364–369 Nucleic Acids Research, 1994, Vol. 22, No. 3 © 1994 Oxford University Press

Constant denaturant capillary electrophoresis (CDCE): a high resolution approach to mutational anaylsis

K.Khrapko, J.S.Hanekamp, W.G.Thilly, A.Belenkii[1], F.Foret[1] and B.L.Karger[1,*]
Center for Environmental Health Sciences, MIT, Cambridge, MA 02139 and [1]Barnett Institute, Northeastern University, Boston, MA 02115, USA

Received October 8, 1993; Revised and Accepted December 13, 1993

ABSTRACT

Using a zone of constant temperature and denaturant concentration in capillary electrophoresis, we have devised a simple, rapid, and reproducible system for separating mutant from wild type DNA sequences with high resolution. Important to the success of this method, which we call Constant Denaturant Capillary Electrophoresis (CDCE), has been the use of linear polyacrylamide at viscosity levels that permit facile replacement of the matrix after each run. For a typical 100 bp fragment, point mutation-containing heteroduplexes are separated from wild type homoduplexes in less than 30 minutes. Using laser-induced fluorescence to detect fluorescent-tagged DNA, the system has an absolute limit of detection of 3×10^4 molecules with a linear dynamic range of six orders of magnitude. The relative limit of detection at present is 3×10^{-4}, i.e. 10^5 mutant sequences are recognized among 3×10^8 wild type sequences. The new approach should be applicable to the identification of low frequency mutations, to mutational spectrometry and to genetic screening of pooled samples for detection of rare variants.

INTRODUCTION

Point mutations in DNA are studied in the fields of mutagenesis, human genetics, and cancer genetics. Any of a number of mutations in a particular gene may cause altered cellular behavior. Such mutations are infrequent. To study rare variants in human populations, or new somatic or germ-line mutations in humans, a technology is required which can detect any and all of a variety of point mutations in a given gene sequence at fractions from 10^{-1} to 10^{-7} of the wild-type DNA. A useful technique to separate and identify sequence variants is denaturing gradient gel electrophoresis (DGGE), first introduced in 1983 by Fischer and Lerman (1). This method has wide applicability (2) and can be reliably applied to analysis of point mutations in a 100 bp sequence at mutant fractions of the wild-type DNA as low as 5×10^{-3}. A recent modification of this method, constant denaturant gel electrophoresis (CDGE), was introduced by Hovig *et al.* (3).

Separation by these denaturant gel electrophoretic procedures is based on the fact that the electrophoretic mobility in a gel of a partially melted DNA molecule is greatly reduced as compared to an unmelted molecule. Sequences suitable for separation consist of two domains: a low melting and a high melting one, which, within a certain range of denaturant concentrations and/or temperatures, creates a stable, partially melted intermediate. Most but not all base changes in the low melting domain result in significant changes of melting behavior. When a mixture of molecules, differing by single base changes, are separated by electrophoresis on a gel under partially denaturing conditions, they display different states of equilibrium between the unmelted DNA fragment and the partially melted form. The fraction of time spent by the DNA molecules in the slower, partially melted form and, hence, the mean velocity, varies among specific sequences. The result is efficient separation wherein the less stable species move more slowly than the more stable ones. Unmelted double-stranded species will migrate most rapidly through the gel.

An important variation in this procedure is the conversion of the set of all mutants into heteroduplexes with the wild-type sequence by simply boiling a sample containing a predominance of wild-type sequences admixed with mutant sequences (4). By mass action, all mutant homoduplexes are converted to heteroduplexes containing one wild-type strand. Importantly, the melting temperature of each such heteroduplex is significantly lower than that of the perfectly matched wild-type homoduplex. Thus, all point mutants are converted to a form easily separated from the predominant wild type form.

While DGGE and CDGE both permit the study of mixtures of mutant heteroduplexes, the use of slab gels employing radioactive labels and high temperature (60°C) requires several days for completion and also results in substantial chemical alteration of DNA from hydrolysis and radiolysis (5). We reasoned that these problems could be significantly reduced if one were able to adapt the differential melting process to capillary electrophoresis with nonradioactive DNA labels. Capillary electrophoresis permits high speed separation of biopolymers, in particular DNA (6). Even so, cross-linked gel filled capillaries which yield ultrahigh resolution suitable for DNA sequencing (7,8) do not permit operation above room temperature or easy

* To whom correspondence should be addressed

replacement. Linear polyacrylamide (9) or other polymeric sieving matrices (10–12) are stable at temperatures up to at least 60°C for reasonable periods of time and are now generally substituted for gel-filled capillaries in the separation of dsDNA and even have been used for single stranded fragments in DNA sequencing (13). Furthermore, when working with linear polyacrylamide matrices of moderate viscosities, it is possible to replace the material by the simple expedient of introducing a new aliquot after each run. This procedure permits highly reproducible conditions for serial separations using the same capillary.

We have combined the several elements of replaceable linear polyacrylamide capillary electrophoresis and constant denaturant gel electrophoresis into a new and quite useful approach, which we call constant denaturant capillary electrophoresis (CDCE). The new method is a fast and high resolution procedure with high dynamic range and is automatable. It is well suited to detect mutations at low fraction levels such as required in mutational spectrometry (14), in detection of cancer cells in tissues or in the simultaneous screening of pooled human tissue samples for detection of polymorphisms or rare mutations. The possibility of capillary electrophoresis for mutational analysis was briefly discussed previously (15), but the actual conditions necessary for CDCE operation with the facility reported herein have not been previously reported.

MATERIALS AND METHODS

Equipment

The instrumentation was similar to that described previously (16). Briefly, the power supply was 30 kV dc (Model CZE 1000R-2032, Spellman, Plainview, NY). A low power argon ion laser (Omnichrome, Chino, CA) was run at 5 mW. The laser beam was filtered through a 488 nm (10 nm bandwidth) filter (Corion, Holliston, MA), and focused into the horizontal separation capillary. The focusing lens, capillary and objective were mounted onto 3-D micropositioners, and the system was placed on an optical bench. Emitted light was collected by a microscope objective (Oriel, Stamford, CT) at right angles to both the capillary and the beam and directed through two 520 nm (10 nm bandwidth) filters (Corion) into a photomultiplier (Oriel). The signal from the photomultiplier was amplified (10^8 V/A) by a current preamplifier (Oriel) and recorded by MP100 data acquisition system (Biopac Systems, Goleta, CA).

Capillary electrophoretic conditions

Electrophoresis was performed at various electric fields in 75 μm ID, 350 μm OD capillaries (Polymicro Technologies, Inc., Phoenix, AZ), coated as described elsewhere (17), and filled with 6% linear polyacrylamide, 3.3 M urea, 20% (v/v) formamide in TBE (89 mM Tris, 89 mM boric acid and 1 mM EDTA, pH 8.3). In one example, Fig. 4C, the denaturants, urea and formamide, were omitted.

Acrylamide was polymerized in 10 ml glass syringes under conditions favoring formation of long polymer chains (13). After polymerization was completed, the syringe could be stored at −15°C for several weeks. Linear polyacrylamide from a 10 ml syringe was dispensed into 100 μl high pressure gas-tight syringes (Hamilton, Reno, NV) as needed. The syringes were used to replace the polyacrylamide media before each run (2μl per run). Typically, a capillary was used for several weeks and withstood hundreds of replacements.

In cases where the exact amount of DNA injected into a capillary was important, e.g. for calibration of the detector, samples of 0.2 μl in water or dilute buffer (not higher than 0.1×TBE) were electroinjected into a capillary (1 μA for 2 min) from a 350 μm I.D. Teflon tube mounted onto the injection end of the capillary. Once the detector was calibrated, the samples were loaded directly from a larger sample volume (typically 5 μl) by applying currents of the order of 1 μA for several seconds. As much as 85% of the DNA material from 2 μl samples could be injected in one injection (1 μA for 2 min).

DNA

Total DNA was isolated from frozen human male TK-6 lymphoblasts (18). The DNA fragment of interest was amplified with *Pfu* DNA polymerase (Stratagene, La Jolla, CA) according to the manufacturer's protocol, purified by standard 8% PAGE and quantified by ethidium bromide staining. The primers CW7 (5′-ACCGTTAACTTCCAATTAAC), CW7mut (5′-ACCGTTAACTTCCAATTAACTAGTTTTGATAACATTCAAA) and 5′-fluorescein labelled, as well as non-labelled J3 (5′-ATGGAGAAAGGGACGCGGGC) were obtained from Synthetic Genetics (San Diego, CA). The melting map of the wild type DNA fragment and the positions of the primers are shown in Fig.1. In the case of radioactive detection on DGGE, J3 was 5′ labelled with ^{32}P prior to PCR amplification. To introduce a GC to AT substitution, the primer CW7mut, which formed a T:C mismatch with the template at position 30, was substituted for CW7 in a PCR reaction. The introduction of the point mutation was confirmed by sequencing of the PCR product, as previously described (19). For boiling and reannealing, DNA in 5 μl of water was heated to 100°C for 10 sec, then adjusted to 200 mM NaCl, 10 mM Tris HCl pH8, 2 mM EDTA and incubated at 60°C for half an hour.

RESULTS AND DISCUSSION

DNA sample

A 206 bp DNA sequence, see Fig.1, identified in the human mitochondrial genome, has a contiguous low melting domain (112 bp) and a high melting domain (94 bp), which upon partial melting permits the separation of heteroduplexes from wild-type homoduplexes (20). Two sequences differing by a single bp were used to demonstrate the process: the first, designated GC, had a GC base pair in position 30 bp from the terminus of the low melting domain, while the second designated AT had an AT base pair in this position. Both of these sequences are PCR amplified by the same set of primers, CW7 (low melting domain) and J3 (high melting domain).

Boiling and reannealing an equimolar mixture of GC and AT sequences created four species, the two homoduplexes plus two mismatched heteroduplexes, the latter resulting from the cross hybridization of GC and AT. These two heteroduplexes are called GT and AC, respectively. In some cases single stranded DNA was also included in the sample. Figure 2 shows the separation of the four species by DGGE using radioactive detection with ^{32}P. The single-strand fragment (ss) migrated the furthest, followed by the reannealed fragments in the order of thermal stability of the low melting domain of GC > AT > GT > AC.

The identity of GC and AT homoduplexes and single stranded DNA was determined by running pure species in separate lanes. To identify the two heteroduplex peaks, asymmetrically labelled AT (only the strand of the homoduplex containing A at position

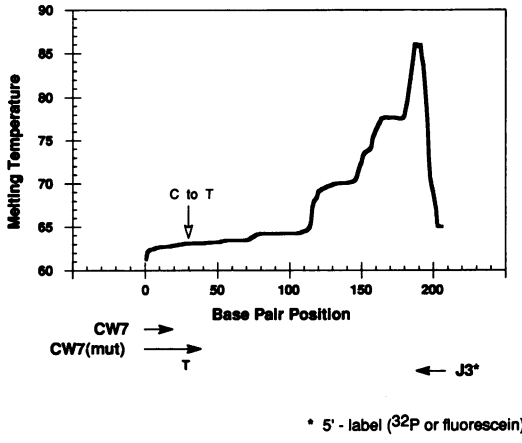

Figure 1. Melting map of the wild-type (GC) DNA fragment calculated according to the melting algorithm of Lerman and Silverstein (20). CW7, CW7(mut) and J3: positions of PCR primers. The vertical arrow indicates the position of the single base pair difference in the sequences used. Base pair one on the map corresponds to bp 10,011 of the human mitochondrial genome.

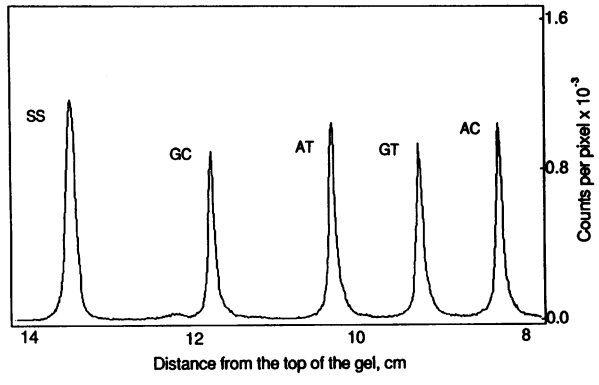

Figure 2. Example of typical slab DGGE separation. An equimolar mixture of two homoduplexes (GC and AT) and two heteroduplexes (GT and AC) was separated under standard DGGE conditions optimized for the separation of these particular sequences: 16 hours, 60°C; gel: 12% acrylamide, 0.4% bis–acrylamide, 1×TAE; 20 cm linear denaturant gradient: top: 6% (v/v) formamide, 1.05 M urea, bottom: 14% (v/v) formamide, 2.8 M urea. DNA was end labelled with ^{32}P; the gel was dried and scanned with a Phosphoimager (Molecular Dynamics). SS indicates a single stranded fragment showing that it has migrated to a position beyond the focused dsDNA duplexes.

30 was labelled) was reannealed with unlabelled GC. This procedure yielded labelled AT and AC and unlabelled GC and GT. The DGGE separation of such partially labelled mixture showed that the AC heteroduplex migrated as the least stable DGGE band.

Behavior of the sample on CDCE

While in principle a denaturing or temperature gradient could be created within a capillary column, we found it simpler to use constant denaturing conditions. Constant denaturant conditions were achieved by the combination of a column temperature (36°C) and a denaturant buffer (3.3M urea plus 20% (v/v) formamide). The temperature was elevated by a water jacket over a 10 cm portion of the capillary (the 'denaturing zone'). The column regions outside the denaturing zone were close to ambient

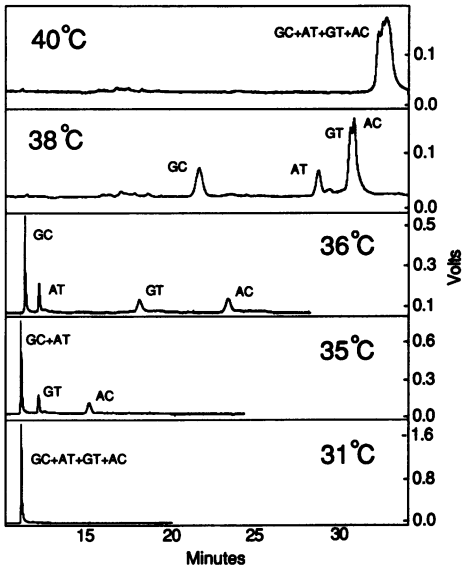

Figure 3. CDCE separation as a function of column temperature. The sample of Fig. 2 was prepared using fluorescein labelled DNA fragments and run on a capillary at the several temperatures indicated. An equal amount of sample was injected per run. Column: 6% T linear polyacrylamide, 3.3 M urea, 20% (v/v) formamide in TBE. Electric field: 250 V/cm.

temperature. In these regions all four double-stranded DNA species were unmelted and migrated with identical velocities.

Figure 3 demonstrates important aspects of CDCE through an examination of the role of column temperature on the peak pattern. At 31°C, a single peak was observed. This peak contains all four sample duplexes in the unmelted form. At 35°C, two extra peaks were obtained that migrated more slowly than dsDNA. These peaks were the more unstable of the four duplexes, the heteroduplex AC and the more stable heteroduplex GT. (The identities of the peaks in CDCE were determined as in Fig. 2).

At 36°C four peaks were observed, the two homoduplexes GC and AT, as well as the two heteroduplexes GT and AC. As the temperature rose to 38°C and 40°C, the separation varied until all four species had identical low velocities. This we interpret to mean that all four were fully converted to the partially melted form in the denaturing zone and thus had identical velocities in the electrophoretic field.

Assuming that the partial melting/reannealing process occured rapidly, the migration time of each band will be dependent on the equilibrium constant K of the partial melting process.

$$K = \frac{X_{pm}}{X_{um}} \quad (1)$$

where X is the mole fraction of a fragment and subscripts pm and um refer to the partially melted and the unmelted fragments. The electrophoretic velocity of a species n in the denaturing zone can be written as

$$\nu = X_{um}\nu_{um} + X_{pm}\nu_{pm} \quad (2)$$

where ν_{um} and ν_{pm} are velocities of unmelted and partially melted fragments, respectively. It is clear from eqns (1) and (2) that as K increases with temperature in the denaturing zone, the

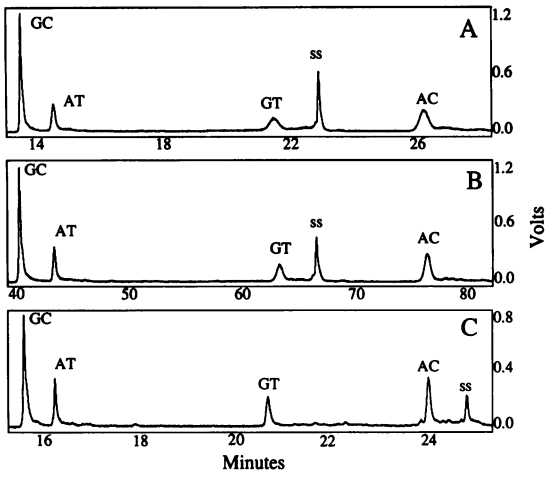

Figure 4. Effects of electric field strength and temperature on CDCE resolution. (A) 3.3 M urea, 20% formamide, 36°C, 250 V/cm. (B) 3.3 M urea, 20% formamide, 36°C, 83 V/cm. (C) 0 M urea, 0% formamide, 63°C, 125 V/cm.

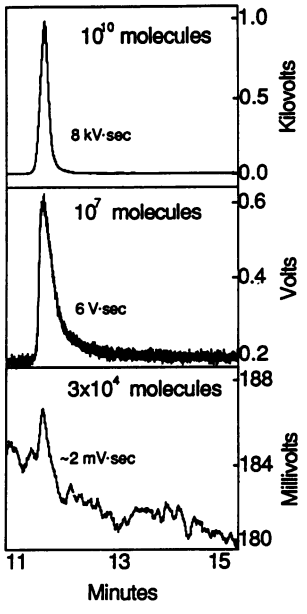

Figure 5. Dynamic range of the system. The indicated amounts of GC homoduplex were injected into the capillary from 0.2 μl samples and run at 22°C. For 10^{10} or more copies, signal amplification was reduced tenfold. For 3×10^{4} copies, the laser power was increased 5 fold, and the signal was averaged over 10 second intervals surrounding each point of the curve. Numbers indicate peak area corrected for differences in laser power or amplification.

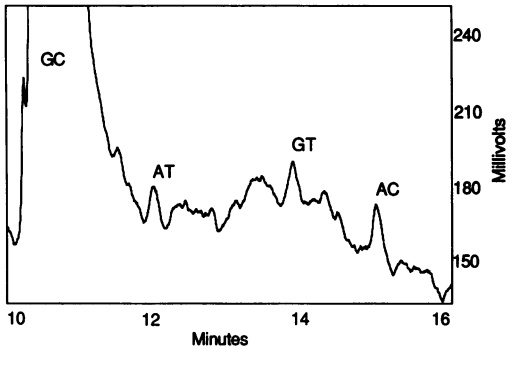

Figure 6. Detection of small mutant fractions in the presence of large excess of wild-type DNA AC and GT mutant heteroduplexes, 10^{5} copies each, were admixed to 3×10^{8} copies of wild-type GC homoduplex and run on a capillary at 36.5°C. The signal was averaged over 10 second intervals.

velocity of a DNA species will decrease from ν_{um} to ν_{pm} (in Figure 3, $\nu_{um} \sim 4\ \nu_{pm}$). It is worth noting that by measuring ν as a function of temperature, along with the values of ν_{pm} and ν_{um}, one can obtain the thermodynamic parameters of DNA melting equilibrium inside the capillary.

Based on the results of Figure 3, a simple procedure defines the conditions necessary to detect the set of all point mutations in a specific DNA sequence. The approximate melting temperature of the low melting domain can be first calculated using Lerman's algorithm, with appropriate correction for the concentration of denaturant (20). This estimated temperature can then be refined by performing test runs of the wild-type sequence at different temperatures. The optimal temperature for the separation of a set of related homoduplexes would be that at which the wild-type sequence is 50% partially melted (as for GC at 38°C in Fig. 3). The optimal temperature for separation of a wild type homoduplex from the set of all related heteroduplexes would be the one at which the wild type is just slightly melted (as for GC at 36°C in Fig. 3). Optimization for a novel sequence is completed in only a few runs. Note that optimization does not require any prior information about the melting behavior of expected mutants.

Resolution characteristics

A striking feature of Figure 3 is the significant increase in peak width of the partially melted DNA fragments relative to the unmelted form (even after correction for mobility differences). Since resolution (defined as the difference in migration times for two species divided by their average peak width) of the partially melted forms is important for the separation of mixtures of mutant species, we explored the role of several operational parameters on resolution in terms of the separation of the two heteroduplexes GT and AC.

Figure 4 presents electropherograms under several conditions. Fig. 4A repeats the separation of Fig. 3 at 36°C and 250 V/cm, while Fig. 4B shows the separation at 1/3 the field, or 83 V/cm. In Fig. 4C, the temperature of the hot zone is elevated to 63°C at a field of 125 V/cm, and no chemical denaturant is in the capillary. To permit visual comparison of the electropherograms, the time axes of the three runs have been adjusted such that the bands appear to coincide.

A three-fold increase in the time spent by the bands in the denaturant zone (Fig. 4B) improves resolution of 1.6 fold over that in Fig. 4A. This surprising result suggests that the band widths for the partially melted forms are controlled by slow kinetics within the separation process, rather than by diffusion. Such kinetics could arise from interactions of the partially melted form with the linear polyacrylamide or from the melting-reannealing process itself.

Based on this finding, we examined the possibility of using a higher temperature for CDCE. Fig. 4C shows the result at 63°C with no denaturant in the capillary. As expected, resolution was

improved by 2.6 fold, primarily due to sharper bands, but with no increase in separation time. Apparently, the higher temperature and lower viscosity accelerated the rate limiting the separation process leading to narrower bands. The position of ssDNA also shifted due to the different operating conditions.

The role of replaceable polyacrylamide

Critical to the practical application of these experiments is the ability to replace the matrix after each run. Using replaceable sieving matrices, as in the case of DNA sequencing (13) or dsDNA separations (21), excellent migration time reproducibility was observed from run-to-run, with about ± 2% relative standard deviation. Since the melting temperature will be quite sensitive to the medium (e.g. ionic strength), linear polyacrylamide/buffer replacement after each run provides a highly reproducible medium for maintaining a constant fraction of partially melted species.

This point cannot be over-emphasized. If samples were injected a second time into the column under the conditions of Fig. 3 without matrix replacement, only a single-stranded peak was obtained, as confirmed by injection of a predenatured sample. Upon matrix replacement, the appropriate four peak pattern was again observed.

The reason for the appearance of only a single stranded fragment with the second injection into a 'used' matrix may be the formation of a zone of low conductivity found at the cathodic end of the capillary. The zone is due to transference number differences for buffer ions in the bulk solution and the sieving matrix (21). Since the voltage drop across this small zone can be high, Joule heating can occur, and this heat was likely sufficient under our conditions to melt the DNA fragments fully to single stranded species. Probably, the melting temperature was also reduced in this low conductivity 'hot zone' due to low ionic strength, and this further aided the denaturing of dsDNA. It is possible to reduce this ion depletion effect (22) and thus to avoid this denaturing step; however, ionic strength changes may still be sufficient to result in poor reproducibility in migration times among serial runs. Moreover, in the replaceable format, denaturing buffer conditions can be rapidly changed when desired.

Dynamic range

Laser induced fluorescence provides a means to detect small numbers of DNA molecules labelled with fluorescent dyes. Nevertheless, in some cases (including mutational spectrometry) the ability to load large numbers of DNA copies is also critical. As many as 10^{11} copies could be loaded and measured from a 0.2 μl sample as shown in Figure 5. However, beyond 10^{10} molecules peak distortion was observed. A peak with as few as 3×10^4 copies of dsDNA was visible above the system background using higher laser power (25 mW) and digital filtering. Because fluorescence signals corresponding to more than 10^{10} DNA molecules may exceed the linear dynamic range of our detector, it was necessary to reduce the detector attenuation. Calibration plots of number of molecules vs. peak area were found to be essentially linear throughout the range of 3×10^4 to 10^{11} molecules.

While even lower detection levels are in principle possible (24), a more critical limit of detection is the fraction of a mutant that can be observed in the presence of a large excess of wild-type homoduplexes. Figure 6 demonstrates that as small as 0.03% admixture of mutant heteroduplexes with the wild-type homoduplex can be determined. The background noise which interferes with identification of mutants at low fractions apparently consists of chemically reacted wild-type DNA molecules (5). (A chemical reaction in either strand will cause a drop in wild-type homoduplex melting temperature.) Reduction of this 'chemical noise' will be important to allow lower fractions of mutants to be observed. In our ongoing studies we find CDCE a valuable aid in identifying the sources of these unwanted reaction products. Note also that CDCE can be used as a purification method to remove the major portion of the wild-type homoduplex peak from the mutant fraction. Reinjection of the collected mutant heteroduplex region should in principle allow lower level mutant fraction determinations.

CONCLUSIONS

A new method for mutational analysis, constant denaturant capillary electrophoresis (CDCE), has been presented. The method is rapid with excellent resolution of wild-type homoduplexes from mutants converted to wild-type/mutant heteroduplexes in less than 30 minutes. CDCE is sensitive with a wide linear dynamic range using laser induced fluorescence detection of 3×10^4 to 10^{11} sequence copies. Such column operations are readily automated, and throughput could be increased by parallel processing of multiple capillary arrays (25).

CDCE, though useful for the identification of inherited mutations and polymorphisms, should be particularly suitable for detection of low fraction somatic mutants, known and unknown, at specific DNA loci, such as in mutational spectrometry (14), and for screening of tissues for mutant cancer cells and related areas. Equally, the method could be applied to the detection of rare genetic alterations in pooled samples. In this case hundreds of samples which are to be screened for the presence of unknown point mutations in a particular DNA locus could be pooled, amplified and examined by CDCE. If the pool were positive, the bearer of each alteration may be rapidly identified by the screening of subpools. Finally, the speed and simplicity of the method ought to allow studies on the thermodynamics and kinetics of melting of DNA and protein–DNA interactions governed by cooperative equilibria. Work is continuing on the development and application of CDCE.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Barnett Innovative Research Fund (BLK) and grants from the National Institute of Environmental Health Sciences and The Office of Health and Environmental Research (U.S. Dept. of Energy) (WGT) for support of this work. The authors further acknowledge Dr A.Cohen and Dr P.Keohavong for early studies in this field. Stimulating discussions with Dr J.Berka are also acknowledged. Contribution #580 from the Barnett Institute.

REFERENCES

1. Fisher, S.G. and Lerman, L.S. (1983) *Proc. Natl. Acad. Sci. USA* 80, 1579–1583.
2. Cariello, N.F., Swenberg, J.A., DeBellis, A and Skopek, T.R. (1991) *Environ. Mol. Mutat.* 18, 249–254.
3. Hovig., E., Smith-Soresen, B., Brogger, A. and Borresen, A.-L. (1991) *Mutat. Res.* 262, 63–71.
4. Thilly, W.G. (1985) *Carcinogenesis* (Raven Press, New York), Vol. 10, pp. 511–528.

5. Hanekamp, J.S. (1993) Ph. D. Thesis (MIT).
6. Cohen, A.S., Najarian, D.R., Paulus, A., Guttman, A., Smith, J.A. and Karger, B.L. (1988) *Proc. Natl. Acad. Sci. USA* 85, 9660–9663.
7. Drossman, H., Luckey, J.A., Kostichka, A.J., D'Cunha, J. and Smith, L.M. (1990) *Anal. Chem.* 62, 900–903.
8. Swerdlow, H., Zhang, V.Z., Chen, D.Y., Harke, H.R., Grey, R., Wu, S. and Dovichi, N.J. (1991) *Anal. Chem.* 63, 2835–2841.
9. Heiger, D.N., Cohen, A.S. and Karger, B.L. (1990) *J. Chromatogr.* 516, 33–48.
10. Schwartz, H.E., Ulfelder, K., Sunzeri, F.J., Busch, M.P. and Brownlee, R.G. (1991) *J. Chromatogr.* 559, 267–283.
11. Bocek, P. and Chrambach, A. (1992) *Electrophoresis* 13, 31–34.
12. Zhu, M., Hansen, D.L., Burd, S. and Gannon, F. (1989) *J. Chromatogr.* 480, 311–319.
13. Ruiz-Martinez, M.C., Berka, J., Belenkii, A., Foret, F., Miller, A.W. and Karger, B.L. (1993) *Anal. Chem.*,65, 2851–2858.
14. Thilly, W.G. (1991) *Carcinogenesis* (Raven Press, New York), Vol.16, 127–133.
15. Cohen, A.S., Karger, B.L., Keohavong, P. and W.G. Thilly (1991) HPLC '91, Basel, Switzerland (Abstract).
16. Cohen, A.S., Najarian, D.R. and Karger, B.L. (1990) *J. Chromatogr.* 516, 49–60.
17. Hjerten, S. (1985) *J. Chromatogr.* 347, 191–198.
18. Skopek, T.R., Liber, H.L., Penman, P.W. and W.G. Thilly (1978) *Biochem. Biophys. Res. Commun.* 84, 411–416.
19. Keohavong, P. and W.G. Thilly (1989) *Proc. Natl. Acad. Sci. USA* 86, 9253–9257.
20. Lerman, L.S. and K. Silverstein. (1987) *Meth. Enzymol.* 155, 482–501.
21. Pariat, Y.F., Berka, J., Heiger, D.N., Schmitt, T., Vilenchik, M., Cohen, A.S., Foret, F. and Karger, B.L. (1993) *J. Chromatogr.*,652, 57–66.
22. Spencer, M. (1983) *Electrophoresis* 4, 36–41.
23. Swerdlow, H., Dew-Jager, K., Brady, K., Grey, R., Dovichi, N.J. and Gesteland, R. (1992) *Electrophoresis* 13, 475–483.
24. Castro, A., Fairfield, F.R. and Shera, E.B. (1993) *Anal. Chem.* 65, 849–852.
25. Kambara, H. and Takahashi, S. (1993) *Nature* 361, 565–566.