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Cae I: an endonuclease isolated from the African green monkey with properties indicating site-specific cleavage of homologous and heterologous mammalian DNA

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ABSTRACT

Component α DNA is a highly repetitive sequence that comprises nearly a quarter of the African green monkey (Cercopithecus aethiops) genome. A previous microbial restriction enzyme analysis showed that the repeat structure of component α DNA is based upon a monomeric unit of 176 ± 4 base-pairs. An endonuclease, provisionally termed Cae I, has been isolated from African green monkey testes that cleaves component α DNA into multimeric segments based upon the same repeat periodicity as that revealed by microbial restriction enzymes. The primary sites of Cae I cleavage in the component α sequence appear to be 120 ± 6 base-pairs distant from the Hind III sites and 73 ± 6 base-pairs distant from the Eco RI* sites. Cae I has been partially characterized with special reference to the effects of ATP and S-adenosylmethionine on the cleavage of component α DNA. Cae I may be a member of a class of similar site-specific nucleases present in mammalian cells.

Cae I also cleaves mouse satellite DNA into a multimeric series of discrete segments: the periodicity of this series is shorter than that revealed by Eco RII restriction analysis of mouse satellite DNA.

INTRODUCTION

A restriction enzyme analysis of component α DNA of the African green monkey (Cercopithecus aethiops) and of five other highly repetitive mammalian DNAs reveals recurrent periodicities in the long-range repeat structure (1,2). A basic repeat unit of 176 ± 4 base pairs underlies a superimposed, higher order sequence arrangement which is characteristic of each mammalian species. Other findings (3,4) suggest a phasing between the nucleosomal proteins of constitutive heterochromatin and the sequence structure of highly repetitive DNA and an essential role of the nucleosome in modulating the repeat periodicities. These observations are consistent with recombinations occurring at defined sites in the nucleosome structure of constitutive heterochromatin and within the sequences themselves. The transmission of the altered sequences to progeny and their rapid evolutionary development require that such recombination events occur in the germ line cells. Site-specific endonucleases seem eminently suitable agents for the initiation of recombination events at

defined sites in repetitive DNA sequences: accordingly, we have looked for such enzymes in mammalian germ line tissues. In this report, we describe a mammalian endonuclease isolated from African green monkey testes with properties indicating site-specific endonucleolytic activity on homologous and heterologous mammalian DNAs.

MATERIALS AND METHODS

Preparation of Testes Extract

The enzyme, provisionally termed Cae I from the Linnean name of the African green monkey, was obtained from testes of adult monkeys. In a typical preparation, the tunica albuginea was trimmed from 4 testes (16 grams of tissue before trimming) and each testis was minced in 3 ml of ice-cold extraction buffer (20 mM Tris-HCl, pH 7.65, 7 mM 2-mercaptoethanol, 1 mM dithiothreitol). Two-ml aliquots of the minced tissue were further homogenized in a Virtis model 60K tissue homogenizer at 17,000 rpm for 45 sec at 4°. The aliquots were pooled, the total volume brought to 23 ml with extraction buffer, and solid NaCl was added with stirring to 1 M final concentration. The homogenate was sonicated until it was no longer viscous (5 times for 30 sec each). The temperature was not allowed to rise above 8° during sonication. The sonicate was centrifuged at 120,000 x g for 45 min at 4°. The pellet, which contained nearly all of the DNA in the extract, was discarded and the supernatant (crude testes extract) was saved for the following purification steps.

Enzyme Purification: Method A

The crude testes extract was loaded onto a Biogel A-0.5 m column (200-400 mesh, 3 X 47 cm) previously equilibrated with extraction buffer containing 1 M NaCl. Column elution was with 500 ml of this buffer at 4°, and the eluate was continuously scanned at 280 nm with a UV monitor (Fig. 1). Column fractions (3.5 ml) were assayed for nuclease activity as described below. The peak Cae I activity eluted at about one-half the bed volume (see arrow, Fig. 1). Four to five nucleases that degraded component α nonspecifically were also resolved: one of these eluted just ahead of the peak of Cae I activity. Fractions containing Cae I activity were pooled and dialyzed against two 3-liter changes of 20 mM Tris-HCl, pH 7.65, 7 mM 2-mercaptoethanol and 15% (vol/vol) glycerol (DEAE buffer).

A DEAE-cellulose column (Whatman DE 52, 2 X 10 cm) was equilibrated with DEAE buffer and washed with 100 ml of the buffer of the second dialysate immediately before loading the sample. After loading, the column was washed with 40 ml of DEAE buffer and eluted with 120 ml of a linear salt gradient

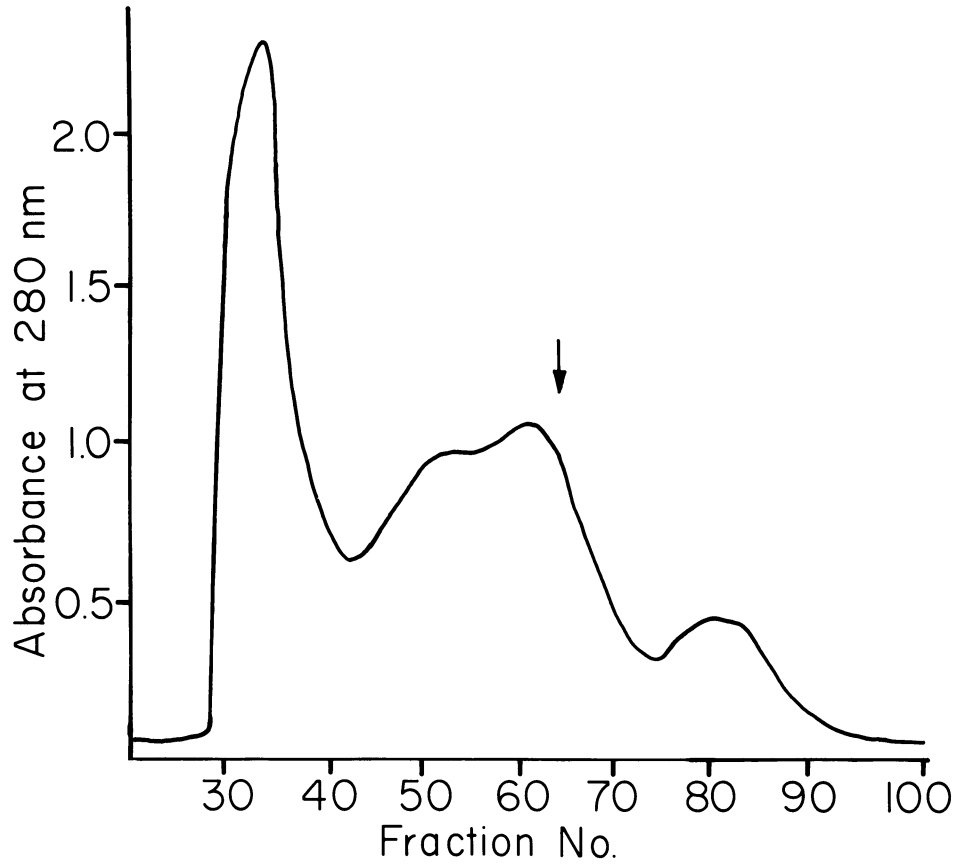

Figure 1. Chromatography of crude testes extract on a Biogel A column. The extract was prepared and subjected to chromatography on the Biogel A column as described in Materials and Methods. The arrow indicates the peak of the Cae I activity (fraction 65), but activity was also detected in four adjacent fractions eluting immediately before and after fraction 65. These nine fractions were pooled, dialyzed, and subjected to DEAE-cellulose chromatography as shown in Fig. 2.

(0 to 0.7 M NaCl) and finally with 10 ml of 0.7 M NaCl in DEAE buffer. The elution was continuously scanned at 280 nm with a UV monitor (Fig. 2) and 1-ml fractions were collected and assayed as described below. The Cae I activity eluted between 65 and 125 mM NaCl (the arrow in Fig. 2 indicates the peak of activity). The active fractions were pooled, dialyzed against DEAE buffer containing 20 mM NaCl, and then solid NaCl was added to 1 M final concentration. Solid ammonium sulfate was then added (85 mg/ml) and the solution stirred for 45 min in an ice-water bath. The supernatant was collected after centrifugation at 17,000 x g for 30 min at 4° C and the pellet discarded.

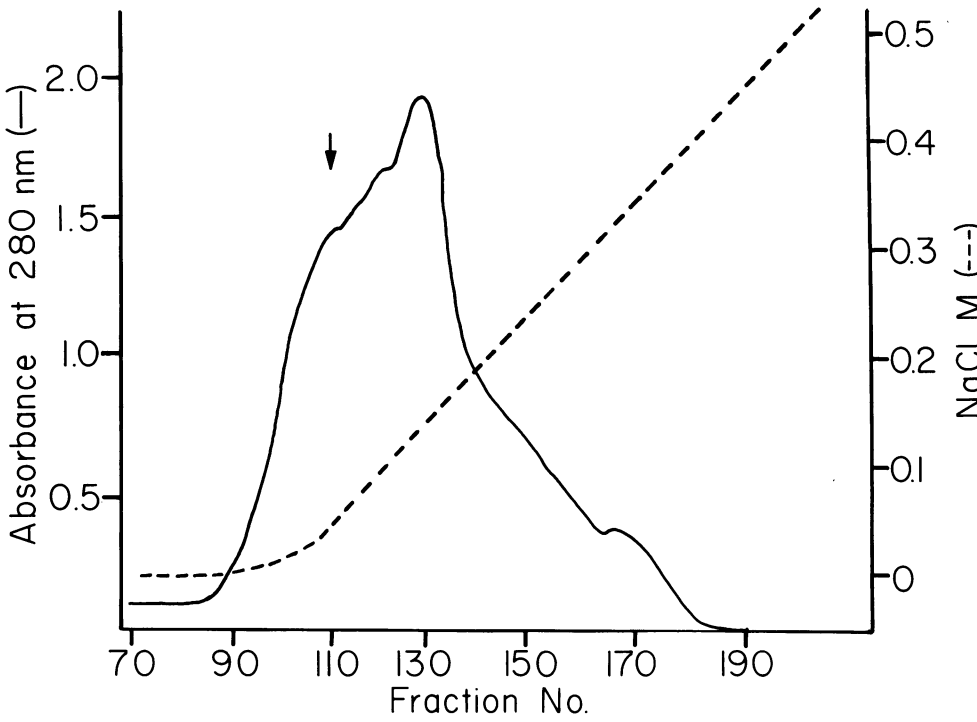

Figure 2. DEAE-cellulose chromatography of the Cae I activity. The active fractions from a Biogel A column (Fig. 1) were pooled and prepared for DEAE-cellulose chromatography as described in the text. The arrow marks the peak of enzyme activity which eluted abruptly at about 65 mM NaCl. There was trailing of enzyme activity as the salt concentration increased to about 125 mM.

Solid ammonium sulfate (121 mg/ml) was added to the supernatant and the solution was stirred for 45 min in an ice-water bath. The precipitate was collected by centrifugation, the pellet dissolved in DEAE buffer containing 20 mM NaCl, and dialyzed overnight against 2 liters of this buffer. At this stage in the purification, the enzyme is stable for weeks when stored at -20°.

Enzyme Purification: Method B

The crude testes extract was prepared, and enzyme purifications by Biogel A and DEAE-cellulose were carried out as described in Method A except that the ammonium sulfate fractionations were omitted. The post-DEAE-cellulose activity was concentrated by ultrafiltration through Amicon PM 10 Diaflow membranes.

Enzyme Purification: Method C

The two ammonium sulfate fractionations described in Method A were

carried out with the crude testes extract before the Biogel A and DEAE-cellulose column chromatography steps.

DNA Sources

Total nuclear DNA was purified from primary explant cultures of African green monkey kidney (AGMK) cells. High molecular weight (> 10^7 daltons) component α DNA (5) and mouse satellite DNA were isolated from the total AGMK or mouse liver DNA as described previously (6,7).

Enzyme Assay

The basic reaction buffer is 20 mM Tris-HCl, pH 7.65, 5 mM $MgCl_2$ and 7 mM 2-mercaptoethanol. Reaction mixtures for column assays contained 1.2 µg of component α DNA or 2 µg of AGMK total nuclear DNA in 100 µl of basic reaction buffer. For economy, the column fractions were first assayed with total AGMK nuclear DNA: however, the nuclease activity described here was often difficult to detect with this substrate because extensive cleavage of bulk DNA sequences obscured the distinctive agarose gel patterns produced by cleavage of component α. Consequently, fractions containing nuclease activity were reassayed for the presence of Cae I with pure component α DNA. Reactions were started by the addition of 2 µl portions of the column fractions and incubation was at 37° for 30 min. Reactions were terminated by the addition of 20 µl of a solution containing 0.25 M EDTA and 5% (wt/vol) sodium dodecyl sulfate. DNAs in the reaction mixtures were precipitated overnight at -20° in 1 volume of 0.5 M ammonium acetate and 4 volumes of absolute ethanol, sedimented by centrifugation at 175,000 x g for 45 min and resuspended in 30 µl of electrophoresis buffer for agarose and acrylamide slab gel electrophoresis. Microbial restriction enzyme digestions, gel electrophoresis and molecular weight calculations of DNA segments were carried out as previously described (1,2). All gels were post-stained for 30 min with ethidium bromide (0.5 µg/ml in electrophoresis buffer). We provisionally define Cae I unit activity as that amount of enzyme required to convert 1.2 µg of component α DNA to decamers or shorter segments of the multimeric series (1,2) in 2 hr at 37°.

RESULTS

Properties of the Enzyme

With pure component α DNA as substrate, the Cae I activity was readily detectable in the crude testes extract before column chromatography. To eliminate large amounts of interfering, nonspecific nucleases, it was necessary to subject the extract to a preliminary ammonium sulfate fractionation as described in Method C. However, the enzyme prepared by Method C appeared

unstable during the purification through column chromatography and nearly all of the activity was lost after the DEAE-cellulose step. Unless noted otherwise, the results described here were obtained with enzyme prepared by Method A. The Cae I activity has a pH optimum near 7.65 and Ca^{2+}, Mn^{2+}, and Zn^{2+} do not substitute for Mg^{2+} in producing the characteristic cleavage patterns with component α DNA. The activity is also inhibited by NaCl at concentrations above 25 mM.

Cae I Cleavage of Component α DNA

Component α DNA contains cleavage sites for four microbial restriction enzyme activities, Eco RI, Eco RI*, Hae III and Hind III. The spacing of the restriction sites reflects a basic repeat periodicity of 176 base-pairs in the sequence (1,2). Cae I also cleaves component α into a multimeric series based upon this monomer length and the set of sharp, multimeric segments are indistinguishable in length and band width from segments produced by Eco RI* (Fig. 3a,b). Cae I digestion of monkey bulk DNA previously stripped of component α sequences produced a heterogeneous collection of DNA fragments which formed a smear when subjected to agarose gel electrophoresis (gels not shown).

Cae I Cleavage of Mouse Satellite DNA

These findings prompted us to examine the action of Cae I on a highly repetitive DNA from a heterologous mammalian source, namely, mouse satellite DNA. Agarose gel electrophoresis of the mouse satellite/Cae I digests revealed a complex, multimeric series of bands in which the DNA segments differed by length increments of about 60 base pairs (Fig. 3d). Thus, the multimeric series differed radically from the series produced by the microbial restriction enzyme, Eco RII (Fig. 3c). A previous restriction enzyme analysis with Eco RII indicated that mouse satellite DNA evolved in four stages or hierarchies to its present long-range repeat organization of 235 base pairs (8). Southern has proposed (8) that the second stage in the development of mouse satellite involves repeat units on the order of 36 to 72 base pairs in length. Possibly, the Cae I activity detects an internal repeat in the modern sequence that reflects this ancestral second-stage organization. We have recently detected an activity in mouse testes with properties of site-specific cleavage similar to Cae I. With homologous mouse satellite DNA as substrate, this activity, provisionally termed Mmu I, generates discrete segments with a periodicity differing from those generated by Cae I (9). These preliminary observations suggest that each mammalian

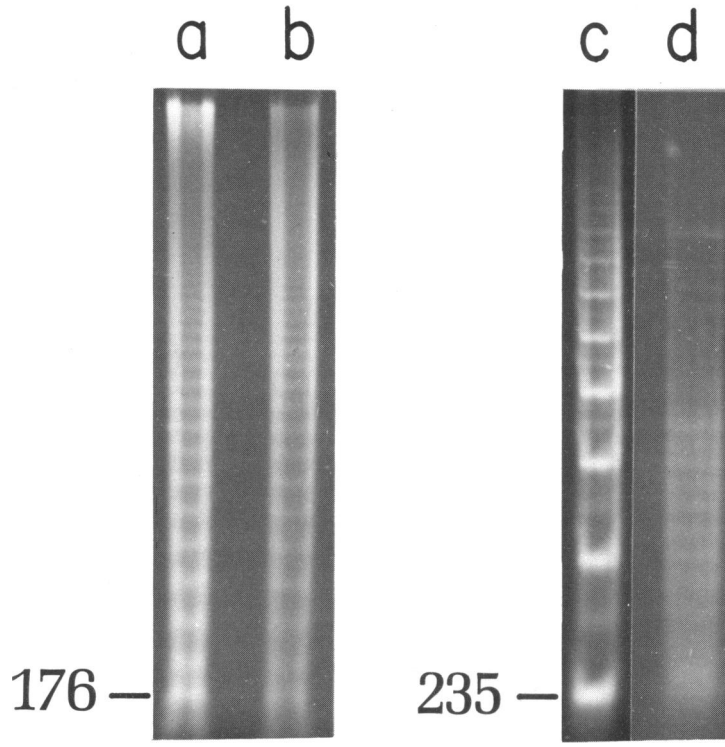

Figure 3. Cae I cleavage of component α and mouse satellite DNAs. Panel (a); 1.2 µg of component α DNA were treated with 1 unit of Cae I activity for 2 hr. Panel (b); 1.2 µg of component α DNA were treated with 280 units of Eco RI* for 4 hr. Panel (c); 1.2 µg of mouse satellite DNA were treated with 8 units of Eco RII for 8 hr. Panel (d); 1.2 µg of mouse satellite DNA were treated with 2 units of Cae I for 2 hr. All incubations were at 37°. Electrophoresis of the ethanol-precipitated DNAs was in 1.4% agarose gels which were post-stained with ethidium bromide. Numbers refer to DNA segment lengths in base-pairs.

species or higher taxonomic order has evolved distinctive site-specific nucleases.

Effect of ATP and S-Adenosylmethionine (SAM) on Cae I Activity

Conceivably, eukaryotic nucleases with site-specific activity may resemble either microbial type I restriction enzymes or type II restriction enzymes. In this section, we describe experiments in which we prepared Cae I from African green monkey testes by Method A (with ammonium sulfate precipitation) and by Method B (without ammonium sulfate precipitation). We observed these differences between the two enzyme preparations:

1) The enzyme prepared by Method B was greatly stimulated by the presence of ATP or SAM in the reaction mixture. In the presence of ATP, compo-

nent α DNA was cleaved almost entirely into monomer segments and oligonucleotides shorter than 176 base pairs which formed a smear in the agarose gels (Fig. 4b). With the same amount of enzyme, SAM had a similar effect (Fig. 4d). However, monomers, dimers and trimers of the component α repeat sequence were still in evidence after Cae I digestion. A high-energy, phosphodiester compound, nicotinamide adenine dinucleotide (NAD) had no effect on the nuclease activity and the cleavage of component α DNA into multimeric

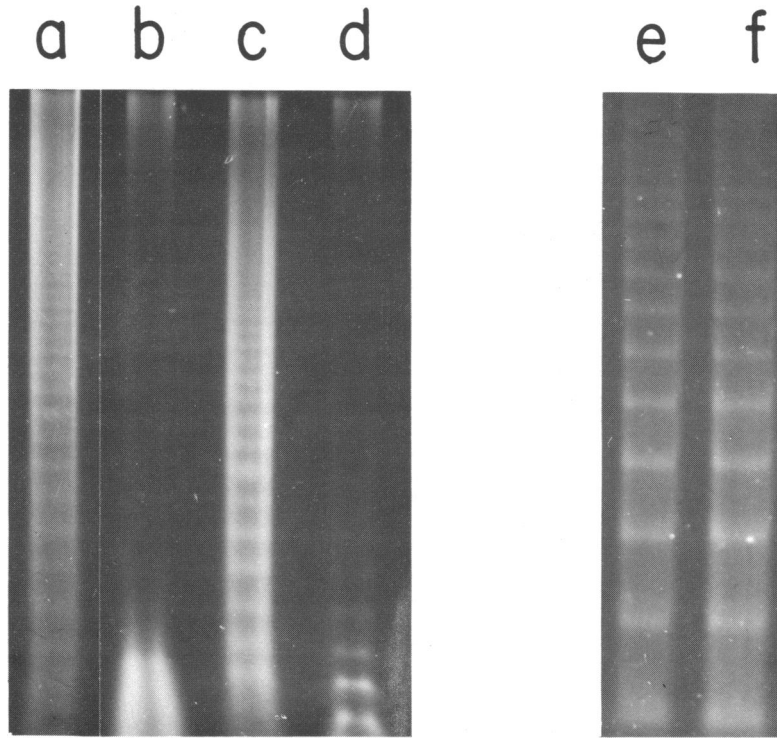

Figure 4. Effect of ATP, NAD and SAM on the cleavage of component α DNA by Cae I. The reaction mixtures contained 1.2 μg of component α DNA in the basic reaction buffer plus additions as indicated. Incubations were for 1 hr at 37° and electrophoresis of all digests was in 1.4% agarose gels. In panels (e-f), the reaction mixtures contained 1 unit of Cae I activity isolated according to Method B (Materials and Methods). (a) Control, no additions. (b) ATP, 3.6×10^{-3} M. (c) NAD, 3.6×10^{-3} M. (d) SAM, 3.6×10^{-4} M. Note: the smear of DNA at the bottom of the gel in panel (b) resolved into monomer segments and DNA fragments shorter than 176 base-pairs upon electrophoresis in 6% polyacrylamide gels (gel photographs not shown).

In panels (e,f), the reaction mixtures contained 1 unit of Cae I activity isolated according to Method A (Materials and Methods). (e) Control, no additions. (f) ATP, 3.6×10^{-3} M.

segments resembled that of the control digests in the basic reaction buffer (Fig. 4a,c).

2) In contrast, the enzyme prepared by Method A was not stimulated by added cofactors: thus, the presence or absence of ATP had no effect on the enzyme activity with component α DNA as substrate (Fig. 4e,f). We do not yet know with certainty whether 1) ATP or SAM stimulates the activity of a contaminating ATP- or SAM-dependent nuclease which copurifies with Cae I in Method B, or 2) the ammonium sulfate precipitations of Method A remove an auxiliary component of the Cae I activity with the consequence that the specificity of its attack in the presence of ATP or SAM is altered.

Position of the Cae I Cleavage Sites Within the Component α Repeat Sequence

Previous restriction mapping of component α showed that nearly 100% of the repeat sequences have either an Eco RI* or a Hind III site and at least 80% of the sequences have both sites (2). The Eco RI* and Hind III sites are 36 base-pairs apart at the near spacing in a repeat unit of 176 ± 4 base-pairs. A secondary Eco RI* site about 62 base-pairs distant from the primary site was also identified. From these data, it was possible to obtain a tentative map of the Cae I cleavage sites with respect to the Hind III and Eco RI* sites. We isolated the monomer segments released by Hind III and by Eco RI* (2) by preparative elution from polyacrylamide gels (10). The Hind III and the Eco RI* monomers were then treated with Cae I and the digestion products subjected to electrophoresis in polyacrylamide gels.

Digestion of the Hind III monomers with Cae I produced secondary segments less than 176 base-pairs in length (Fig. 5a). Polyacrylamide gel electrophoresis resolved two prominent segments which differed in length by about 6 base-pairs. The segment lengths indicated that there are two Cae I cleavage sites located 117 to 123 base pairs from the Hind III site: the two sites are not more than 6 base-pairs apart. A third, faint band of DNA segments 102 base-pairs long was also detected. Possibly, this band arose from a minor Cae I site in the sequence: such minor sites in component α have also been observed with microbial restriction enzymes (1,2).

Digestion of the Eco RI* monomers with Cae I produced the polyacrylamide gel patterns shown in Fig. 5(c,d). DNA segments 69, 73 and 79 base-pairs in length were the major products. A faint segment, 108 base pairs in length, was released from the Eco RI* monomer population during the early stages of digestion with Cae I: upon continued digestion this segment disappeared with the concomitant appearance of a segment 54 base-pairs in length. These patterns are more complex than those observed with the Hind III monomers. We

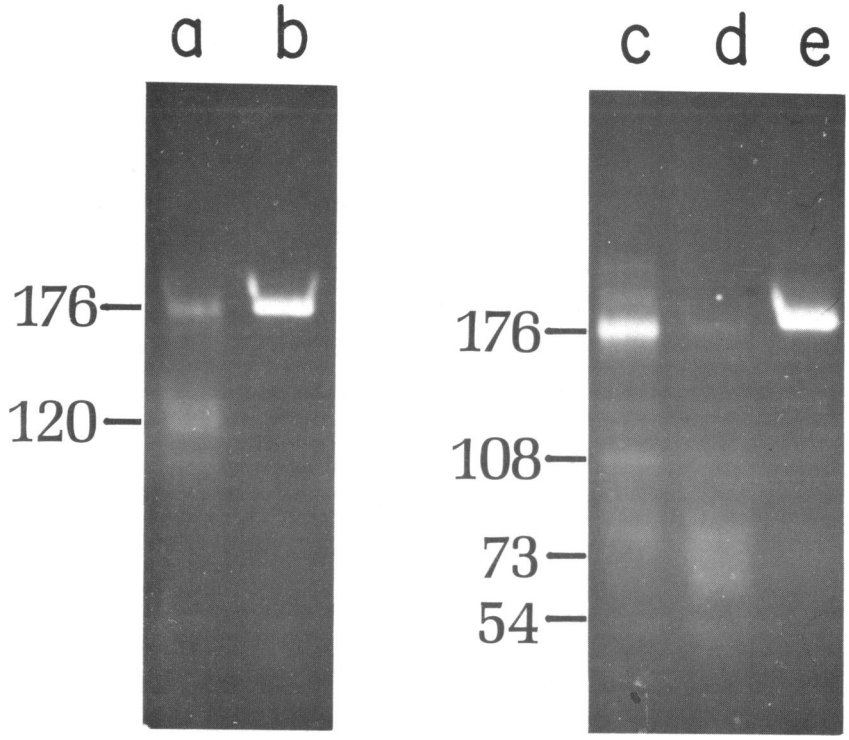

Figure 5. Cae I cleavage of component α monomers released by Hind III and Eco RI* digestion. Component α DNA was extensively digested with Hind III or Eco RI* (2) and the monomer segments were isolated by elution from preparative 5% polyacrylamide gels (10). The Cae I reaction mixtures contained 2.4 µg of the Hind III or Eco RI* monomers in basic reaction buffer and 4 units of the Cae I activity isolated according to Method A (Materials and Methods). Incubation was at 37° for the times indicated. The DNA was then ethanol precipitated, electrophoresed in 5% polyacrylamide gels and the gels post-stained with ethidium bromide. Molecular weight calculations were based upon segments released by Hae III digestion of PM2 bacteriophage DNA and the submonomer segments of component α DNA that are released by Eco RI* as described elsewhere (2). Numbers refer to DNA segment lengths in base-pairs.
(a) Hind III monomers, treated with Cae I for 8 hr. (b) Hind III monomers, untreated control. (c) Eco RI* monomers, treated with Cae I for 4 hr. (d) Eco RI* monomers, treated with Cae I for 8 hr. (e) Eco RI* monomers, untreated control.

attribute this greater complexity to the two Eco RI* sites in the component α repeat sequence which produce two permuted populations of monomer segments 176 base-pairs in length. With this consideration, the gel patterns are consistent with those obtained with the Hind III monomers, suggesting that there are two closely spaced, major sites (about 6 base-pairs apart) for

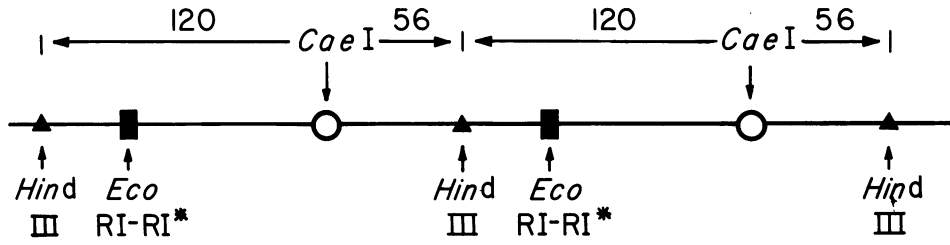

Figure 6. Tentative map of the position of the Cae I cleavage sites with respect to the Hind III and Eco RI* sites in component α DNA. This map is based upon the Cae I digests of the Hind III and Eco RI* monomers of the component α sequence as shown in Fig. 5. In the repeat sequence, a primary Eco RI* site is located 36 base-pairs distant from the Hind III site (1-4) as shown here. In addition, a secondary site of Eco RI* cleavage is present which is located 62 ± 4 base-pairs distant from the primary site (2). For simplicity, the secondary Eco RI* site is not shown on this map. The primary Cae I site is shown by a large open circle to indicate that there may be two closely spaced Cae I sites located only 6 base-pairs apart at this position. These sites appear to be 120 ± 6 base-pairs distant from the Hind III site as shown.

Cae I attack in the component α sequence in addition to a minor site that is attacked less frequently. In Fig. 6 we present a simplified scheme for the arrangement of the major Cae I sites with respect to the Hind III site and one of the Eco RI* sites in the component α repeat sequence.

Cleavage of component α DNA by S1 nuclease and micrococcal nuclease

The apparant site-specificity of cleavage of component α by Cae I could be due to some structural feature of the DNA rather than to the recognition of a specific sequence. For example, S1 nuclease makes specific breaks in SV40 DNA as a result of the superhelical nature of that DNA (11). Conceivably, Cae I recognizes some single-stranded region of component α occurring with a periodicity of 176 base-pairs in the sequence. We therefore treated high-molecular weight, native component α DNA with S1 nuclease to see if the action of this nuclease duplicated the cleavage patterns produced by Cae I. Fig., 7 (a,b) shows that high concentrations of S1 nuclease reduced only slightly the molecular weight of the component α sequences. No specific segments could be detected either in the high-molecular weight DNA or elsewhere in the gel. Under the same incubation conditions, over 90% of the heat-denatured component α DNA was rendered acid-soluble by the action of S1 nuclease (Fig. 7(c,d) and Legend). Similar results were obtained with mouse satellite DNA.

Micrococcal nuclease shows a well-known preference for attacking AT base-pairs (12). Conceivably, Cae I shows a similar preference for attacking

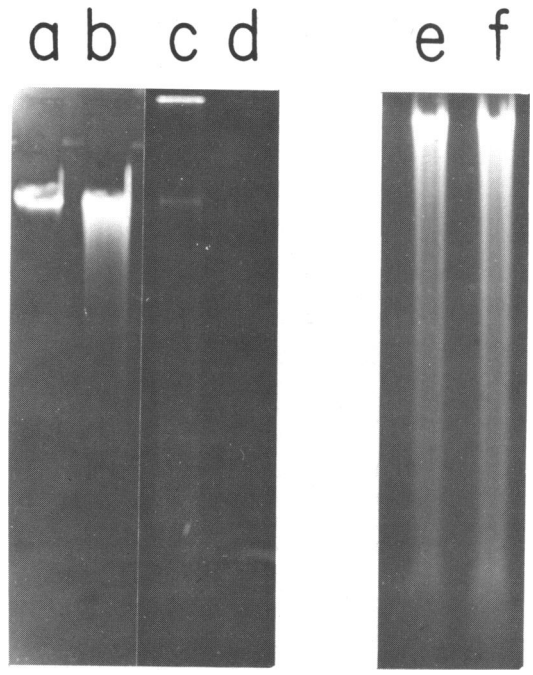

Figure 7. Effect of S1 nuclease and micrococcal nuclease on component α DNA. AGMK DNA was radioactively labelled by growing the primary kidney culture for 6 cell generations in the presence of ^{3}H-thymidine (20 Ci/mmol., 0.005 μCi/ml). Component α DNA with a specific radioactivity of 4,100 cpm/μg was isolated as previously described (6). Reaction mixtures (100 μl) contained 1.5 μg of component α DNA in S1 nuclease buffer (0.05 M acetate buffer, pH 4.5, 0.3 mM $ZnCl_2$ and 0.2 M NaCl) or micrococcal nuclease buffer (0.005 M sodium phosphate buffer, pH 6.8, and 0.25 mM $CaCl_2$). Enzyme activity units of S1 nuclease (Miles Laboratories) and micrococcal nuclease (Worthington) are as defined by the manufacturers. All incubations were carried out at 37°. Heat-denatured component α was prepared by heating the DNA in water for 3 min. at 96° and fast-cooling in ice. Electrophoresis of all samples was in 1.4% agarose gels and the gels were post-stained with ethidium bromide.

Panel (a); Native DNA, untreated control. (b); Native DNA, treated with 400 units of S1 nuclease for 30 min. (c); Heat-denatured DNA, untreated control. (d); Heat-denatured DNA, treated with 400 units of S1 nuclease for 30 min. Radioactivity measurements showed that none of the native DNA was rendered acid-soluble (10% trichloroacetic acid) by treatment with S1 nuclease (panel b) whereas 92% of the heat-denatured DNA (panel d) was rendered acid-soluble under the same incubation conditions.

Panel (e); Native DNA, treated with 0.003 units of micrococcal nuclease for 5 min. (f); Native DNA, treated with 0.01 units of micrococcal nuclease for 5 min.

periodically recurring AT-rich regions in component α DNA. We therefore treated native component α DNA with low concentrations of micrococcal nuclease such that the DNA would not be completely degraded. Thus, if discrete seg-

ments were produced, they could be detected as DNA bands in agarose gels. Fig. 7(e,f) shows that limited digestion of component α DNA by micrococcal nuclease produced a smear of heterogeneous fragments throughout a broad range of DNA molecular weights. However, no discrete segments were detected at any of the graded concentrations of micrococcal nuclease employed.

DISCUSSION

The choice of testes as a tissue likely to contain site-specific endonucleases was dictated largely by theoretical and speculative reasons. The Cae I activity was readily detected in the ammonium sulfate fractions of the crude testes homogenates. However, there were extensive enzyme losses during the subsequent purification. We also tested several mammalian and viral DNAs as substrates in the column assays, but pure component α DNA remained the most reliable substrate for the unambiguous identification of the Cae I activity. Unfortunately, the isolation in bulk of pure component α DNA is both tedious and expensive (6). For these reasons, we have been exploring other purification techniques and other sources of enzyme and substrate to ensure the elimination of all nonspecific nuclease activity. In a preliminary screening, we found that CV-1 cells, a stable, heteroploid cell line derived from AGMK, also contain Cae I activity. Cell fractionation with aqueous media indicated that the enzyme activity was about equally distributed between nuclear and cytoplasmic fractions (unpublished observations). It seems that in CV-1 cells, the enzyme is not associated with mycoplasma contaminants or with cytoplasmic organelles. It may, in fact, be predominantly nuclear in origin.

We note that the Cae I activity described here may represent a mammalian equivalent of the site-specific endonuclease that has been recently described in the unicellular eukaryote, Chlamydomonas (13). This endonuclease, which may be associated with chloroplasts, generates discrete segments with adenovirus-2 DNA as substrate. A description of the action of Cae I on mammalian viral DNAs, with special reference to the SV40 genome, will be presented elsewhere (9). If Cae I and the Chlamydomonas enzyme are indeed similar in their mode of action, then recognition sites for Cae I occur in close proximity at regular periodicities on the complementary strands of component α and mouse satellite DNA. In the case of component α DNA, the Cae I recognition sites occur 176 ± 4 base-pairs apart and generate DNA segments that are indistinguishable from those generated with microbial restriction enzymes.

The cleavage of component α DNA by Cae I preparations obtained by Method B (Materials and Methods) was greatly stimulated by the presence of ATP or

SAM in the basic reaction buffer. In the presence of ATP in particular, component α DNA was rapidly degraded to monomers and low molecular weight fragments. These effects were not observed with enzyme preparations obtained by Method A; thus in the presence or absence of ATP, component α was cleaved into discrete, multimeric segments of the basic repeat periodicity. It is not known with certainty whether these effects are due to an intrinsic property of Cae I, or whether they are due to a contaminating nuclease which is activated by ATP or SAM. It is interesting to note that the recB+C+ genes in Escherichia coli code for two subunits of a nuclease which exhibits ATP-dependent and ATP-independent activities. In the absence of ATP, the enzyme can act as an endonuclease which attacks single-strand DNA: in the presence of ATP, the enzyme can act as an exonuclease and degrade double-stranded DNA sequentially (14). The possibility that the Cae I activity reported here may bear analogies with the recB+C+ system of E. coli is under investigation.

Finally, the existence of mammalian endonucleases such as Cae I poses several questions of basic biological interest:

1) Preliminary observations indicate that the sites recognized by Cae I differ from those recognized by Mmu I (a mouse enzyme exhibiting similar properties). Has each mammalian species evolved characteristic site-specific nucleases?

2) Are there multiple site-specific nucleases within a single species and can these be distinguished in organisms which contain multiple satellite DNAs such as the calf (15)? Or, is the presence of such enzymes in eukaryotes indirectly related to satellite DNAs and directly related to other, unknown functions?

3) Do the properties of these endonucleases depend upon secondary modifications of the DNA such as methylation, rather than upon the primary DNA sequences? Do eukaryotes contain restriction-modification systems analogous to those found in prokaryotes and if so, do such systems function in the selective silencing of genes or sets of genes (16)?

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