Modifications of guanine bases during oligonucleotide synthesis

Anthony T.Yeung*, William J.Dinehart and Beverly K.Jones

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111, USA

Received November 13, 1987; Revised and Accepted April 14, 1988

ABSTRACT

Guanine bases are sensitive to modification during automated DNA synthesis and processing reactions. Methods for the detection of two types of guanine modifications are described. The first method uses the higher reactivity of the modified G base to KMnO_4 oxidation than T bases, and thus allows detection by chemical DNA sequencing. The second method makes use of the *Escherichia coli* nucleotide excision repair enzyme UvrABC endonuclease which can detect "bulky" base modifications at each nucleotide in the synthetic DNA. Though the chemical structures of the two modifications are not known, they may be related. Both types of G modifications are often found in oligonucleotides synthesized by the methoxy-diisopropyl-phosphoramidite (MEDP) chemistry but non-detectable in the products of the beta-cyanoethyl-diisopropyl-phosphoramidite (CEDP) chemistry. The Rubin and Schmid pyrimidine-specific chemical DNA sequencing procedure (Rubin, C.M., and Schmid, C.W. (1980) *Nucleic Acids Res.* 8, 4613-4619) was found to be applicable to oligonucleotides synthesized by the CEDP chemistry, and to oligonucleotides synthesized by the MEDP chemistry if precautionary measures are taken to destroy the signals produced by the highly KMnO_4 sensitive modified guanine bases. We also show how chemical DNA sequencing might be useful for diagnosing other chemical modifications in synthetic oligonucleotides.

INTRODUCTION

The automation of chemical DNA synthesis has dramatically increased the applications of synthetic oligonucleotides in research and clinical chemistry. In spite of the amount of automation in DNA synthesis reaction cycles, the efficiency of full length oligonucleotide synthesis is still dependent on the skills of the operator in the maintenance, programming, and operation of the instrument. An oligonucleotide which appears as a single band on a DNA sequencing gel may contain different amounts of undetected base modifications. The presence of 3-methylthymidine, 5-methylcytidine and 2-aminoadenine have been detected (1,2,3). The sources of the various G modifications (4,5,6,7) during automated DNA synthesis are not well understood. When oligonucleotides, usually in the range of 17 to 24 mers, are used as DNA sequencing

primers and as hybridization probes, the presence of less than 0.5% of the bases being modified does not usually present a problem to these experiments. On the other hand, molecular cloning often requires oligonucleotides longer than 40 bases. In these longer oligonucleotides, the fraction of molecules containing at least one modified base is quite significant. As a result, it is important to have convenient methods of monitoring the quality of an oligonucleotide beyond the appearance of a major peak on high performance liquid chromatography (HPLC) or a major band in polyacrylamide gel electrophoresis.

The most common method used for the analysis of base modifications in a synthetic oligonucleotide involves hydrolysis of the oligonucleotide into free bases and subsequent analysis of the products with HPLC (1,8). The average modification of each of the four deoxynucleotides in the DNA is measured in such an approach. Because the rate of modification of a base may be affected by the flanking sequences, the amount of modification of each base may vary. In this study, we directly tested for the presence of base modification in each position in synthetic oligonucleotides by the use of the *E. coli* nucleotide excision enzyme UvrABC endonuclease. The UvrABC endonuclease is an enzyme complex which recognizes "bulky" DNA lesions and incises the DNA bimodally, on the modified DNA strand, at the 8th phosphodiester moiety 5' to the modified base as well as at the 5th phosphodiester moiety 3' to the modified base (9,10,11,12,13). Lesions which are substrates of UvrABC endonuclease are usually mutagenic and induce recombination (14,15). Base modifications by small chemical groups like methyl and ethyl are not detected by UvrABC endonuclease (16).

Bases which are modified have different chemical reactivities from the unmodified bases. For example, methylation at N7 position of guanine is used in the G-specific reaction in the Maxam and Gilbert procedure for DNA sequencing (18). In the studies in this paper, we tested whether some modified guanine bases might be sensitive to oxidation by KMnO_4 . This method also examines each guanine position in the DNA instead of measuring the average modification of all the guanine positions. Because oxidation of thymine bases by KMnO_4 is used in the T-specific sequencing reaction in the Rubin and Schmid procedure (17), we examined whether modified bases which are sensitive to oxidation may pose a problem in that reaction.

MATERIALS AND METHODS

Preparation of the oligonucleotides.

An Applied Biosystems 380A synthesizer with extended programmability was used to synthesize the oligonucleotides with the MEDP chemistry. A 380B and a

381A synthesizer were used occasionally, in addition to the 380A, for synthesis with the CEDP chemistry. The synthesizers were meticulously maintained. All DNA synthesis reagents were supplied by Applied Biosystems and used as recommended. Acetonitrile was from Baker Chemicals. Applied Biosystems thiophenol and deprotection cycle were used for the demethylation of MEDP chemistry synthesized DNA. The oligonucleotides were routinely deblocked at 55°C for 16 hours in fresh ammonium hydroxide and dried in a Speedvac. A second cycle of deblocking was used for oligonucleotides longer than 24 bases before the DNA was extracted twice with 70% ethanol and dried. The DNA was stored in 10 mM Tris, 0.4 mM EDTA pH 7.6 in aliquots at -20°C. The coupling efficiency was usually over 98.4% as judged by the percentage of full length oligonucleotide on Mono Q anion-exchange FPLC chromatography (Pharmacia) at pH 12.5. The oligonucleotides were usually purified by lightly labeling with ^{32}P (about 100,000 dpm/ μg) in the presence of unlabeled ATP to completion, and gel purified on 7 M urea, 20% polyacrylamide DNA sequencing gels as described (18). Alternatively, UV-shadowing with minimal UV exposure, using the fluorescence of the back of a lightening-plus enhancer screen, was used to locate the DNA band within the gel. The DNA was then labeled at the 5' end with T4 polynucleotide kinase, and gel purified as above.

DNA sequencing reactions

DNA sequencing was performed by the Maxam and Gilbert protocol for the G and G+A reactions (18) and the Rubin and Schmid (17) protocol for the pyrimidine reactions. The C reaction was improved by the inclusion of 0.45 M NaCl in the initial reaction to suppress the cross-reactivity with T bases. The T reaction used 40 mM KMnO_4 . Both the C and T reactions contained 3 μg of heat denatured sonicated purified calf-thymus DNA as carrier DNA. A summary of the protocols we use is presented in Fig. 1. There is a reasonable amount of flexibility in the conditions used in these sequencing reactions.

DNA sequencing gels.

All polyacrylamide gels used in this study were denaturing gels. The gels were 0.5 mm x 30 cm x 40 cm, containing 10 or 20% acrylamide, 7 urea, 1 X TBE. The ratio of acrylamide to bisacrylamide was 37.5:1. All gels were run between 43 and 49°C at 2200V. The tracking dye was 95% formamide and 0.025% bromophenol blue in 1 X TBE. Samples were 2-3 μl containing about 3000 cpm as measured by a Ludum hand-held survey meter. For an 18 mer, the gel was run until the tracking dye was 18 cm from the wells. The gel was then removed from the plates by adhesion to a used autoradiogram, covered with plastic

SEQUENCING

1.5 X 10⁶ dpm of oligonucleotides, dissolved in water, are added to each of four microcentrifuge tubes and dried down in speedvac. Individual reactions are as follows:

T only reaction

10 μ l dH₂O + 3 μ g CT DNA
 20 μ l 0.04 mg/ml KMnO₄
 *15' at 22°C
 STOP w/ 10 μ l Allyl alcohol
 *dry in speedvac

G only reaction

10 μ l DNA + dH₂O
 160 μ l DMS Buffer
 1 μ l DMS
 *10' at 20°C
 40 μ l STOP Solution
 5 μ l CT DNA (1 mg/ml)
 1 ml 100% ETOH
 *15' at 4°C (Eppendorf)
 250 μ l 0.3 M NaAc pH 7.0
 1 ml 100% ETOH
 *15' at 4°C (Eppendorf)
 *wash w/ 80% ETOH
 15' at 4°C (Eppendorf)
 *dry pellet in speedvac

G+A reaction

10 μ l dH₂O + 1 μ g CT DNA
 3 μ l Sodium formate
 *25' at 37°C
 STOP w/ 150 μ l 1 M Piperidine
 *-20°C until cleavage rxn.

C only reaction

10 μ l dH₂O + 3 μ g CT DNA
 3 μ l 5 M NaCl
 20 μ l NH₂OH.HCL
 *30' at 22°C
 STOP w/ 1 ml ETOH
 *15' at 4°C (Eppendorf)
 *wash w/ 80% ETOH
 *15' at 4°C (Eppendorf)
 *wash w/ 80% ETOH
 *15' at 4°C (Eppendorf)
 *dry pellet in speedvac

*denotes incubation or manipulation.

CLEAVAGE REACTION

T, C, and G reactions are treated with 150 μ l, 1 M Piperidine. All caps are sealed w/teflon tape and the tubes secured in sequencing racks. After 30 minutes at 90°C, the microcentrifuge tubes are dried in speedvac, rewet with 50 μ l water, dried, rewet with 20 μ l water, and redried.

Reagents:

Sodium formate: 1 M pH 2.0 w/NaOH, Fisher, stored at -70°C.
 Hydroxylamine: 4 M pH 6.0 w/Diethylamine, Sigma, stored at -70°C.
 KMnO₄: diluted from 4 mg/ml stock w/water for use. Stock is stored at -20°C.
 DMS stop solution: 1.5 M NaAc pH 7.0, 1 M 2-Mercaptoethanol (4°C).
 DMS Buffer: 50 mM Sodium Cacodylate, 1 mM EDTA, pH 7.0 stored at 4°C.
 DMS: Dimethyl sulfate, Aldrich, stored desiccated at room temperature.
 CT DNA: purified calf-thymus DNA, sonicated, heat denatured.
 NaAc: Sodium Acetate, pH 7.0.
 Piperidine: Redistilled, stored at 4°C over molecular sieve, Fisher.
 1X TBE: 50mM Tris-borate, 1 mM EDTA, pH 8.3.
 dH₂O: deionized water.

Fig. 1. Protocol of the DNA sequencing reactions used in this study. The G- and G+A specific reactions were adapted from Maxam and Gilbert (18). The pyrimidine reactions were adapted from Rubin and Schmidt (17), improved with the addition of 0.5 M NaCl in the C-specific reaction.

Fig. 2. G- and T-specific DNA sequencing reactions of oligonucleotides synthesized by the MEDP chemistry. These oligonucleotides were 5' labeled with ^{32}P and purified on denaturing polyacrylamide gels. Lanes A-I were nine different oligonucleotides

wrap, and placed in a film cassette at -70°C . Exposure was overnight with an enhancer screen, or three days without the use of an enhancer screen.

UvrABC endonuclease reactions

The *E. coli* UvrA, UvrB, and UvrC proteins used in these studies were purified as described (13) and their purities, judging from SDS polyacrylamide gel electrophoresis, were better than 95%. For the UvrABC endonuclease incision reaction, an oligonucleotide labeled at the 5' termini with ^{32}P was made double-stranded by hybridization to a complementary strand of oligonucleotide. About 20 fmol of this DNA was treated with 530 fmol of UvrA protein, 750 fmol of UvrB protein and 263 fmol of UvrC protein under standard assay conditions for 1 hour at 37°C (10). The reaction was stopped by the

addition of EDTA to 50 mM and incubated at 37°C for another 20 minutes. The mixture was extracted once with phenol, twice with ether, precipitated with ethanol, washed with ethanol and dried. The dried sample was dissolved in 10 μ l of tracking dye and 2 μ l was loaded into a well of the DNA sequencing gel.

RESULTS AND DISCUSSION

Detection of the G-modifications by chemical DNA sequencing reaction.

We routinely use the G and G+A reactions of the Maxam and Gilbert procedure (18) and the pyrimidine reactions of Rubin and Schmid (17) for the chemical DNA sequencing of plasmid DNA as well as synthetic oligonucleotides. When natural DNA is used, the KMnO_4 thymine-specific reaction is usually and clearly thymine-specific, and the cross-reactivities with either A, G or C bases were no higher than the usual background caused by piperidine. However, with oligonucleotides synthesized by the MEDP chemistry, we have often observed cross-reactivity with G bases in the thymine reaction. Fig. 2 shows the G and the T reactions for some of the oligonucleotides we synthesized with MEDP chemistry. In some of these oligonucleotides (Panels A,E,H,I), the T reactions appeared as G+T reaction. In others, the T lanes were more T-specific. This G reactivity was not the result of over-reaction by KMnO_4 . To the contrary, the experiment in Fig. 3 clearly demonstrated that shorter reaction times with KMnO_4 only produced the G bands without the T bands. Thus, we conclude that modified G bases were present and they were much more reactive with KMnO_4 than the T bases. The reaction with the modified G bases essentially completed within seconds. At 45 minutes of reaction, the T reaction has consumed all the KMnO_4 present in the reaction and the G-specific bands initially generated have broken down into shorter lengths at the T sites, thus appearing as T bands. KMnO_4 has long been known to oxidize the 5,6 double bond of the thymine base (19), but the guanine base is most resistant to KMnO_4 oxidation especially in the conditions of these reactions (19). We considered the possibility that the isobutyryl group used to protect the exocyclic amine of guanine may not have been completely removed in these oligonucleotides. However, we do not believe this to be true because multiple deblocking reactions do not decrease the reactivity of these guanines to KMnO_4 (data not shown). Because of the speed of the oxidation of the modified G bases, one might expect the $\text{C}_8=\text{N}_9$ double bond to have become highly reactive, as may be the case if a strongly electron withdrawing group is attached at the C_8 position. A possible candidate in this case may be the

Fig. 3. Time course of KMnO_4 T-specific reaction with an oligonucleotide synthesized by the MEDP chemistry. Lane 1, dimethylsulfate G-specific reaction as reference. Lane 2, the gel purified, untreated oligonucleotide. Lane 3, T-specific reaction mock treatment without the addition of KMnO_4 , the piperidine steps were included. Lanes 4-12: T-specific reaction time with KMnO_4 for less than 10 seconds, 30 seconds, 1,2,3,5,10,20, and 90 minutes at 23°C , respectively. Notice the initial time points were G-specific while later time points were more T-specific. All lanes contained an equal number of counts.

Fig. 4. G- and T-specific DNA sequencing reactions of oligonucleotides synthesized by the CEDP chemistry. The oligonucleotides were 5' labeled with ³²P and purified on denaturing polyacrylamide gels. Lanes A-F were six oligonucleotides which have been subjected to the G-specific and the T-specific sequencing reactions, and then analyzed on a DNA sequencing gel as described in Materials and Methods.

thiophenol group, transferred there by the substitution of methyl iodide. The methyl iodide may have been formed in small amounts by the reaction of iodine with the methyl protection group on the phosphate of oligonucleotides synthesized by the MEDP chemistry. The reactions are probably favored by the high temperature and alkaline conditions used in the deprotection reactions of the crude oligonucleotide. The source of these chemicals may be from the expected contamination that exist in the chemical delivery lines, perhaps trapped within the oily substance formed by the solvents used in the iodine solution. In contrast, oligonucleotides synthesized by the CEDP chemistry on three different Applied Biosystems DNA synthesizers did not show guanine base sensitivity to the KMnO_4 T-specific sequencing reaction (Fig. 4). The reason may be the avoidance of thiophenol and the methyl blocking group in the design of the CEDP chemistry. We would like to know what kinds of modifications in guanine can make it reactive to KMnO_4 . Instead of trying to proof the structure of the trace by-product observed in this work, we shall be studying the reactivities of specifically modified guanine compounds.

Detection of G-modification by UvrABC endonuclease.

The use of UvrABC endonuclease in the quality control of synthetic oligonucleotide requires double-stranded DNA of about 40 basepairs or longer. In Fig. 5, Panels A and B, a pair of complementary 54 mers (named A and B) synthesized by MEDP chemistry were hybridized, treated with the purified UvrABC endonuclease and analyzed on a DNA sequencing gel with the DNA sequencing reactions as reference. Strand A was 5' labeled and examined in Panel A while Strand B was 5' labeled and examined in Panel B. In Panel B, the UvrABC endonuclease produced several incision bands in Strand B (lane 5), indicating the presence of "bulky" lesions in this oligonucleotide. These incision bands can be traced to reveal the G bases as the modified bases in the oligonucleotide. In order to estimate the extent of the modification of the guanine bases in the oligonucleotides, we used as a reference, the same 54 mer except that a psoralen monoadduct has been placed in every molecule of Strand B. The psoralen is covalently bonded to the T in the single 5'TpA sequence in this oligonucleotide (20). The relative amount of incision at the psoralen monoadduct versus that of the G bases is estimated by densitometer tracing. Because of the UvrABC endonuclease has some sequence specificity in its repair incision efficiency, the extend of G modification in the oligonucleotide can only be estimated as about 0.5 to 3% at a given G base. In contrast, Strand A showed no UvrABC incisions in lane 5 of Panel A, suggesting the absence of "bulky" modifications. The oligonucleotide in Strand A was

Fig. 5. UvrABC endonuclease detection of G modifications in oligonucleotides synthesized with MEDP chemistry. A pair of complementary 54 mers (named A and B in the text) synthesized by MEDP chemistry were hybridized, treated with the purified UvrABC endonuclease and analyzed on a DNA sequencing gel with the DNA sequencing reactions as reference. Strand A was 5' labeled and examined in Panel A while Strand B was 5' labeled and examined in Panel B. The DNA sequence of Strand B was resynthesized with CEDP chemistry and named Strand C. In Panel C, Strand C is hybridized to Strand A and subjected to the same analyses as in the experiment in Panel B. In each panel, Lanes 1-4 are the sequencing lanes as indicated. Lane 5 in each panel is the UvrABC endonuclease incision of the labeled DNA strand in the DNA duplexes. Lane 6 in Panels B and C are incision by the UvrABC endonuclease in the labeled DNA strand in which a psoralen monoadduct was covalently bound to the thymine in the unique 5'TpA site. In each case, a line is drawn from the UvrABC endonuclease incision band, through the phosphodiester bond broken by the enzyme, to the base with which the UvrABC incision is correlated.

chosen to show that UvrABC endonuclease detectable base modifications are not found in all oligonucleotides synthesized by the MEDP chemistry. When Strand B was resynthesized with CEDP chemistry (named Strand C), hybridized to the same Strand A and treated with the UvrABC endonuclease, no incisions were

observed (Panel C) in Lane 5. The UvrABC endonuclease incision at the psoralen monoadduct was again included in Panel C, Lane 6, as a control. Comparing Lane 6 in Panel B with Lane 6 in Panel C, one can see that the oligonucleotides synthesized by the CEDP chemistry do not contain non-psoralen lesions which can be recognized by the UvrABC endonuclease. Interestingly, the KMnO_4 reactivity of the G bases described above was present only in Strand B and absent in Strand A and C (Fig. 5), suggesting the possibility that the G modifications observed by the two methods we described may be related.

Since there is a remote possibility that some of the isobutyryl groups used to protect the N_2 of guanine may still be on the oligonucleotide during our UvrABC reaction, and that such a blocked G base will likely be a good substrate for UvrABC endonuclease, we repeated the experiment in Fig. 5, Panel B, after deblocking the DNA with another 24 hours of ammonium hydroxide reaction at 55°C . The results (not shown) were indistinguishable from the results illustrated in Fig. 5. Thus, we conclude that the isobutyryl groups were successfully removed in our deblocking reactions and were not the substrates for UvrABC endonuclease incision seen in these experiments.

Application of the Rubin and Schmid pyrimidine-specific reactions to the chemical sequencing of oligonucleotides.

Maxam and Gilbert were first to establish pyrimidine-specific reactions for chemical DNA sequencing (18). Their procedure uses hydrazine to open the pyrimidine ring, with the T reaction suppressed by the presence of salt. Rubin and Schmid (17) described an alternative procedure for the pyrimidine-specific reactions for chemical DNA sequencing. Their procedures make use of the sensitivity of the thymine base to the oxidation by KMnO_4 , and the reactivity of the cytosine base with hydroxylamine at pH 6.0. The benefits of the Rubin and Schmid reactions are speed, avoidance of flammable hydrazine, absence of T reaction suppression commonly encountered in the hydrazine reaction due to salt contamination, absence of C cross-reactivity in the T reaction, and the near absence of T cross-reactivity in the C reaction. The drawback is the sensitivity of the T reaction to the secondary structures in the DNA (19). Initially, we found the KMnO_4 T-specific reaction to be G and T-specific for synthetic oligonucleotides but highly T-specific for plasmid derived DNA. We believe the problem was due to the KMnO_4 sensitive modified G bases in oligonucleotides synthesized by the MEDP chemistry. As seen in Fig. 6, the Rubin and Schmid method for pyrimidine-specific reaction yields good results if the oligonucleotides are synthesized by the CEDP chemistry.

Fig. 6. Other putative base modifications in oligonucleotides synthesized by the CEDP chemistry, as suggested by chemical DNA sequencing. Panels A and B are the DNA sequencing reactions as indicated. The phantom bands about 1 1/2 bases above C bases are indicated by triangles on the side. The phantom bands about half a base spacing above G bases are indicated by arrows on the side. The A lanes were weak in these sets of reactions.

Application of chemical DNA sequencing to quality control of synthetic oligonucleotides.

While DNA sequencing is useful for confirming the DNA sequence of an oligonucleotide, it can also be useful for the diagnosis of minor base modifications. The chemical reactivities of the bases in the DNA have been extensively characterized (for review, see 21). As shown in Fig. 6, when freshly distilled piperidine is used (or piperidine kept over molecular sieve after distillation), the G,C and T reactions can be expected to be specific. Cross-contamination of phosphoramidites due to valve problems or programming errors usually results in abundant distortion of all four sequencing lanes (data not shown). Base modifications may appear in two different ways in a sequencing gel. In the lane normally specific for that base, the modification of the base may interfere with the ring opening reaction, or the beta-elimination reactions for the cleavage of the phosphate backbone, resulting in the consistent appearance of phantom bands at a discrete spacing above where the base-specific bands would appear. Examples for possible C and G modifications in the DNA synthesized by the CEDP chemistry are shown in Fig. 6. The putative modified bases are not recognized by the KMnO_4 method or the UvrABC endonuclease method of detection (data not shown). The phantom bands for C were seen about 1 1/2 base spacings above the C bands (indicated by triangles on the side) while the phantom bands for G were half a base spacing, or sometimes 2 1/2 to 3 base spacings, above the G bands (indicated by arrows on the side). One possible explanation may be that the modified G base exaggerates the normally slower beta-elimination at the 5' side of the base when compared to the 3' side of the modified base, and the resultant oligonucleotide fragment, having the same charge as the band from the completed G reaction but a base larger, moves about half a base slower on the DNA sequencing gel. Thus, the phantom bands for the G bases are absent when the DNA is labeled at the 3' end instead of the 5' end (data not shown). These modified bases were not detected in the oligonucleotides we synthesized with MEDP chemistry. Alternatively, the base may become non-reactive to DNA sequencing reactions, as shown for psoralen-modified thymine bases (20). If the modification group is large, all DNA sequencing bands above the modified base may be displaced upwards by about one base spacing in all sequencing lanes as in the case of a psoralen monoadduct on a thymine (20). In the other lanes in which a normal base should not react, a modified base may acquire new reactivity, resulting in bands that may or may not run to the same position as a specific cleavage reaction. An example is the modified G bases which are

detected in the T reaction as described above. Unlike problems caused by impure piperidine, these bands do not appear in all four lanes. Chemical DNA sequencing is a rather artful procedure that is at times problematic. Bands in an autoradiogram can be made to appear or disappear even without photographic enhancement. For example, enhancing screens tend to exaggerate major bands and make minor bands disappear. We have tried to avoid such distortions in our autoradiograms, and photographed our autoradiograms on white background without transillumination, at low contrast, to preserve the natural appearance of the autoradiograms. In skillful hands, chemical DNA sequencing can reveal base modifications at specific bases in manners that cannot be accomplished by other methods.

While our data suggests that CEDP chemistry produces DNA that does not contain some of the the G modifications introduced by the MEDP chemistry, it is not our intention to endorse instruments using CEDP chemistry over instruments using the MEDP chemistry. Problems of hardware, software, the source of the reagents, and proper maintenance of the DNA synthesizer are also important issues. We have stopped using the MEDP chemistry because it is inconsistent in our hands in the normal operation of a DNA synthesizer, in spite of our efforts to properly use the DNA synthesizer, short of thoroughly flushing out the whole instrument and loading all new reagents before each synthesis. Because examination of our thorough log for the synthesis operation and quality control has not linked the G modifications to any particular event in the maintenance and operation of the synthesizer or to specific lots of synthesis chemicals, the problem may be inherent in the current designs of DNA synthesizers and chemicals. MEDP chemistry may permit a smaller margin of error than the CEDP chemistry. The DNA synthesized by either chemistries, in our hands, functions very well as DNA sequencing primers without need for post-synthesis purification. DNA synthesized by the MEDP chemistry is good enough for many applications, while the DNA synthesized by the CEDP chemistry has its own problems such as putative new forms of base modifications and difficulty in deblocking to completion. However, we regard DNA lesions which are recognized by UvrABC endonuclease as the more serious base modifications because they are likely to be mutagenic and induce recombination, as has been previously demonstrated for all known substrates of UvrABC endonuclease (14,15). They may even induce SOS functions in the host cells and thus lead to unexpected mutation events (14). These lesions are not repaired as rapidly as other less "bulky" lesions like uracil,

3-methyladenine, formamidopyrimidine, etc, that are efficiently repaired by abundant DNA glycosylases in the cell (22,14). DNA synthesized by the CEDP chemistry does not contain detectable levels of UvrABC recognizable lesions. These DNA also showed biological activity close to that of biological DNA while the DNA synthesized by the MEDP chemistry was about 30 times lower in biological activity (3). However, our findings may not have any bearing on that latter observation because variations in similar DNA synthesis protocols can have dramatic effects on base modification.

Recently, Eadie and Davidson (23) reported the observation of a fluorescent dG adduct in oligonucleotides synthesized with the CEDP chemistry. The adduct was believed to be caused by the attachment of N,N-dimethylamino-pyridine (DMAP) to the 6 position of guanine on the oligonucleotides. This adduct can be converted in ammonium hydroxide to 2,6 diaminopurine. Conceivable, this adduct may be present in the oligonucleotides we used for the studies in this report, and its bulkiness suggests that it should be a substrate for the UvrABC endonuclease. However, the amount of this fluorescent adduct was reported to be only 0.07% with respect to deoxyguanine (23). Because the modified dG in Eadie and Davidson's experiments was made by 10 to 50 cycles of DNA synthesis chemistry on a capped dG controlled pore glass support, the dG was not allowed to increase in number to form an oligonucleotide. Thus the 0.07% value for modified dG with respect to dG may be 10 to 50 times higher than what would be observed in a normal oligonucleotide synthesis. We believe that our methods of analysis cannot detect such low levels of guanine modification. However, we do not know if the level of this fluorescent adduct may be higher in oligonucleotides synthesized with the MEDP chemistry and whether it contributed to the modified guanines we detected.

In summary, we have shown that some base modifications that occur in synthetic oligonucleotides can be detected with the E. coli UvrABC endonuclease. We showed that a specific chemical reaction can be used to detect a guanine modification in synthetic oligonucleotides. These modifications are often observed in oligonucleotides synthesized by the MEDP chemistry but not in oligonucleotides synthesized by the CEDP chemistry. We also showed that the Rubin and Schmid C- and T-specific reactions can be used for the chemical sequencing of oligonucleotides, especially those synthesized by the CEDP chemistry. Finally, we suggest that chemical DNA sequencing is a useful tool for monitoring sequence-specific base modifications in synthetic oligonucleotides.

ACKNOWLEDGEMENTS

These studies were supported, in part, by a grant from the National Science Foundation (DMB84-17419) to A.Y., Institutional grants from the National Institute of Health to the Fox Chase Cancer Center (CA06927, RR05539), an appropriation from the Commonwealth of Pennsylvania, and a grant from the Glenmede Trust to the FCCC.

*To whom reprint requests should be sent

REFERENCES

1. Gao, X., Gaffney, B.L., Senior, M., Riddle, R.R., and Jones, R.A. (1985) Nucleic Acids Res. **13**, 573-584.
2. Gaffney, B.L., Markey, L.A., and Jones, R.A. (1984) Tetrahedron Letters **40**, 3-13.
3. Urdea, M.S., Ku, L., Horn, T., Gee, Y.G., and Warner, B.D. (1985) Nucleic Acids Res. **16**, 257-260.
4. Reese, C.B., and Ubasawa, A. (1980) Tetrahedron Letters **21**, 2265-2268.
5. Reese, C.B. (1978) Tetrahedron Letters **34**, 3143-3179.
6. Shapiro, R. (1968) Progress in Nucleic Acid Res. and Mol. Biol. **8**, 73-112.
7. Singer, B. (1975) Progress in Nucleic Acid Res. and Mol. Biol. **15**, 218-284.
8. Zon, G., Gallo, K.A., Samson, C.J., Shao, K.L., Summers, M.F., and Byrd, R.A. (1985) Nucleic Acids Res. **13**, 8181-8196.
9. Yeung, A.T., Mattes, W.B., and Grossman, L. (1986) Nucleic Acids Res. **14**, 2567-2582.
10. Yeung, A.T., Mattes, W.B., Oh, E.Y., Yoakum, G.H., and Grossman, L. (1983) Proc. Natl. Acad. Sci. USA **80**, 6157-6161.
11. Sancar, A., and Rupp, W.D. (1983) Cell **33**, 249-260.
12. Yeung, A.T., Jones, B.K., Capraro, M., and Chu, T. (1987) Nucleic Acids Res. **15**, 4957-4971.
13. Yeung, A.T., Mattes, W.B., Oh, E.Y., Yoakum, G.H., and Grossman, L. (1986) Nucleic Acids Res. **14**, 8535-8556.
14. Walker, G.C. (1985) Ann. Rev. Biochem. **54**, 425-457.
15. Howard-Flanders, P. (1973) Br. Med. Bull. **29**, 226-235.
16. Schendel, P.F., Defais, M., Jeggo, P., Samson, L., and Cairns, J. (1978) J. Bacteriol. **135**, 466-475.
17. Rubin, C.M., and Schmid, C.W. (1980) Nucleic Acids Res. **8**, 4613-4619.
18. Maxam, A.M., and Gilbert, W. (1980) Methods Enzymol. **65**, 498-560.
19. Hayatsu, H., and Ukita, T. (1967) Biochem. Biophys. Res. Comm. **29**, 556-561.
20. Jones, B.K., and Yeung, A.T. (Submitted for Publication).
21. Organic Chemistry of Nucleic Acids, Part B. eds. N.K. Kochetkov and E.I. Budovskii. Plenum Press. London and New York. 1972.
22. Lindahl, T. (1982) Ann. Rev. Biochem. **51**, 61-87.
23. Eadie, J.S., and Davidson, D.S. (1987) Nucleic Acids Res. **15**, 8333-8349.