EVIDENCE FOR PALINDROMIC SEQUENCES NEAR THE TERMINI OF ADENOVIRUS 2 DNA

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SUMMARY

When the kinetics of Escherichia coli exonuclease III digestion of adenovirus 2 DNA were studied by DNA polymerase I-catalyzed repair synthesis at 5°C, there was an indication of the formation of hairpin structure in the single-stranded template, exposed by exonuclease III. The hairpin structure results from a sequence with an inverted repetition of the type, a b c d···d'c'b'a'. The location of these sequences was determined to be about 180 nucleotides from each terminus of adenovirus 2 DNA with the use of specific restriction endonucleases. The possible role of this region in the replication of the adenovirus 2 genome is discussed.

INTRODUCTION

Human Ad 2* DNA is a linear, duplex molecule of 23 x 10^6 daltons (2). Ad 2 DNA molecules do not have terminal redundancy of the type a b c···a b c that is present in many bacteriophage DNAs, since they do not circularize after treatment with E.coli exonuclease III followed by annealing (2). But Ad 2 DNA molecules possess a unique inverted terminal repetition of the type a'b'c'···c b a, which permits the formation of single-stranded DNA circles after denaturation and reannealing (3,4). The length of this inverted terminal repetition has been estimated at 100 to 140 nucleotides by the use of specific endonucleases (5). In this communication, we report the presence of an unusual structural feature - a long palindromic DNA sequence located approximately 180 nucleotides from each terminus of Ad 2 DNA.

MATERIALS AND METHODS

Viral DNA. Ad 2 was grown in suspension cultures of human KB cells and virus DNA was purified as reported earlier (6). As a final step, zonal centrifugation in a neutral sucrose density gradient was included (7).

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*Abbreviations used: Ad 2, Adenovirus 2; EcoR1, HaeIII and HpaII-restriction endonucleases from E.coli, Hemophilus aegyptius and Hemophilus parainfluenza, respectively (see Ref. 1).

Enzymes. E.coli exonuclease III, purified as described (8,9), was a gift from Dr. Ray Wu. DNA polymerase I was purified as described by Jovin et al. (9). EcoR1, HaeIII and HpaII restriction endonucleases were purchased from Biolabs (Boston, Mass.).

Exonuclease III digestion of unlabeled Ad 2 DNA followed by DNA polymerase I-catalyzed repair. Ad 2 DNA was digested with E.coli exonuclease III as follows. The incubation mixture (0.68 ml) containing 27 µg of Ad 2 DNA in 60 mM Tris·HCl(pH 7.6), 70 mM NaCl, 10 mM dithiothreitol, 5 mM Mg^{2+}, and 5 units of exonuclease III (10), was incubated at 37°C. Aliquots (25 µl) were removed at indicated time intervals and the reaction was stopped by the addition of 1 µl of 0.2 M EDTA and heating at 74°C for 10 min. The enzymatic repair synthesis was performed in a reaction mixture (35 µl) containing 70 mM potassium phosphate (pH 6.9), 68 mM NaCl, 10 mM dithiothreitol, 15 mM Mg^{2+} (7), all four [α-^{32}P] labeled deoxyribonucleoside triphosphates, and 2 units of DNA polymerase I for 6 hr at 5°C. Acid-insoluble radioactivity of 1 µl aliquots was determined as the reaction progressed (7), which reached a plateau after 5 hr.

Endonuclease Digestion. Ad 2 DNA was labeled by treatment with exonuclease III to four different extents followed by enzymatic repair as described above. Endonuclease digestion with EcoR1 (11), HaeIII (12) or HpaII (13) was carried out as described (5).

Gel electrophoresis. Electrophoresis on polyacrylamide or agarose slab gels (14) was used. Analyses of the EcoR1 restriction endonuclease digest of end labeled Ad 2 DNA were performed on a 1.4% agarose gel and those of HpaII or HaeIII endonuclease digests on a 4% polyacrylamide gel.

RESULTS

Exonuclease III digestion and DNA polymerase I-catalyzed repair of Ad 2 DNA. The kinetics of exonuclease III digestion from the 3'-termini of Ad 2 DNA was measured by DNA polymerase I-catalyzed repair which uses the exposed DNA single-strands as template. The results shown in Fig. 1 indicate that the kinetics are biphasic. The repair synthesis was linear until about 180 nucleotides were incorporated into each 3'-terminus of Ad 2 DNA. When Ad 2 DNA was digested further with exonuclease III, there was a slight drop in the total incorporation of nucleotides, presumably due to a decrease in the available template. A 2.5 fold decrease in the amount of exonuclease III used in the experiment gave virtually identical results, i.e. a decrease in the total incorporation during the subsequent DNA-polymerase I-catalyzed repair occurred again when about 180 nucleotides had been removed from each 3' end. These data indicate that exonuclease III digests Ad 2 DNA molecules synchronously (15). The reproducible biphasic kinetics (similar results were obtained in 5 experiments) suggest the possible formation of secondary structure within the single-stranded template exposed by exonuclease III. We considered the possibility that a structure with a hairpin loop could arise from a sequence with an inverted repetition, a b c d e.t.e'd'c'b'a', where t represents

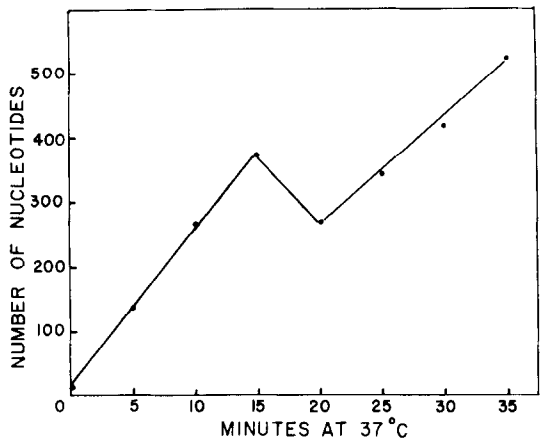

Fig. 1. The kinetics of E.coli exonuclease III digestion of Ad 2 DNA. The extent of enzyme digestion was measured by DNA polymerase I-catalyzed repair, as described in the text. The specific activities of all four [α-^{32}P]deoxynucleoside triphosphates (New England Nuclear, Boston, Mass.) were approximately equal. The number of nucleotides incorporated were calculated using a molecular weight of 23 x 10^6 for Ad 2 DNA (2) and an absorbance of 6.6 at 260 nm being equal to 1 μmole nucleotide/ml.

the nucleotides in the "turn around" region of the loop (16). During the initial and intermediate stages of exonuclease III digestion (when 75 to 150 nucleotides from each 3' end were removed) the DNA molecule will probably exist as shown in structure A (see Fig. 2). The enzymatic repair on molecules A would be expected to reach both termini of native Ad 2 DNA, giving rise to completely duplex molecules. At more extensive levels of digestion (when greater than 180 nucleotides per 3' end were removed) by exonuclease III, structure B or C can be formed. The DNA polymerase I-catalyzed repair synthesis would probably be incomplete in DNA molecules in the form of structure B or C. If all the molecules are in form B or C at these levels of exonuclease III digestion, one would expect a more pronounced decrease in total number of nucleotides incorporated during subsequent repair (see Fig. 1). An equilibrium between structures B (or C) and D may account for the incomplete drop in the total number of nucleotides incorporated due to the formation of a hairpin structure. In addition, prolonged incubation with exonuclease III which might contain slight amounts of contaminating nucleases (15) could result in the addition of a small number of nucleotides to internal regions of Ad 2 DNA during subsequent repair synthesis (suggested by the presence of minor bands in Fig. 3 A and C).

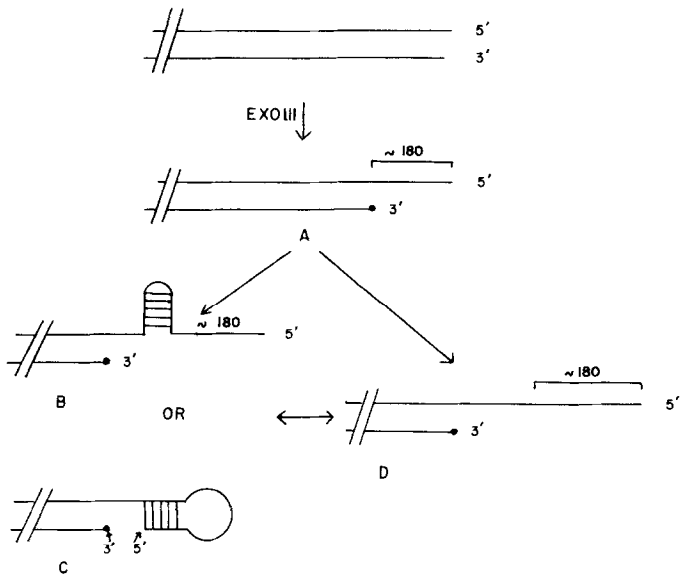

Fig. 2. A model for the exonuclease III reaction on Ad 2 DNA. During the initial and intermediate stages of reaction, (75 to 150 nucleotides removed from each 3' terminus), Ad 2 DNA molecules are represented by form A. As the exonuclease III digestion becomes more extensive (over 180 nucleotides removed per end), Ad 2 DNA molecules may exist in form B or C which is probably in equilibrium with form D. The filled circle at the 3' terminus represents exonuclease III enzyme.

Analysis of end labeled Ad 2 DNA by digestion with restriction endonucleases EcoRl, HaeIII and HpaII . In order to test our suggested interpretation of the data, Ad 2 DNA was digested with exonuclease III to 4 different levels and subsequently repaired with DNA polymerase I under conditions described in Fig. 1. The number of nucleotides added to the 3' end of exonuclease III treated Ad 2 DNA preparations, 1 to 4, were about 75, 150, 225 and 350 nucleotides respectively. The labeled DNA was digested with EcoRl which cuts Ad 2 DNA into 6 fragments (11). The labeled end fragments, A and C, after separation by gel electrophoresis on 1.4% agarose were further purified by hydroxyapatite chromatography (17). The A fragment was further digested with HaeIII and HpaII (see Fig. 3, A and C, respectively) and the C fragment with HaeIII (B). From independent electrophoretic data, we estimate the sizes of the HaeIII end-labeled fragments to be about 265 and 315 base pairs from EcoRl A and C termini, respectively. Ad 2 DNA at initial and intermediate stages of exonuclease III digestion (see form A in Fig. 2), followed by

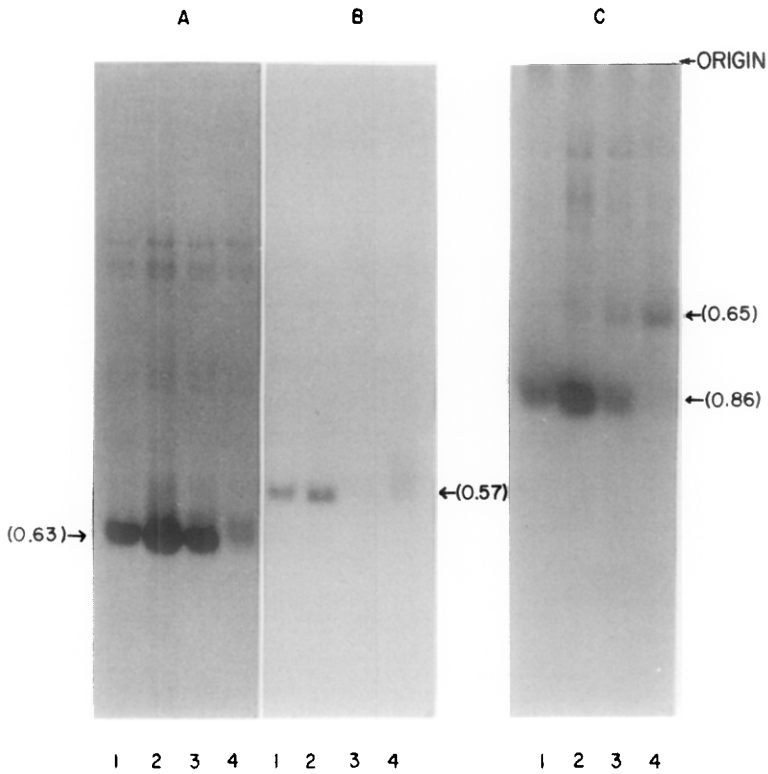

Fig. 3. Polyacrylamide gel electrophoresis of restriction endonuclease digests. Ad 2 DNA was labeled at 3' end by digesting with exonuclease III to 4 different levels followed by enzymatic repair as described in the text. The number of nucleotides incorporated to Ad 2 DNA preparations, 1 to 4 were about 75, 150, 225 and 350 nucleotides, respectively. EcoR1 terminal DNA fragments, A and C were obtained from these DNAs and further digested with HaeIII (Fig. 3A and 3B) or HpaII (Fig. 3C). The substrate used in Fig. 3A and 3C was EcoR1 A fragment and in Fig. 3B, C fragment. The relative mobilities of the electrophoretic bands indicated by an arrow with reference to the bromphenol blue dye as the marker are given in the parenthesis.

DNA polymerase I repair, each yielded a major HaeIII terminal fragment (Fig. 3, lanes 1 and 2 in A and B). In DNA molecules that were more extensively digested with exonuclease III (see forms B or C in Fig. 2), this fragment was less dense and more diffuse, indicating that only a fraction of the molecules were repaired completely by DNA polymerase I (see Fig. 3, lanes 3 and 4 in A and B). The existence of an equilibrium between structures B (or C) and D may account for the appearance of a smaller amount of HaeIII terminal fragments from these DNA molecules. The fact that extensive exonuclease III digestion creates Ad 2 DNA structures that are incompletely repaired by E.coli DNA polymerase I is further supported by digestion of EcoR1 A fragment from these DNA molecules

with HpaII endonuclease (see Fig. 3C). HpaII cleaves Ad 2 DNA at approximately 140 base pairs from the EcoR1 A terminus (5). HpaII treatment of the EcoR1 A fragment (obtained from Ad 2 DNA treated with exonuclease III at initial and intermediate levels, followed by enzymatic repair) yields the terminal fragment (lane 1 and 2), whereas at more extensive levels of digestion, this fragment arises only from a portion of the DNA molecules (lane 3), or completely absent (lane 4). A new band appears with a relative mobility of 0.65 which might be the HpaII cleavage product adjacent to the terminal fragment.

DISCUSSION

We have identified the presence of DNA sequences with two-fold symmetry located approximately 180 nucleotides from each terminus of Ad 2 DNA. Our data (unpublished results) obtained by digesting uniformly [^{32}P] labeled Ad 2 DNA with exonuclease III to remove about 350 nucleotides per end, followed by single-stranded DNA specific nuclease S_1, indicate that the length of the duplex region of the hairpin structure (B or C in Fig. 2), is approximately 48 base pairs long. An interesting observation which resulted from these studies is the fact that DNA polymerase I could not "melt" the secondary structure of 48 base pairs during the repair synthesis carried out at 5°C and in 180 mM salt. When the temperature of repair synthesis was 37°C the enzyme was able to copy the region with the secondary structure as suggested by the appearance of the two end fragments after digestion with HaeIII enzyme (unpublished data).

Sequences with two-fold symmetry have been found in the binding sites of E.coli RNA polymerase on SV40 DNA (18), ribosome binding site (19), the lac operator (20,21) and the promoter region of the gene for an E.coli tyrosine tRNA (22). The palindromic sequences present in Ad 2 genome may serve a function as a recognition signal for some biologically important protein. Our preliminary experiments show that Ad 2 DNA-associated protein which circularizes the genome (23) is bound to the two HindIII terminal fragments of Ad 2 DNA (unpublished data). The question of whether these palindromic sequences are involved in the binding of this protein must await further studies on the location of its binding site with the use of other restriction endonucleases.

Recent data (24,25) are consistent with a mechanism of Ad 2 DNA replication involving initiation at either end of viral DNA. Another possibility is that these palindromic sequences at about 180 nucleotides from the terminus of each strand might serve as initiation sites of replication. Cavalier-Smith proposed a model for replication of eukaryotic chromosomes involving sequences with two-fold symmetry present at the termini of DNA

(see Fig. 2 of Ref. 26). His model would predict that when Ad 2 DNA is subjected to exonuclease III digestion, the newly exposed 5'-terminal sequences of single-stranded DNA would form a hairpin, represented by structure C in Fig. 2. Our present data do not distinguish between the two possible hairpin arrangements (B or C in Fig. 2). Further studies are required in order to delineate between these two structures and to establish the biological role of this unique feature of the Ad 2 genome.

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