4998–5003 *Nucleic Acids Research, 1996, Vol. 24, No. 24*

Detection of all single-base mismatches in solution by chemiluminescence

Norman C. Nelson*, Philip W. Hammond, Eiji Matsuda, Anil A. Goud and Michael M. Becker

Gen-Probe Incorporated, 9880 Campus Point Drive, San Diego, CA 92121, USA

Received July 25, 1996; Revised and Accepted November 6, 1996

ABSTRACT

A rapid in-solution method for the detection of all 12 single-base mismatches is described. The technique is based on the hybridization protection assay (HPA) format that utilizes oligonucleotide probes labeled with a highly chemiluminescent acridinium ester (AE). Hydrolysis by weak base renders AE permanently non-chemiluminescent. When an AE-labeled probe hybridizes to an exactly complementary target, AE is protected from hydrolysis relative to the unhybridized conformation. Single-base mutations in the duplex adjacent to the site of AE attachment disrupt this protection resulting in rapid AE hydrolysis and loss of chemiluminescence. The discrimination effect was seen in both DNA and RNA. Studies of T_m values revealed that this effect is not due to a decrease in the overall stability of the duplex, suggesting the AE is responding to local structural changes in the double helix induced by mismatches. Using this principle all 12 single mismatches were clearly discriminated from the corresponding matched sequences. The assay is homogeneous, simple, sensitive, applicable to both amplified and non-amplified targets, and is completed in 30–60 min. An example showing discrimination between wild-type and mutant sequences corresponding to the reverse transcriptase coding region of HIV-1 is given.

INTRODUCTION

Rapid advances in the field of molecular genetics coupled with the large amount of sequence data made available through the Human Genome Project have led to a dramatic increase in the identification of genetic mutations which are associated with human disease. Often the genetic mutation is simple, consisting of a single base substitution or the insertion or deletion of a small number of bases. The need for effective methods to detect and identify such known mutations as well as to screen for unknown mutations has also increased dramatically. As a result, many new techniques for mutational analysis have emerged in recent years.

The single-stranded conformational polymorphism (SSCP) procedure (1,2) for detecting unknown mismatches relies on differences in electrophoretic mobility between fragments which differ by as little as a single nucleotide. Related methods include constant denaturant capillary electrophoresis (CDCE; 3), temperature gradient gel electrophoresis (TGGE; 4,5) and directed heteroduplex analysis (DHDA; 6,7). Chemicals (8) or enzymes (9–11) which cleave preferentially at mismatches or proteins (12–14) which preferentially bind mismatches have also been used to detect unknown mutations.

A number of techniques for detecting known mutations, including direct sequencing of DNA (15,16), have also emerged. The amplification refractory mutation system (ARMS; 17), mutagenically separated PCR (MS–PCR)(18) and the mismatch amplification mutation assay (MAMA; 19) utilize differences in PCR amplification efficiencies to discriminate between mutant and wild-type targets. Other methods have utilized hybridization with sequence specific oligonucleotides, such as ASOs (allele-specific oligonucleotides) in a conventional dot blot format (20) or in a dot blot followed by direct sequencing format (21), to identify known mutations.

We present a sensitive method for detecting all single-base mismatches which is simple, rapid and performed completely in solution. The method is based on the hybridization protection assay (HPA) format which utilizes the highly chemiluminescent acridinium ester (AE) molecule as a reporter group (22). Alkaline hydrolysis of the ester bond of AE renders it permanently non-chemiluminescent. When covalently attached to a single-stranded oligonucleotide probe, hydrolysis of AE is rapid. In contrast, when the probe hybridizes to a complementary target nucleic acid, the hydrolysis rate of AE is greatly reduced. Detection of a nucleic acid target by the HPA format therefore entails hybridizing an AE-labeled probe in solution to target nucleic acid under non-hydrolyzing conditions, adjusting the solution to mildly alkaline pH to hydrolyze AE associated with unhybridized probe, and detecting in a luminometer the non-hydrolyzed, hybrid-associated AE as a measure of the target nucleic acid present.

We previously reported that a single T·G mismatch in a duplex immediately adjacent to the site of AE attachment abolished the ability of the duplex to protect AE from hydrolysis. Thus, the HPA format was capable of rapidly distinguishing matched and T·G mismatch-containing duplexes by virtue of differences in AE hydrolysis rates (23). In the present study we examined whether

*To whom correspondence should be addressed. Tel: +1 619 546 7963; Fax: +1 619 452 5848

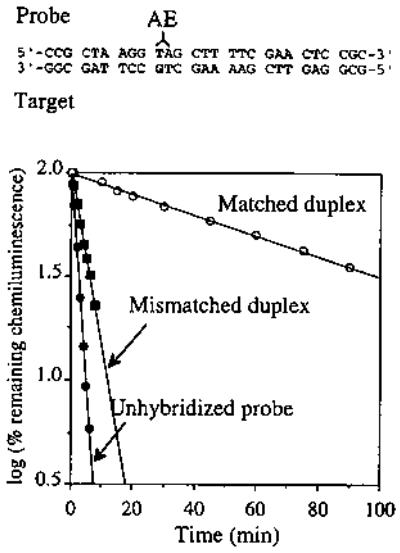

Figure 1. Kinetic analysis of ester hydrolysis for matched and T·G mismatched duplexes and unhybridized acridinium ester-labeled probe. The loss of chemiluminescence was measured (Methods and Materials) and plotted as the log of the percent remaining chemiluminescence versus time in minutes. A line was fitted to the points and a slope determined using standard linear regression analysis. The sequence of the AE-probe and the mismatched target used for this example are given for reference.

all single-base mismatches could be detected by the HPA format and found that all 12 single-base mismatches could be clearly distinguished from the corresponding matched duplex. The assay format consists of three steps: hybridization in solution, hydrolysis of AE linked to unhybridized probe and mismatched duplex and detection of remaining chemiluminescence (corresponding to matched duplex) in a luminometer. This assay is rapid and simple to perform, rendering the assay a practical method for detection and identification of known mismatches. The method is exemplified by detection of wild-type and mutant sequences corresponding to the codon 181 reverse transcriptase coding region of HIV-1.

MATERIALS AND METHODS

Synthesis and purification of acridinium ester-labeled DNA probes

DNA and RNA oligomers were synthesized using standard phosphoramidite chemistry and purified using standard polyacrylamide gel electrophoresis. For probe sequences, amine-terminated linker arm was incorporated at a predetermined position in each oligomer during synthesis using an abasic linker arm chemistry described previously (24). The oligomers were then labeled with acridinium ester (AE) and purified using high performance liquid chromatography as described previously (25).

Measurement of AE hydrolysis rates

The rates of hydrolysis for AE attached to oligonucleotide probes were determined as described previously (22). Briefly, 0.1 pmol AE-labeled probe and 1 pmol target nucleic acid (matched or mismatched, DNA or RNA) were combined in 30 µl of hybridization buffer [0.1 M lithium succinate, pH 5.0, 5% lithium lauryl sulfate (w/v), 400 mM LiCl, 1 mM EDTA and 1 mM EGTA] and incubated at 50°C for 30 min. The reaction mixture was then diluted with 270 µl of the same buffer, and 10 µl aliquots were placed in replicate 12 × 75 mm polystyrene tubes. To each tube was added 100 µl hydrolysis buffer (0.19 M $Na_2B_4O_7$, pH 7.6, 5% Triton X-100) and the tubes were placed at 50°C. At various time points 200 µl of 0.4 N HCl, 0.1% H_2O_2 were added to one of the replicates and the chemiluminescence was measured in a LEADER® I luminometer (Gen-Probe Incorporated, San Diego, CA) with the automatic injection of 1 N NaOH followed by a 5 s read time. The data were plotted as the log of the percent remaining chemiluminescence (compared with time zero) versus time and hydrolysis rates were calculated from the slope using standard regression analysis.

Determination of the thermal stability of mismatched duplexes

AE-labeled probes were hybridized with various mismatched targets as described above, then diluted to 1 ml with hybridization buffer. Aliquots of 50 µl were placed in replicate 12 × 75 mm polystyrene tubes, and each replicate incubated for 7 min at one of several temperatures between 52 and 80°C (2°C increments). The samples were then placed on ice until all replicates at all temperatures had been incubated. Hydrolysis buffer (150 µl) was added to each sample followed by incubation at 50°C for 17.5 min. The chemiluminescence of each sample was then measured as described above. The resulting data were plotted as temperature versus percent remaining chemiluminescence, and the temperature at which 50% of the hybrid signal was lost (T_m) was determined graphically. To confirm that this chemiluminescence method yielded accurate melting temperatures, optical melts were performed on several duplexes with the result that both methods yielded similar T_m values (data not shown).

Detection of HIV wild-type and mutant sequences in a full assay format

Probes specific for wild-type and mutant sequences corresponding to the HIV-1 reverse transcriptase coding region (see text) were synthesized with the linker placed at the site of the mutation. The wild-type and mutant AE-probes were hybridized with decreasing amounts of either the wild-type or mutant target sequences, respectively, in the absence or presence of a constant amount of the other target, in 100 µl 0.1 M lithium succinate, pH 5.2, 8.5% lithium lauryl sulfate (w/v), 1.5 mM EDTA and 1.5 mM EGTA at 62.5°C for 30 min. Hydrolysis buffer (300 µl) was added and the samples were incubated at 62.5°C for an additional 12.5–15 min. The chemiluminescence of each sample was then measured in a LEADER® 50 luminometer (Gen-Probe Incorporated, San Diego, CA) with the automatic injection of 0.1% H_2O_2, 1 mM HNO_3, followed by automatic injection of 1 N NaOH and a 5 s read time.

RESULTS AND DISCUSSION

Hydrolysis of AE in mismatched DNA duplexes

Acridinium ester is attached to oligonucleotide probes through use of an abasic linker-arm chemistry (24). The linker adds one ethylene phosphate group to the backbone of the probe which bulges out from the double helix upon hybridization to target nucleic acid, thus allowing complementary bases to hydrogen bond to one another.

Table 1. Detection of all 12 single mismatches in DNA by AE chemiluminescence

Basepair Identity		Match/Mismatch S/N[a]		Basepair Identity		Match/Mismatch S/N	
Mismatch	Neighbor[b]	3'[c]	5'[c]	Mismatch	Neighbor	3'	5'
G•G	G•C	72.6	73.1	T•G	G•C	27.9	77.8
	C•G	91.9	55.8		C•G	36.8	64.1
	T•A	81.2	71.7		T•A	24.2	70.1
	A•T	75.6	89.4		A•T	32.1	88.8
G•T	G•C	76.3	45.4	T•C	G•C	14.0	14.0
	C•G	85.2	39.5		C•G	27.9	7.4
	T•A	75.6	55.8		T•A	12.2	10.0
	A•T	77.3	69.6		A•T	14.0	10.0
G•A	G•C	70.1	59.9	T•T	G•C	15.0	13.1
	C•G	91.9	42.3		C•G	10.4	10.5
	T•A	80.0	55.8		T•A	1.0	10.5
	A•T	72.1	76.8		A•T	2.7	16.0
C•C	G•C	13.1	12.2	A•G	G•C	48.6	70.6
	C•G	32.1	6.0		C•G	61.5	29.9
	T•A	16.0	5.3		T•A	15.0	48.6
	A•T	11.3	32.1		A•T	21.2	67.8
C•T	G•C	24.2	42.3	A•C	G•C	31.2	13.1
	C•G	22.7	18.5		C•G	43.8	17.2
	T•A	2.7	24.2		T•A	24.2	9.2
	A•T	15.0	36.8		A•T	9.9	10.5
C•A	G•C	36.8	45.4	A•A	G•C	39.5	11.3
	C•G	39.5	26.0		C•G	49.3	8.1
	T•A	17.2	26.0		T•A	14.0	10.0
	A•T	16.0	34.4		A•T	6.1	14.0

[a]Signal-to-noise (S/N) ratio for every single-base mismatch was determined by dividing the percentage of chemiluminescent signal remaining for a matched hybrid by the percentage of chemiluminescent signal remaining for the corresponding mismatched target after both were hydrolyzed for time (t), where (t) is equal to seven times the hydrolysis half-life of the mismatched hybrid.
[b]Each mismatch is neighbored by one of four possible matched base pairs on the opposite site of the linker arm.
[c]Each mismatch is located either on the 3′ or the 5′ side of the linker arm as indicated.

Table 2. Detection of mismatches in RNA versus DNA

Basepair Identity			Match/Mismatch S/N[a]	
Mismatch	Neighbor	Position	RNA	DNA
G•A	G•C	5'	46.6	59.9
C•A	G•C	5'	28.1	45.4
T•G	G•C	5'	77.3	77.8
T•T	G•C	5'	13.4	13.1
G•A	T•A	3'	52.4	80.0
C•C	T•A	3'	14.0	16.0
T•C	T•A	3'	16.9	12.2
A•C	T•A	3'	22.7	24.2
G•G	T•A	3'	55.0	81.2
G•T	T•A	3'	50.3	75.6

[a]Signal-to-noise (S/N) ratio for each of the indicated single-base mismatches was determined as described in Table 1.
[b]Each mismatch is neighbored by one of four possible matched base pairs on the opposite site of the linker arm.
[c]Each mismatch is located either on the 3′ or the 5′ side of the linker arm as indicated.

The presence of the linker (with or without AE) has a negligible effect on the T_m of the double helix (data not shown), suggesting minimal structural defects caused by the linker.

To examine the ability of AE-labeled probes to detect all 12 single-base mismatches, 16 AE-labeled DNA probes consisting of the oligonucleotide model sequence

5′-CCGCTAAGG**N** (AE) **N**GCTTTTCGAACTCCGC-3′

were synthesized where **N** represents any of the four bases and AE represents the site of attachment of AE. The corresponding 16 exact DNA complements were also synthesized. By hybridizing each AE-labeled probe with various complementary targets, 96 duplexes containing a single mismatch immediately adjacent to the AE were generated. Half of the duplexes contained a mismatch on the 5′ side of the linker, each mismatch neighbored by one of four possible matched base pairs on the 3′ side of the linker arm, and half of the duplexes contained a mismatch on the 3′ side of the linker, each mismatch neighbored by one of four possible matched base pairs on the 5′ side of the linker arm. The 16 perfectly matched duplexes were also generated. Mismatches more than one base away from the linker yielded smaller effects on AE hydrolysis rates (results not shown) and therefore only those bases immediately adjacent to the linker were varied.

Following hybridization, the hydrolysis rate of AE for each matched and mismatched duplex as well as each corresponding unhybridized probe was measured in borate buffer (pH 7.6) at 50°C as described in Materials and Methods. Under these conditions, the hydrolysis of AE is pseudo first-order with respect to hydroxide concentration, as is exemplified in Figure 1 with a T·G mismatch. From the observed kinetics, half-lives of hydrolysis were determined. To express the effect of a mismatch on the hydrolysis of AE in the duplex, the percentage of chemiluminescent signal remaining for a matched duplex (e.g. G·C) after the corresponding mismatched duplex (e.g. G·G) had been hydrolyzed to 0.78% (seven half-lives) of its initial value before hydrolysis was computed. The percentage signal remaining for the matched duplex was then divided by the percentage signal remaining for the mismatched duplex (i.e. 0.78%) to yield a matched/mismatched signal-to-noise ratio (S/N) for every single-base mismatch.

To be useful as an assay to discriminate matched and mismatched duplexes, the HPA technique should meet the following two criteria: (i) signal from mismatched duplex should be reduced to ≤1% of its initial value, and (ii) matched/mismatched S/N should be at least 10. Criterion 1 was satisfied by hydrolyzing AE associated with a given mismatched duplex for seven half-lives, yielding 0.78% remaining signal. As summarized in Table 1, each single-base mismatch yielded a matched/mismatched S/N of at least 10, thereby satisfying criterion 2. A few mismatches yielded S/N ratios of < 10 when placed on one side of the linker or the other, but no mismatches yielded S/N ratios of < 10 on both sides of the linker. Therefore, by judicious placement of the linker relative to the site of the mismatch, the goal of S/N ratios of ≥ 10 was achieved for all single-base mismatches. To determine if similar results would be observed in other sequence contexts, mismatch discrimination was examined in several different probe/target systems, with the result that the discrimination patterns were essentially the same as those given in Table 1 (results not shown).

The degree of discrimination (i.e. the magnitude of the S/N ratio) was dependent upon the identity of the bases in the mismatched pair. In general, mismatched base pairs containing one or more purines, particularly guanine, yielded higher S/N ratios than mismatches lacking purine bases, although mismatches

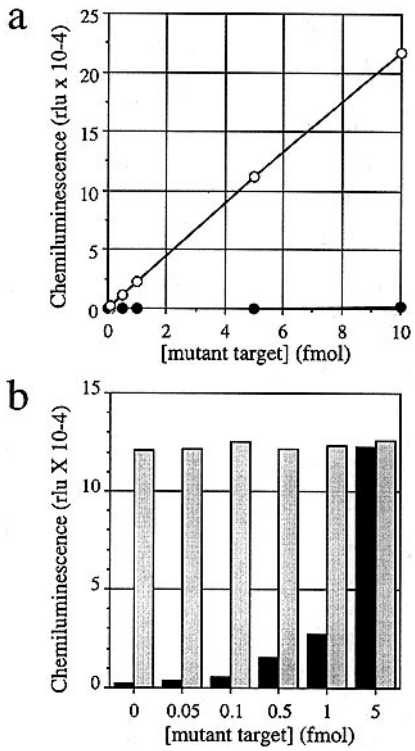

Figure 2. Analysis of a mutant HIV-1 reverse transcriptase coding region sequence containing a single A to G point mutation using the HPA format. (a) Various amounts of mutant target were hybridized with either a mutant-specific (○) or a wild-type-specific (●) AE-labeled probe (see text). Following hybridization, borate buffer (pH 7.6) was added, AE linked to unhybridized probe or mismatched duplex was hydrolyzed and the chemiluminescence of each sample measured. The data for mutant probe/mutant target were fit with a line using standard linear regression analysis (R^2 = 1.000). (**b**) Various amounts of mutant target and a constant amount of wild-type target were hybridized with either mutant-specific (■) or wild-type-specific (shaded) AE-labeled probe, then assayed as described in (a) above.

lacking purine bases were still detected at S/N ratios of ≥10. A second factor which affected the degree of discrimination was whether the mismatch was on the 5′ or the 3′ side of the linker. For example, for a C·T mismatch adjacent to a flanking T·A match, better discrimination was observed when the mismatch was on the 5′ side of the linker (S/N = 24.2) than when it was on the 3′ side (S/N = 2.7). This orientation factor was also observed for C·C mismatches. Lower S/N ratios were observed when the C·C mismatch was on the 5′ side of the linker and the 3′ flanking base of the probe strand was a pyrimidine (S/N = 5.3–6.5), but higher S/N ratios were observed when the C·C mismatch was on the 3′ side of the linker with any 5′ flanking base (S/N = 11.3–32.1) (Table 1).

Thermal stability of mismatched duplexes

To determine if the increased AE hydrolysis rates observed for the mismatched hybrids were due to thermal instability of the duplexes caused by the mismatches, the T_m's of 13 different mismatched duplexes described above were measured using a chemiluminescent method as described in Materials and Methods. Under the conditions of our experiments all T_m values ranged between 70 and 76°C, which is well above the 50°C temperature used for the AE hydrolysis rate measurements (results not shown). Thus, mismatches do not appear to enhance the hydrolysis of AE by destabilizing the overall double helix. We propose that the mismatch introduces a local structural defect in the double helix that renders AE more susceptible to hydrolysis. In support of this hypothesis, when the site of the AE linker was moved nine bases away from the mismatch site, the T_m and thus the overall stability of the mismatched hybrid remained the same but the ability of AE to detect the mismatch disappeared (results not shown).

Mismatch detection in RNA

Current diagnostic assays target not only DNA but also RNA, such as ribosomal RNA (26) and RNA amplification products (27). In order to compare the degree of mismatch discrimination seen in RNA with that seen in DNA using the HPA method, selected probe or target strands of the oligonucleotide model sequence described above were synthesized as RNA. AE hydrolysis rates were determined and the effect of each mismatch was calculated as described above. The results are shown in Table 2 with the corresponding data for DNA from Table 1 included for comparison. In most cases the results for RNA and DNA were essentially the same. In a few cases mismatches were detected in RNA less readily than in DNA and in one case a mismatch was detected more readily in RNA than DNA. Each mismatch, however, was detected with a S/N of > 10, demonstrating that the method described here can be used to detect single-base mismatches in RNA as well as DNA.

Detection of wild-type and mutant HIV-1 sequences

The differences in AE hydrolysis rates observed for matched and mismatched duplexes described above provide the basis for an assay format to rapidly detect and quantitate nucleic acid sequences containing known mutations. An AE-labeled probe exactly complementary to the mutant sequence is constructed such that the linker-arm is placed immediately adjacent to the site of the mismatch. This probe will yield a chemiluminescent signal when hybridized to the mutant sequence since AE will be protected from hydrolysis, but will not yield a signal when hybridized with the wild-type sequence since the resultant heteroduplex will contain a mismatch adjacent to the linker site and AE will be hydrolysed. An AE-probe exactly complementary to the wild-type sequence and therefore mismatched with the mutant sequence can also be constructed and used in a similar fashion. In this way both mutant and wild-type can be quantitated in a sample containing both sequences.

As a demonstration of principle, this method was utilized to detect mutant and wild-type sequences in HIV-1. A single A to G point mutation in the HIV-1 reverse transcriptase coding region at codon 181 causes broad resistance to non-nucleoside inhibitors such as nevirapine. Probes to this region of HIV-1 (28) were synthesized for wild-type as well as mutant HIV-1 sequences with the linker placed at the site of the mutation [5′-AT(AE)A-3′ for wild-type and 5′-AC(AE)A-3′ for mutant]. Wild-type probe with mutant target produces a 5′ T·G mismatch and mutant probe with wild-type target produces a 5′ C·A mismatch. Mutant probe or wild-type probe were hybridized with increasing amounts of mutant target under mildly stringent, non-hydrolyzing conditions. Borate buffer (pH 7.6) was added, AE linked to unhybridized probe or mismatched duplex was hydrolyzed and the remaining chemiluminescence of each sample measured in a luminometer. The results (Fig. 2a) demonstrate that the matched duplex (mutant probe/mutant target) was readily detected and accurately quantified, whereas the mismatched duplex

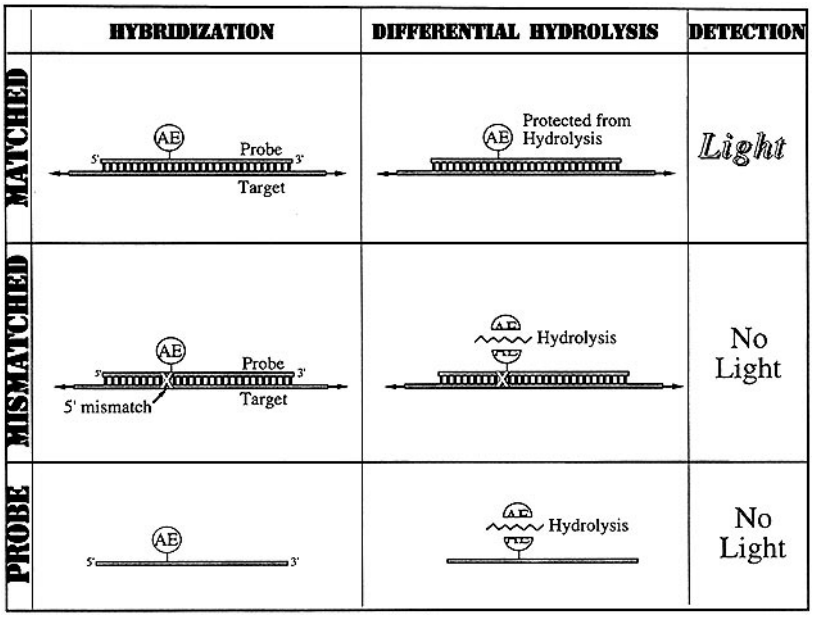

Figure 3. Schematic representation of mismatch detection using the hybridization protection assay. An acridinium ester-labeled probe (AE-probe) is synthesized to be exactly complementary to the desired target sequence with the AE placed immediately adjacent to the site of the mismatch (a 5′ mismatch is shown; a 3′ mismatch is on the other side of the linker). In step one of the assay, AE-probe is hybridized in-solution with target nucleic acid under non-hydrolyzing conditions. In step two, mildly alkaline buffer is added and AE linked to unhybridized probe and mismatched duplexes is rapidly hydrolyzed whereas AE linked to matched duplexes is protected from hydrolysis. In step three, chemiluminescence of non-hydrolysed AE is detected in a luminometer as a measure of the matched target present.

(wild-type probe/mutant target) yielded minimal signal. When the experiment was repeated with wild-type target, the matched duplex (wild-type probe/wild-type target) was readily detected and accurately quantified, whereas the mismatched duplex (mutant probe/wild-type target) yielded minimal signal (results not shown).

To determine whether a small amount of matched target could be detected in the presence of a large amount of mismatched target, various amounts of mutant target and a constant amount of wild-type target were hybridized to mutant probe. The results (Fig. 2b) demonstrate that the matched target (mutant) was readily detected and accurately quantified in the presence of the mismatched target (wild-type). A replicate set of samples was also hybridized with wild-type probe as a control to demonstrate the presence of a constant amount of wild-type target in each sample. When the experiment was repeated with wild-type probe and various amounts of wild-type target and a large amount of mutant target, the matched target (wild-type) was readily detected and accurately quantified in the presence of the mismatched target (mutant) (results not shown). Therefore, small amounts of matched target could be accurately detected in the presence of large amounts of mismatched targets. Using the HPA technique in an alternate assay format, these mutant and wild-type HIV sequences were simultaneously detected in a single tube (29).

CONCLUSION

When an AE-labeled probe hybridizes to a complementary target, AE is protected from hydrolysis relative to the unhybridized conformation. Single-base mismatches in the duplex adjacent to the site of AE attachment disrupt this protection resulting in more rapid AE hydrolysis, thus providing a basis for discrimination between matched and mismatched targets. This disruption is not caused by a decrease in the overall stability of the duplex, but most likely by a local alteration of the structure of the double helix which renders AE more susceptible to hydrolysis. All 12 single-base mismatches displayed this effect, yielding a matched to mismatched target signal-to-noise ratio of at least 10 for all possible single-base mismatches.

A simple assay format utilizing this difference in AE hydrolysis rates between matched and mismatched duplexes consists of the following three steps: (i) hybridization in solution of an AE probe to target nucleic acid; (ii) hydrolysis in solution of AE associated with unhybridized probe and mismatched duplex; (iii) reading of the remaining chemiluminescence in a luminometer as a measure of the amount of matched target present (Fig. 3). AE probes can be designed to be complementary to either normal sequences or mutant sequences such that either sequence can be detected with a positive signal. The utility of this method was demonstrated by quantitatively detecting both wild-type and mutant sequences associated with the HIV-1 reverse transcriptase coding region, either separately or in a mixture of the two.

Differences in the hydrolysis rates of AE linked to matched and mismatched duplexes account for all of the discrimination (S/N) described in Table 1. However, hybridization stringency can also be used in the HPA format to discriminate between matched and mismatched targets. Therefore, discrimination obtained from AE hydrolysis alone can be further enhanced by carrying out hybridization and differential hydrolysis under more stringent conditions.

When the mutation associated with disease is not known, often relatively large sequences must be scanned for any potential base change at any location. Techniques such as SSCP (1,2), DHDA (6,7) and mismatch cleavage or binding (8–14) are particularly well-suited for analysis of unknown mutations. The HPA technique, as with any sequence-specific probe approach, is not well-suited for scanning large sequences and would not be the method of choice for this type of analysis. However, when the

mutation associated with disease is known, it is important to have a rapid, simple, accurate, low-cost method of analysis so that large numbers of samples can be quickly screened for the mutation in a cost-effective manner. The HPA technique is particularly well-suited for the detection of known mutations. It is simple, fast, sensitive, quantitative and can be used with both DNA and RNA targets. It can also be used to detect multiple mismatches, insertions, deletions and translocations (results not shown). The method can be used directly when the target is relatively abundant or in conjunction with virtually any amplification procedure. It is not adversely affected by the presence of the clinical specimen material, it can be used in high throughput applications and it is amenable to automation.

The HPA technique yields several advantages over existing methodologies for the analysis of known mismatches. Direct sequencing is effective but is tedious, time-consuming and can require expensive, complex instrumentation. The HPA technique is simple to perform, rapid, and does not require complex instrumentation. A commonly used technique for the analysis of known mutations is dot blots with radiolabeled allele-specific oligomers. This technique is also tedious and time-consuming and suffers from the drawbacks of radioisotopes. In addition to being simple and rapid, the HPA technique is non-isotopic. Other techniques, such as ARMS and MS-PCR, rely on differences in PCR amplification efficiencies to distinguish mutant and wild-type sequences. The products are typically analyzed using gel electrophoresis, again increasing the time and complexity of the assay. Also, these techniques are limited to PCR reactions. The HPA technique is not limited to PCR, and can be used directly for targets that are relatively abundant and with virtually any amplification procedure. The attributes and advantages of the HPA technique therefore render it an attractive method for the rapid detection of known mismatches in both research and clinical settings.

REFERENCES

1 Walsh,T.J., Francesconi,A., Kasai,M. and Chanock,S.J. (1995) *J. Clin. Microbiol.*, **33**, 3216–3220.
2 Sarkar,G., Yoon,H. and Sommer,S.S. (1992) *Nucleic Acids Res.*, **20**, 871–878.
3 Khrapko,K., Hanekamp,J.S., Thilly,W.G., Belenkii,A., Foret,F. and Karger,B.L. (1994) *Nucleic Acids Res.*, **22**, 364–369.
4 Ke,S. and Waartell,R.M. (1993) *Nucleic Acids Res.*, **21**, 5137–5143.
5 Chen,X.G., Baumstark,T., Stager,G. and Riesner,D. (1995) *Nucleic Acids Res.*, **23**, 4524–4525.
6 Zimmerman,P.A., Carrington,M.N. and Nutman,T.B. (1993) *Nucleic Acids Res.*, **21**, 4541–4547.
7 Zimmerman,P.A., Shapiro,M., Tang,J., Nutman,T.B. and Unnasch,T.R. (1995) *BioTechniques*, **19**, 972–977.
8 Bhattacharyya,A. and Lilley,D.M.J. (1989) *J. Mol. Biol.*, **209**, 583–597.
9 Babon,J.J., Youil,R. and Cotton,R.G.H. (1995) *Nucleic Acids Res.*, **23**, 5082–5084.
10 Youil,R., Kemper,B.W. and Cotton,R.G.H. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 87–91.
11 Rojas,J.M., Sanchezpalomino,S., Santana,M., Lopezgalindez,C. and Tabares,E. (1995) *J. Infect. Dis.*, **172**, 1602–1605.
12 Wagner,R., Debbie,P. and Radman,M. (1995) *Nucleic Acids Res.*, **23**, 3944–3948.
13 Miret,J.J., Parker,B.O. and Lahue,R.S. (1996) *Nucleic Acids Res.*, **24**, 721–729.
14 Takamatsu,S., Kato,R. and Kuramitsu,S. (1996) *Nucleic Acids Res.*, **24**, 640–647.
15 Phelps,R.S., Chadwick,R.B., Conrad,M.P., Kronick,M.N. and Kamb,A. (1995) *BioTechniques*, **19**, 984–989.
16 Kovach,J.S., Hartmann,A., Blaszyk,H., Cunningham,J., Schaid,D. and Sommer,S.S. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 1093–1096.
17 Newton,C.R., Graham,A., Heptinstall,L.E., Powell,S.J., Summers,C., Kalsheker,N., Smith,J.C. and Markham,A.F. (1989) *Nucleic Acids Res.*, **17**, 2503–2516.
18 Rust,S., Funke,H. and Assmann,G. (1993) *Nucleic Acids Res.*, **21**, 3623–3629.
19 Cebula,T.A., Payne,W.L. and Feng,P. (1995) *J. Clin. Microbiol.*, **33**, 248–250.
20 Petersen,K.B., Kølvraa,S., Bolund,L., Petersen,G.B., Koch,J. and Gregersen,N. (1988) *Nucleic Acids Res.*, **16**, 352.
21 Shuber,A.P., Skoletsky,J., Stern,R. and Handelin,B.L. (1993) *Hum. Mol. Genet.*, **2**, 153–158.
22 Nelson,N.C., Reynolds,M.A. and Arnold,L.J.,Jr. (1992) In Kricka, L.J. (ed.), *Nonisotopic DNA Probe Techniques.* Academic Press, San Diego, CA, pp. 275–310.
23 Nelson,N.C., Hammond,P.W., Wiese,W.A. and Arnold,L.J.,Jr. (1990) In Van Dyke,K. and Van Dyke,R. (eds), *Luminescence Immunoassay and Molecular Applications.* CRC Press, Boca Raton, FL, pp. 293–309.
24 Arnold,L.J.,Jr, Reynolds,M.A. and Bhatt,R.S. (1989) European Patent Application, Bulletin 89/17, Publication No. 0 310 312
25 Arnold,L.J.,Jr and Nelson,N.C. (1993) US Patent 5,185,439
26 Hale,Y.M., Melton,M.E., Lewis,J.S. and Willis,D.E. (1993) *J. Clin. Microbiol.*, **31**, 451–453.
27 Jonas,V., Alden,M.J., Curry,J.I., Kamisango,K., Knott,C.A., Lankford,R., Wolfe,J.M. and Moore,D.F. (1993) *J. Clin. Microbiol.*, **31**, 2410–2416.
28 Richman,D., Shih,C., Lowy,I., Rose,J., Prodanovich,P., Goff,S. and Griffen,J. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 11241–11245.
29 Nelson,N.C., Cheikh,A.B., Matsuda,E. and Becker,M.M. (1996) *Biochemistry*, **35**, 8429–8438.