMT for each allele. The results are found in Table 4. Other individuals' DNA of known alleles were PCR amplified and the products (unknown concentration) diluted 1:4000 with deionized water prior to CE–LIF analysis. To all 20 μl samples 1 μl of the $2.0 \cdot 10^{-6}$ *M* FAM standard was added. The NMT was calculated and the allele assignment was made.

The bins were calculated based on a 4 base pair repeat. The individual 5 electropherogram contained a peak with a shoulder (Fig. 5). This individual's alleles are 9 and 9.3 where the 0.3 indicates a microvariant of a three base pair repeat instead of four base pair repeat. This sample's resolution between peak and shoulder was 0.49 indicating the presence of an additional allele, where the peak of the homozygote (single) allele was symmetrical with no shoulders (data not shown). Using the resolution data and bin assignment, this individual alleles were assigned correctly.

Table 4
Typing of THO1 alleles

Allele	6	7	8	9	10
NMT range	1.7463–1.7566[a]	1.7567–1.7716	1.7717–1.7818	1.7819–1.7918	1.7919–1.7964[a]
Individual 1 (8,10)			1.7753		1.792
Individual 2 (7,9)		1.7674		1.7858	
Individual 3 (6,6)	1.7543				
Individual 4 (9,10)				1.7880	1.7947
Individual 5 (9,9.3)				1.7838	1.7937
Individual 6 (6,9)	1.7556			1.7871	

[a] These NMTs were the value of the calculated allele and not an averaged value.

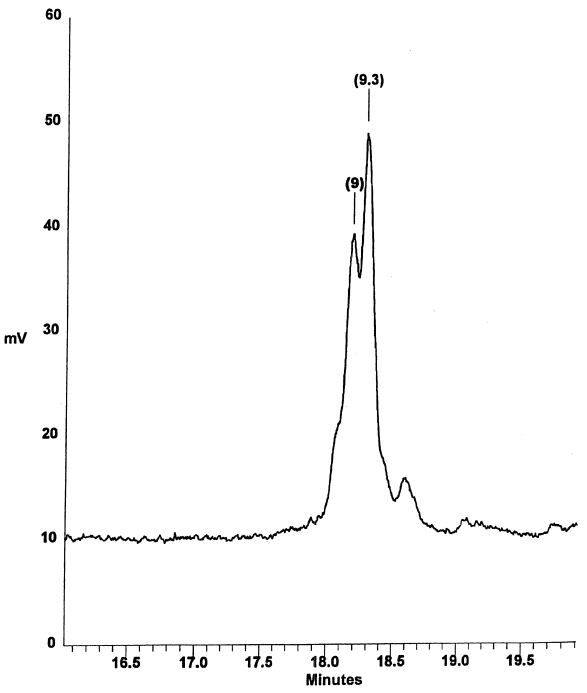

Fig. 5. Analysis of locus THO1 alleles (9,9.3) intercalated on-column with TOTO-1. The sample of THO1 (9,9.3) with 1 μl of a 10 n*M* solution of FAM-labeled oligonucleotide added was analyzed by CE. The alleles were intercalated on-column with TOTO-1. The analysis parameters and data acquisition were the same as in Fig. 1 except the applied field was 230 V/cm. The resolution for the allele was 0.49. The numbers above each peak indicates the allele.

4. Conclusion

Alleles of the short tandem repeat loci, THO1, F13A01 and vWFA31 were PCR amplified and HPLC purified for use in this intercalation study. The alleles ranged in size from 151 (vWFA allele 17) to 199 base pairs (F13A01 allele 8). The single alleles of homozygote individuals of each loci were mixed with two sets of intercalation dyes, each set consisting of a monomeric dye and the corresponding dimer dye. Electrophoresis of the DNA fragments intercalated before injection onto the CE column resulted in loss of resolution and sensitivity when compared to the on-column intercalating technique. The bisintercalators offer greater sensitivity of detection of intercalated DNA over the corresponding monomeric dyes. They exhibit consistent detection and resolution when the DNA is present in the low pg/μl range and the dye concentration is greater than $100 \cdot 10^{-6}$ m*M*. The study of dye–DNA complexes during electrophoresis required the development of a fluorescently labeled single-stranded DNA internal standard. The information provided in this manuscript can be applied to any assay involving double-stranded DNA regardless of the primers used for PCR amplification. The results of the electrophoresis experiments were then applied to a STR typing assay. Using the HPLC-purified alleles and the single-stranded fluorescently labeled standard, bins of normalized migration times for each of the THO1 alleles were calculated. These data were used to correctly type individuals (determine alleles assignments). This CE–LIF analysis, using on-column intercalation with TOTO-1, was completed in less than 20 min, and only required dilution of the PCR product prior to injection.

5. Disclaimer

The opinions or assertions herein are those of the

author and do not necessarily reflect the views of the Department of Army of the Department of Defense.

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