Detection of single-base mutations by reaction of DNA heteroduplexes with a water-soluble carbodiimide followed by primer extension: application to products from the polymerase chain reaction

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Received February 28, 1990; Revised and Accepted May 5, 1990

ABSTRACT

A new method was developed for the detection of single-base mutations in DNA. The polymerase chain reaction was used to prepare DNA fragments of up to 1 kb. Fragments that differed by a single-base were combined, denatured and renatured to generate heteroduplexes. The heteroduplexes were reacted with a water-soluble carbodiimide under conditions in which the carbodiimide modified Gs and Ts that were not base paired. The DNA was then used as a template for primer extension with Taq DNA polymerase under conditions in which extension terminated at the site of the carbodiimide-modified base and generated a ^{32}P-labeled fragment that was identified by polyacrylamide gel electrophoresis as a fragment smaller than the full length product. The procedure detected all four general classes of single-base mutations in several different sequence contexts. The site of the mutation was located to within about 15 bp. Extension with both a 5'- and a 3'-primer made it possible to confirm the site of the mutation in most DNA samples or detect a mutation in heteroduplexes even if a G or T in one strand was unreactive because of its sequence context. The procedure appears to have several advantages over previously published techniques.

INTRODUCTION

Methods for the rapid detection of single-base mutations in DNA are clearly important for the study of both genetic diseases and many problems of basic biology. Three general strategies have been pursued to develop such methods (for recent review, see ref. 1). One was to prepare DNA:DNA, DNA:RNA or RNA:RNA heteroduplexes of wild type and mutated sequences and to cleave the heteroduplexes with an enzyme specific for single-base mismatches in the heteroduplexes (2–5). A second strategy was to detect single-base mutations by differential melting and therefore migration of DNA homoduplexes or heteroduplexes during electrophoresis in denaturing gels (6–10). A third general strategy was to use chemical methods either to modify unpaired bases or both to modify and cleave unpaired bases in DNA heteroduplexes (1, 11–16). A number of specific variations on each of the three general strategies were developed. As recently noted by Cotton (1), however, each of the available methods has limitations. For example, cleavage of heteroduplexes with S1 nuclease was not sensitive enough to detect single-base mismatches (15). Digestion of RNA:RNA heteroduplexes with ribonucleases A or T1 was more sensitive (3–5) but detected only 60 to 70% of all possible single-base mismatches (4). Identification of single-base mutations by electrophoresis in denaturing gels was limited to detection of mutations in low melting domains of DNA fragments (6–9), and therefore required introduction of GC-rich clamps or other modifications of the technique to detect changes in high melting domains of the same fragments (1, 10). Chemical modification of DNA heteroduplexes with a water-soluble carbodiimide (CDI), a reagent that reacts with unpaired Gs or Ts, was shown to modify the electrophoretic behavior of DNA heteroduplexes containing single-base mismatches (12), but the effects on electrophoretic migration were often small and not readily predictable (12, 14). A modification of the CDI procedure was to use antibodies specific for CDI-modified DNA and to locate the site of single-base mismatches by immuno-electron microscopy of DNA heteroduplexes (14). The procedure, however, required time-consuming electron microscopy. A more recent chemical method was to modify unpaired Cs in DNA heteroduplexes with hydroxylamine and unpaired Ts and Cs with osmium tetroxide (1, 11, 16). The DNA was then cleaved at the modified bases by treatment with piperidine and analyzed by gel electrophoresis to detect the site of the mismatch. The procedure was shown to detect mismatched bases in many contexts. It required, however, preparation of radioactive probes to detect the cleaved fragments and two separate chemical reactions (1, 11). Also, a recent report

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indicated that the combination of hydroxylamine and osmium tetroxide did not detect all single-base mismatches in some sequence contexts (17).

Here we describe a method for detection of single-base mutations by reaction of DNA heteroduplexes with a water-soluble CDI followed by primer extension with Taq DNA polymerase under conditions in which extension terminates at the site of the CDI-modified base (18). The procedure appears to have several advantages over previously published techniques. As shown here, the technique can readily be used to detect mutations in DNA fragments synthesized with the polymerase chain reaction (PCR) (19).

MATERIALS AND METHODS

Reagents

1-Cyclohexyl-3-[2-(4-morpholinyl)ethyl]-carbodiimidemetho-*p*-toluenesulfonate (carbodiimide; CDI) was purchased from Fluka Chemical Corporation (Ronkonkoma, NY). Synthetic oligonucleotides were prepared on an Applied Biosystems Incorporated synthesizer (Foster City, CA). Taq DNA polymerase was purchased from Perkin-Elmer-Cetus Corporation (Norwalk, CT) or United States Biochemical Corporation (Cleveland, Ohio). ^{32}P-Labeled deoxynucleotide triphosphates were purchased from New England Nuclear Research Products (Boston, MA). Ammonium acetate was purchased from Fisher Scientific (Pittsburgh, PA) and stored in parafilm-sealed bottles.

Preparation of PCR products and heteroduplex formation

PCR products (19) were prepared in an automated thermocycler (Perkin-Elmer-Cetus) using a commercial kit under conditions recommended by the supplier (United States Biochemical Corporation or Perkin-Elmer-Cetus). The denaturing step was at 94°C for 1.5 min, the annealing step at 52°C or 54°C for 1 min, and the extension was at 74°C for 1 min. After 25 cycles, the sample was removed from the tube by inserting a micropipette below the level of the oil droplet used to prevent evaporation and transferred to a separate microcentrifuge tube. An equal volume of 0.1 mM EDTA in 10 mM Tris-HCl buffer (pH 7.4; TE buffer) was added. The sample was extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1) and then once with chloroform:isoamyl alcohol (24:1). The DNA was precipitated for 1 h at −70°C by addition of a one-half volume of 7.5 M ammonium acetate and two volumes of ethanol. The pellet was washed with 70% ethanol, and the pellet was dried in a vacuum centrifuge for about 5 min with care not to dry it so thoroughly as to denature the DNA (20). The DNA was dissolved in 50 μl of TE buffer.

To generate heteroduplexes of the DNA, 20 μl of PCR products in TE buffer were placed in a 650 μl microcentrifuge tube. The amount of DNA varied from 40 to 500 ng as assayed by ethidium bromide staining on agarose gels. To the sample was added 10 μl of hybridization buffer that consisted of 3 M NaCl and 35 mM $MgCl_2$ in 30 mM Tris-HCl buffer (pH 7.4). Seventy μl of water were added followed by a drop of mineral oil to prevent evaporation. The tube was placed in a boiling water bath for 10 min to denature the DNA, and then placed in a heat block set at 42°C overnight for reannealing (11). The sample was recovered with a micropipette inserted under the oil and transferred to a separate microcentrifuge tube. The DNA was extracted once with chloroform: isoamyl alcohol (24:1) and the aqueous upper layer was transferred to a separate tube. A half volume of 7.5 M ammonium acetate and two volumes of ethanol were added, and the DNA was precipitated at −70°C for 1 h. The pellet was washed with 70% ethanol and dried in a vacuum centrifuge for about 5 min with care to prevent over-drying. The heteroduplex DNA was dissolved in 60 μl TE buffer.

CDI modification of the heteroduplexes

A fresh solution of 200 mM CDI in water (84.7 mg/ml) was prepared just before the reaction. The reaction was carried out in a microcentrifuge tube by adding 26 μl of the heteroduplex DNA solution (20 to 200 ng), 4 μl of 1 M sodium borate (pH 8.0), and 10 μl of 200 mM CDI. The sample was incubated at 30°C for 3 h. (As noted below, if no mismatch was detected in the first experiments, the procedure was repeated and the incubation was carried out in 10 mM sodium borate at 37°C for 1 h instead of 100 mM sodium borate at 30°C for 3 h.) To remove unreacted CDI, 40 μl of 10 mM sodium phosphate buffer (pH 7.0) and 40 μl of 7.5 M ammomium acetate were added, and the sample was immediately extracted three times with 480 μl of isoamyl alcohol freshly equilibrated with a solution of 2.5 M ammonium acetate in TE buffer that was adjusted to about pH 7.4 before use. The aqueous lower phase (about 120 μl) was transferred to a siliconized microcentrifuge tube (Marsh Biomedical Products, Rochester, NY) and the DNA precipitated

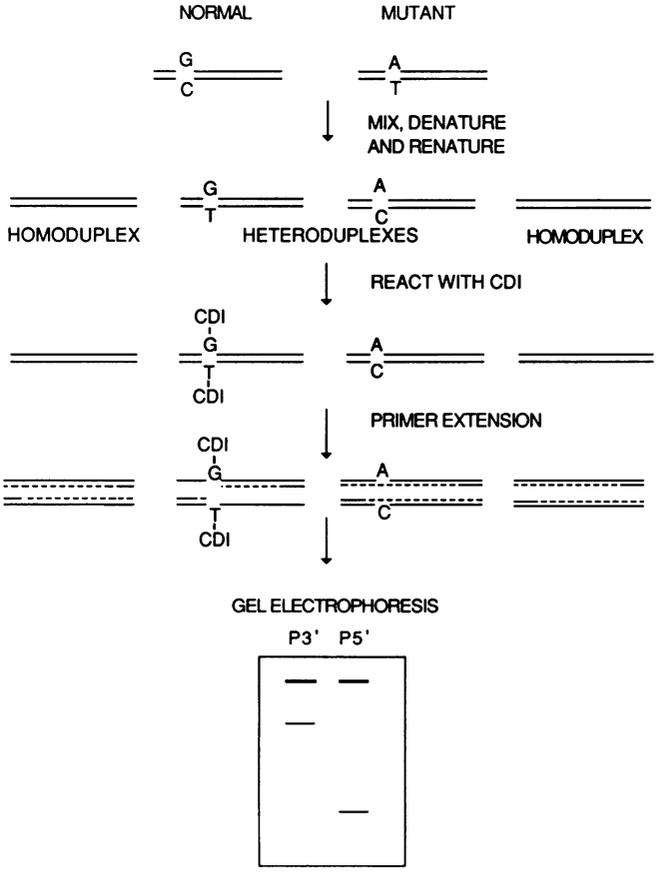

Figure 1. Scheme summarizing the procedure. Symbols: P3', 3'-primer; P5', 5'-primer.

Table I. Detection and location of single base mismatches in PCR products

Heteroduplexes of PCR products					Site of mutation (bp) [a]		
Gene/ Vector	Length (bp)	Mutation	Mismatches	Context	CDI Assay 3′-primer	5′-primer	Known site
M13[b]	810	G⟷A	G:T/A:C	TCCGGGG	330	330	330
M13[b]	810	G⟷T	G:A/T:C	TCCGGGG	330	Non-reactive[c]	330
M13[b]	810	G⟷C	G:G/C:C	TCCGGGG	330	330	330
M13[b]	810	T⟷A	T:T/A:A	TCCTGGG	330	330	330
M13[b]	810	C⟷A	C:T/A:G	CCCCGGA	Non-reactive[c]	340	330
M13[b]	810	C⟷T	C:A/T:G	CCCCGGA	340	340	330
COL1A2[b]	508	T⟷A	T:T/A:A	TGGTCCT	328	320	320
COL1A2[b]	822	C⟷T	C:A/T:G	AGGCATT	470	470	474
		G⟷A	G:T/A:C	CCTGGTC	740	750	748
COL1A1[b]	300	G⟷A	G:T/A:C	CCCGCCG	120	140	137
COL1A1[b]	992	G⟷T	G:A/T:C	CAGGGCC	500	Non-reactive[c]	483
COL3A1[d]	893	G⟷A	G:T/A:C	CGGGGTC	Not detected	540	556
COL3A1[d]	1067	G⟷A	G:T/A:C	CCCGGAC	Not detected	470	456
COL3A1[d]	921	G⟷A	G:T/A:C	GATGGTG	Not detected	450	447
COL3A1[d]	932	G⟷A	G:T/A:C	GAGGCCC	Not detected	320[e]	317

[a] Sites are defined in coding strand. With the CDI assay, the site defined by the 3′-primer was estimated by subtracting the size of the extra band from the full-length of the template. The known site of the mutation was established by nucleotide sequencing of the DNA.
[b] Heteroduplexes prepared by mixing PCR products synthesized with two M13 clones differing by a single base.
[c] Non-reactive because primed strand did not contain a mismatched G or T.
[d] PCR products synthesized using mRNA-derived cDNA as a template. The three of the four mutations in the COL3A1 gene are described in more detail elsewhere (24–26).
[e] Mutation detected only when the CDI reaction ws repeated in 10 mM sodium borate at 37°C for 1 h instead of in 100 mM sodium borate at 30°C for 3 h (see Fig. 4).

by adding 360 μl of ethanol and leaving it at −20°C overnight. The pellet was washed with 70% ethanol, and dried in a vacuum centrifuge for about 5 min with care to avoid over-drying. The pellet was taken up in 26 μl of TE buffer (pH 7.4). The sample was heated at 100°C in a boiling water bath for 5 min to destroy any residual CDI (21) and then cooled on ice for 5 min.

Primer extension using the CDI-modified heteroduplexes as template

The reaction was carried out in a siliconized microcentrifuge tube by adding 4.0 μl of the CDI-modified DNA template, 1.0 μl of primer (5.0 pmole), and 5.0 μl of a cocktail continuing Taq DNA polymerase and the four dNTPs. The cocktail was prepared for 20 tubes by mixing 38 μl of water; 32 μl of an aqueous solution containing 1.25 mM dGTP, 1.25 mM dATP, 1.25 mM dTTP and 0.625 mM dCTP; 10 μl of α-^{32}P-labeled dCTP (0.1 mCi, 3,000 Ci/mmole); and 20 μl of 10×commercial buffer for the PCR (Perkin-Elmer-Cetus Corporation or United States Biochemical Corporation). The solution was heated to 94°C for 5 min to inactivate any proteases, and cooled on ice for 5 min. One μl of Taq polymerase (2.5 units) was then added to complete the cocktail. After adding the cocktail to the CDI-modified DNA and primer, the sample was overlaid with mineral oil and placed in an automated thermocycler programmed for incubation at 94°C for 3 min, 56°C for 1 min, and 74°C for 3 min. After one cycle, the reaction was stopped by adding 6 μl of 95% formamide (Clontech, Palo Alto, CA), 20 mM EDTA, 0.1% (w/v) Bromophenol blue (Fisher Scientific) and 0.1% (w/v) xylenecyanol FF (Fisher Scientific) and heating the sample at 94°C for 2 min. An aliquot of 8.0 μl was immediately loaded on a 5% polyacrylamide DNA sequencing gel for electrophoresis under standard conditions (22). Molecular weight markers for the sequencing gels were prepared by end-labeling HaeIII fragments of ϕX174 with γ-^{32}P-ATP and polynucoleotide kinase (United States Biochemical Corporation).

Controls and precautions for the assay

As noted below, fortuitous bands were occasionally encountered in the assay because of internal priming of the DNA heteroduplexes, non-specific modification of bases by the CDI and other effects. To distinguish bands caused by single-base mismatches from such fortuitous bands, one of two types of controls were employed in each assay. One type of control sample was a homoduplex of the same DNA fragments that was denatured, renatured and treated with CDI under the same conditions as the test samples. A second type of control was a heteroduplex of the same DNA prepared under the same conditions but not modified by CDI. As noted previously (14), CDI interacts non-covalently with DNA and alters its solubility so that CDI-treated DNA is difficult to precipitate with ethanol. Also, CDI-treated DNA adheres to glass and plastic surfaces. Therefore, siliconized tubes were used and care was taken not to over-dry samples. With small DNA fragments, it may be enecessary to concentrate the sample by ultrafiltration instead of by ethanol precipitation. Because of the greater losses during processing of the CDI-treated samples, control samples not treated

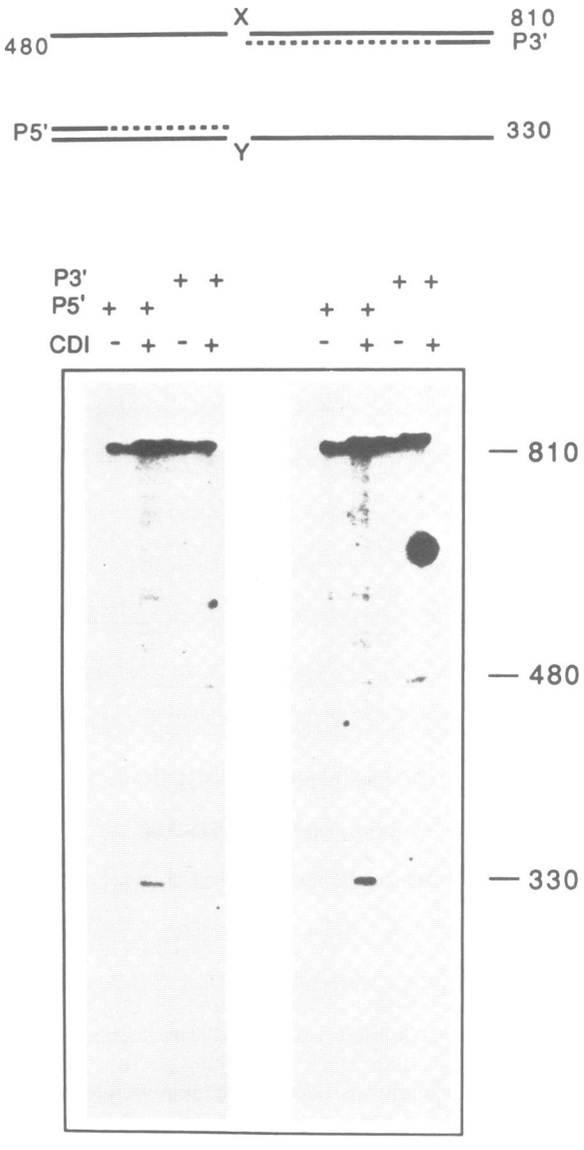

Figure 2. Detection of G to C and G to A mutations in PCR products. M13 clones that differed by a single base were used as templates for PCR with primers that generated fragments of 810 bp. Heteroduplexes containing mismatches were prepared by mixing PCR products synthesized using as template an M13 clone with a G at nt 330 with PCR products synthesized using as template an M13 clone with a C or A at the same site (14). Heteroduplexes of the PCR products were than analyzed as described in text. *Upper panel*: Schematic of the site of the single-base mismatches (X and Y) and the expected fragments. *Lower panel*: Polyacrylamide gel of the products from primer extension with G:G/C:C mismatch (left panel) and G:T/A:C mismatch (right panel).

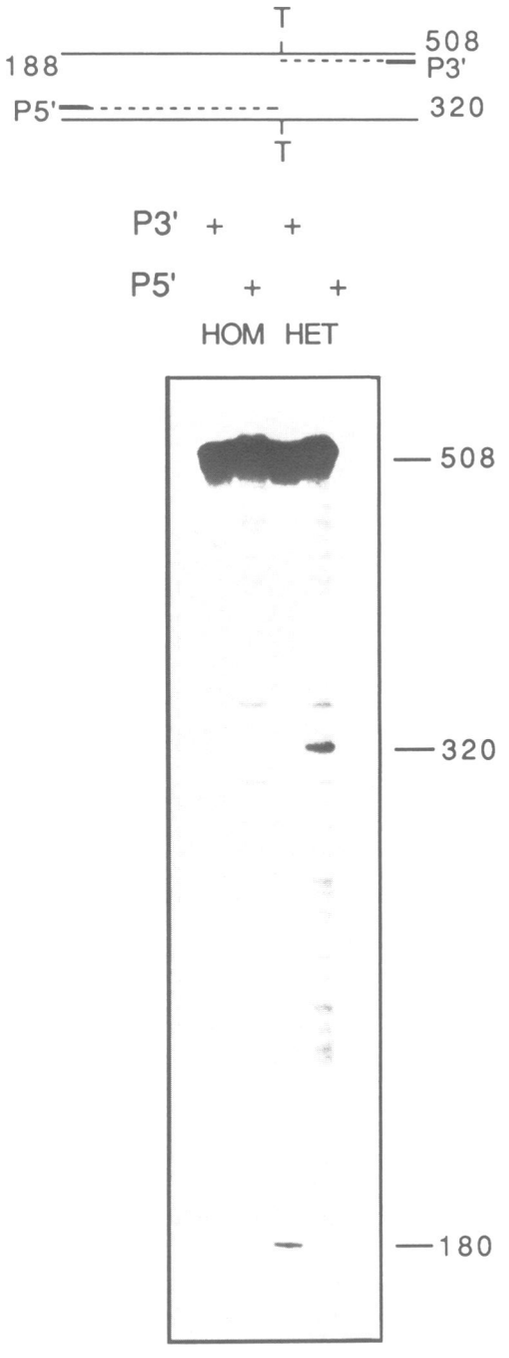

Figure 3. Detection of a T to A mutation in a coding sequence of a collagen gene (COL1A2). PCR products were prepared using two M13 clones as templates and primers that generated a fragment of 508 bp. *Upper panel*: Schematic for site of the single-base mismatch and the expected fragments. *Lower panel*: Polyacrylamide gel analysis of the products from primer extension. Background bands are seen both in homoduplexes (Hom) not containing a base mismatch and in the heteroduplexes (Het).

with CDI were usually diluted two or four times before application to the electrophoresis gel in order to generate bands of comparable intensity.

RESULTS

Detection of all eight possible single base mismatches in M13 constructs

To test the procedure described here (Fig. 1), four variants of the filamentous phage M13 were prepared so as to differ by a single base at the same site (14). The M13 clones were then used as substrate for the PCR using two oligonucleotide primers that generated fragments of 810 bp. The PCR products from pairs of different variants were mixed, denatured and renatured to generate heteroduplexes containing all eight possible single-base mismatched pairs (Table I). The heteroduplexes were modifed with CDI, and the CDI-modified DNA was then used as templates for primer extension. As indicated in Figure 2, primer extension

Table II. Summary of data on detection of mutations with hydroxylamine/osmium tetroxide (HOT) and CDI[a]

Mutation	Mismatches in heteroduplexes	Detection with HOT: Mismatches Ref.1	Mismatches Ref.17	Mutation Ref.1	Mutation Ref.17	Detection with CDI: Mutation
A⟷C	C:T	14.14	2.2			
or	and			14/14	2/2	3/3
G⟷T	A:G					
A⟷G	G:T	12/12	2/2			
or	and			26/26	2/2	7/7
C⟷T	A:C	14/14	1/2			
A⟷T	T:T	3/3	0/2			
or	and			3/3	0/1	2/2
T⟷A	A:A					
C⟷G	G:G					
or	and			5/5	1/1	1/1
G⟷C	C:C	5/5	1/2			

[a] Values indicate number of mismatches or mutations detected (numerator) over number tested (denominator). With data from Bhattacharyya and Lilley (17), their observations with heteroduplexes prepared with single-stranded DNA are interpreted in terms of the class of mutations from which such mismatches can arise. With the CDI assay, it was not possible to define the specific mismatch detected in DNA heteroduplexes under the conditions employed here.

with a 5′-primer generated the expected full-length fragment of about 810 nt and a second fragment 330 nt from heteroduplexes that contained a G:G/C:C mismatch (left panel) and a G:T/A:C mismatch (right panel). Primer extension with a 3′-primer generated the expected full-length fragment of 810 nt and the second fragment of about 480 nt with both heteroduplexes. Therefore, assay with the 3′-primer also located the mismatch at about 330 nt in the coding strand (810 nt −480 nt = 330 nt). The 480 nt fragment, however, was fainter with the G:G/C:C mismatch (lower left panel in Fig. 2), apparently because the G in one sequence context (X position in upper panel) was less reacted with CDI than the G in the other sequence context (Y position).

Analysis of the heteroduplexes formed with the four different M13 constructs detected all eight of the possible single-base mismatches in the heteroduplexes (Table I). The mismatch was detected with both primers in four out of six of the heteroduplexes. As expected, the mismatched base in the other two of the six heteroduplexes was detected with one primer but not the other, because one of the strands did not contain a mismatched G or T and, therefore, was non-reactive with CDI. The site of the mismatches was correctly located within about 10 bp. The T:T mismatch consistently gave the strongest bands and the G:G mismatch the weakest. Time course experiments (not shown) demonstrated that a reaction time of 3 h at 30°C was usually necessary with the G:G mismatch whereas the others were usually discernible at 1 h.

Detection of single-base mutations in collagen genes

In further experiments, the procedure was tested on PCR products of collagen genes that were conveniently available in our laboratory.

In the first series of experiments, a T to A mutuation was detected in coding sequences from the gene for the proα2 chain of type I procollagen (COL1A2). mRNA derived cDNA was used to generate M13 clones. DNA sequencing of one of the M13 clones identified a single-base mutation of T to A that was probably an artifact of the PCR (23). An M13 clone with the normal sequence and a clone with the T to A mutation were used separately to prepare two PCR products of 508 bp each and with the mutation at nt 320 of the coding strand. The PCR products were mixed and heteroduplexes generated. Analysis of heteroduplexes of the PCR products detected the full-length 508 nt band and an extra band about 180 nt with a 3′-primer (Fig. 3). Therefore, the results indicated a mismatch at about 328 nt in the coding strand (508 nt −180 nt = 328 nt). Assay with a 5′-primer detected a band of 320 nt. Therefore, both primers detected the mismatch and correctly located the site to within 10 bp.

Similar experiments were carried out with PCR products containing coding sequences from another region of the gene for the proα2 chain of type I procollagen (COL1A2). Also, the same experiments were carried out on PCR products that were synthesized using mRNA-derived cDNA as a template and primers for a coding sequence of the gene (COL3A1) for type III procollagen (24). As summarized in Table I, the CDI assay detected single-base mutations in eight heteroduplexes of collagen-coding sequences known to contain such mutations. With four of the eight samples, the predicted bands were seen with one primer but not the other. With one of the collagen sequences (G to A, COL3A1; 932 bp fragment in Table I), it was necessary to alter the reaction conditions with CDI and to carry out the reaction in 10 mM sodium borate buffer at 37°C for 1 h instead of in 100 mM sodium borate at 30°C for 3 h (Fig. 4). The altered reaction conditions, however, decreased the yield of ^{32}P-labeled strands, apparently because of increased non-specific interaction of the DNA with CDI (14) and, therefore, losses in processing of the samples. The G to A mutation found in the 932 bp fragment of the COL3A1 gene was not detected (G. Tromp, unpublished observations) in three experiments using the hydroxylamine procedure to assay mRNA:cDNA heteroduplexes under the conditions described by Bateman *et al.* (28). In the same

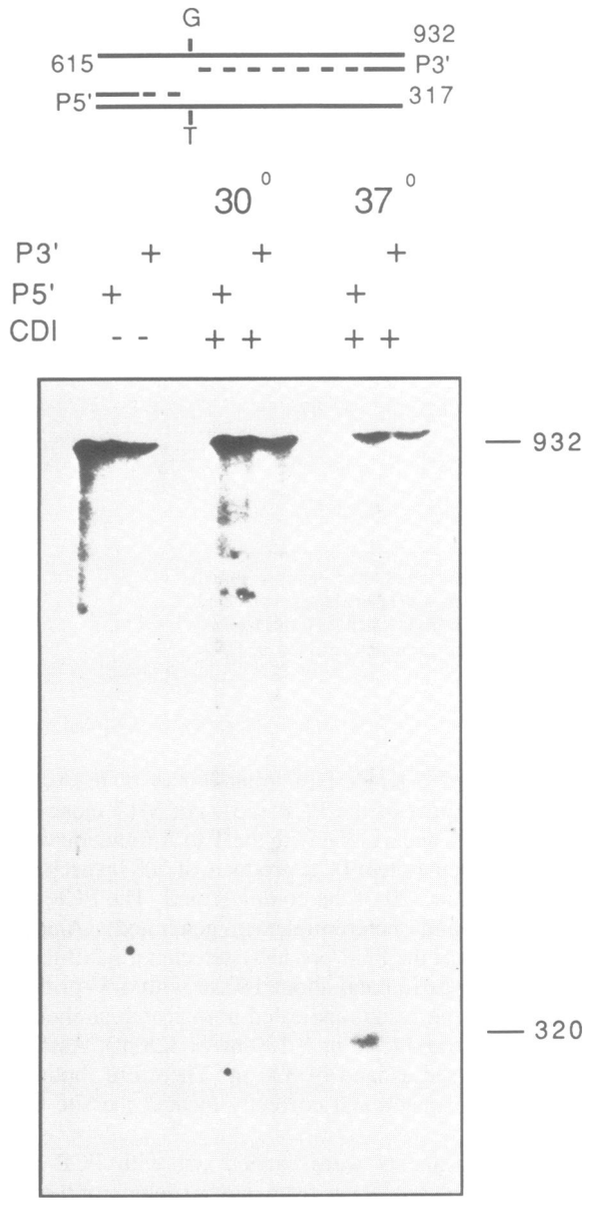

Figure 4. Detection of a G to A mutation in a coding sequence of a collagen gene (COL3A1). *Upper panel*: Schematic for the site of the single-base mismatch and the expected fragments. *Lower panel*: Polyacrylamide gel electrophoresis of the products from primer extension. The mutation was not detected when the CDI reaction was carried out at 30°C for 3 h in 100 mM sodium borate but was detected when the reaction was repeated at 37°C for 1 h in 10 mM sodium borate.

experiments, the hydroxylamine procedure detected two other G to A mutations with varying degrees of sensitivity, i.e. the mutations found in the 893 bp fragment and the 1,067 bp fragment of the COL3A1 gene (Table I).

DISCUSSION

The procedure developed here was based on extensive previous work that demonstrated a water-soluble CDI can react specifically with unpaired Gs and Ts in heteroduplexes of DNA or with unpaired Gs and Us in RNAs (see 12, 20). The procedure was also based on the premise that CDI-modified Gs and Ts in heteroduplexes of DNA will interrupt primer extension of the DNA templates with a DNA polymerase (Fig. 1; 18). All possible single-base mutations will generate at least one unpaired G or T in heteroduplexes formed from wild type and mutant DNA fragments (Table II). Therefore the procedure should be applicable to all single-base mutations. Because extension in both directions is interrupted by appropriately modified Gs or Ts, the mismatches in DNA heteroduplexes generated by three of the four general classes of single-base mutations (Table II) can be primed with both a 3′-primer and a 5′-primer to give two distinctive fragments that confirm the site of a single-base mismatch. Also, priming of CDI-modified heteroduplexes in both directions increases the probability of detecting a mismatch if an unpaired G or T in one of the strands is unreactive under the conditions employed.

It is unlikely that a single chemical reaction with DNA heteroduplexes will detect all four general classes of single-base mutations (Table II) in all sequence contexts. The structures of mismatched base pairs in double stranded DNA have been extensively investigated by a variety of techniques (29–31). X-ray data and other evidence indicate that most mismtched bases are stacked into the DNA double helix in much the same conformation as conventional base pairs and that frequently the mismatched base pairs are hydrogen bonded. As noted by Bhattacharyya and Lilley (17), hydroxylamine is more likely to detect a mismatched cytosine if the pyrimidine moves into the major groove of B-form DNA so as to expose the 5,6-bond that reacts with hydroxylamine. It is less likely to react if the pyrimidine moves into the minor groove. Similar considerations apply to the reaction of osmium tetroxide with the 5,6-bond of thymine. Also, similar considerations are applicable to the reaction of CDI with the 1-position nitrogen of guanine or the 3-position nitrogen of thymine. In addition, it is apparent that reaction conditions such as the concentration of reactants, the temperature, the reaction time, the salt concentration and pH are critical. Conditions that destabilize the double helix or increase chemical reactivity will increase the sensitivity of the assay but are likely to produce modification of bases that are paired but in sequences that undergo breathing, form cruciform structures or otherwise deviate from the conformation of the B-form DNA. Therefore, it will be necessary to test any chemical procedure for detecting single-base mismatches in a variety of sequence contexts and probably with several different reaction conditions to establish both its sensitivity and specificity.

The procedure described here was tested on all four general classes of single-base mutations in one to seven different sequence contexts (Tables I and II). Single-base mutations were detected in all fourteen DNA heteroduplexes tested. In four of the heteroduplexes tested, one of the predicted bands was not obtained by primer extension in one direction but was obtained by priming the same samples in the opposite direction (Table I). In all four instances, the mismatched base not detected was a G but the mismatched T in the opposite strand was readily seen. Therefore, unpaired Ts appeared to be more reactive than unpaired Gs. Detection of one of the G to A mutations in one collagen gene sequence required altering the reaction conditions with CDI. The same mutation was not found by assay of mRNA:cDNA heteroduplexes with hydroxylamine (28). Collagen sequences may be particularly difficult for detection of single-base mutations because they are GC-rich (24–27) and, therefore, likely to be more stably base-paired than other sequences.

Cotton and his associates reported (1, 16) that a combination of one reaction with hydroxylamine and a second reaction with

osmium tetroxide detected all possible single-base mutations in DNA heteroduplexes with a series of different sequence contexts (Table II). They indicated that no single-base mutations they assayed had gone undetected with the combination of the two chemical procedures. Bhattacharyya and Lilley (17), however, failed to detect reaction in four heteroduplexes in which reaction with either hydroxylamine or osmium tetroxide was expected (Table II). In particular, they were unable to detect a T:T mismatch, a result indicating that an A to T or T to A mutation in the sequence they tested was not detectable. The results here indicated that a T:T mismatch generated by such a mutation was the mismatch most readily detected by the CDI assay in two DNA sequences that were tested.

The question of whether the present procedure is more sensitive and specific than other procedures for detection of mutations cannot be resolved until a large number of sequence contexts are tested. The procedure, however, appears to have several advantages. For example, it does not require prior preparation of high specific activity ^{32}P-labeled DNA probes. It involves a single chemical reaction to detect all possible base mismatches, and therefore it is relatively simple to establish optimal conditions for reaction with a given DNA fragment. Also, it does not require any specialized equipment or reagents. With most DNA heteroduplexes, priming of the same fragment in both directions makes it possible to confirm the site of the mutation or detect a mutation even if a G or T in one strand was unreactive under the conditions employed.

ACKNOWLEDGEMENTS

The work was supported in part by N.I.H. grants AR-38188, AR-38923 and AR-39740, and by a grant from the March of Dimes-Birth Defects Foundation. The authors are grateful to Dr. Allen Zeiger for advice and consultation during the course of the work. They are also grateful to Dr. John Rooney, Dr. Helena Kuivaniemi and Dr. Gerard Tromp for assistance in several aspects of the work. In addition, they thank Drs. Loretta Spotila, Andrea Westerhausen and Jiapiao Zhuang and Ms. Caren Kleinert and Ms. Larisa Sereda for providing DNA templates with defined single-base mutations.

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