Characterization of a Restriction Endonuclease from *Ureaplasma urealyticum* 960 and Differences in Deoxyribonucleic Acid Modification of Human Ureaplasmas

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**Uur960I**, a restriction endonuclease from *Ureaplasma urealyticum* 960, cleaved at the sequence 5'-GC/NGC-3' and is thus an isoschizomer of *Fnu4HI*. *Fnu4HI* cleaved deoxyribonucleic acid from human ureaplasma serovars I, III, and VI but not from II, IV, V, VII, VIII (strain 960), and IX. This grouping of serovars, indicative of their deoxyribonucleic acid modification, matches that previously reported by others using different criteria.

The mollicutes are procaryotes which lack a cell wall and have genomes among the smallest (0.5 × 10⁷ to 1 × 10⁹ daltons) known for free-living organisms. Restriction endonucleases have been identified in many procaryote species, but of the known activities listed (17), only one, *SceNI*, is from a mollicute (23). In addition, in vivo restriction activities acting against mycoplasma viral deoxyribonucleic acid (DNA) at defined sites have been identified in *Acholeplasma* strains (22). None has been previously reported for the *Mycoplasmataceae* family, which includes *Ureaplasma urealyticum*, a human pathogen characterized by its ability to hydrolyze urea (21) but which is not well defined in its other biochemical features. Here we describe the characterization of a type II restriction endonuclease, *Uur960I*, from *U. urealyticum* 960*. We then used *Fnu4HI*, an isoschizomer of *Uur960I*, to seek evidence of the nature of DNA modification in different serovars of *U. urealyticum*.

To prepare *Uur960I*, *U. urealyticum* 960* (ATCC 27618*, serovar VIII; supplied by G. Masover) was grown at 37°C without shaking for 16 to 20 h. Growth medium contained (per liter) 20 g of PPLO broth, 5 g of yeast extract (both from Difco Laboratories, Detroit, Mich.), 15 ml of normal horse serum (not inactivated; Commonwealth Serum Laboratories, Parkville, Victoria, Australia), 50 mg of methionine, 50 mg of ampicillin, 0.9 g of urea, 3.1 g of L-histidine, 4 g of dithiothreitol (Calbiochem, La Jolla, Calif.). Cell suspension was adjusted to 5.9. Cells were harvested by centrifugation at 28,000 × g for 50 min at 4°C when the culture had reached late exponential phase, as indicated by a pH of 7. After being washed with 0.25 M NaCl, the cells from 8 liters of medium were suspended in 1 ml of 10 mM Tris hydrochloride (pH 8.5)-1 mM dithiothreitol (Calbiochem, La Jolla, Calif.). Cell extracts were then made as previously described (3). Partial purification of *Uur960I* was achieved by using a Pharmacia Fast Protein liquid chromatography system. Six milligrams of cellular protein was applied to an anion-exchange column (Mono Q) and eluted with an NaCl gradient from 0 to 1 M, buffered with 10 mM Tris hydrochloride, pH 8.5. Sixty 0.5-ml fractions were collected and immediately placed on ice. Within 1 h, the most active fractions, detected previously by their capacity to digest bacteriophage λ or plasmid pBR322 DNA to give a banded distribution of DNA fragments after agarose gel electrophoresis, were used for digesting various DNAs. All *Uur960I* restriction endonuclease digestions were performed at 37°C for 2 to 4 h in medium salt buffer (9).

Determination of the recognition and cleavage sites for *Uur960I* was undertaken by cloning of DNA fragments from the following digest into M13mp9 (12) for sequencing. Five micrograms of pMC1403 (1) DNA was digested with 60 μl of the most active *Uur960I* fraction in a total volume of 100 μl. Figure 1 shows the sequences obtained for the ends of 10 cloned fragments with bases numbered for their position in the plasmid pMC1403 as identified by a computer search for the corresponding sequence. The sequences were the same in clones 2 and 3 and in clones 5 and 6. In each case the three bases ending the sequence were 5'-GCN-3', where N was G in five cases, T in three cases, and A in two cases. These three bases were always identified as part of the five-base palindrome 5'-GCNGC-3' within the plasmid sequence. The fact that three of the five bases of the palindromic sequence were cloned is consistent with the cut site of *Uur960I* being 5'-GC/NGC-3', leaving a 3' recessed end which would have been extended by end filling before blunt-end ligation into the *Smal* site. *Uur960I* has the same recognition and cleavage sites as *Fnu4HI* (7), making the two enzymes isoschizomers (17). Support for this relationship to *Fnu4HI* came from the observation that the two enzymes each produced a fragment of 3.4 kilobases (kb) from the linear streptococcal phage c6A (15), which has one cut site for *Fnu4HI*. Further confirmation that *Uur960I* and *Fnu4HI* were isoschizomers came from evidence that *Uur960I* cut pMC1403 and pBR322 at *Fnu4HI* sites.

A general pattern for bacteria containing restriction endonucleases is that their DNA is protected through modification by a corresponding methylase activity (11). As expected, chromosomal DNA of *U. urealyticum* 960* was not digested by *Uur960I* under conditions where phage λ is digested. DNA from *U. urealyticum* 960* was also resistant to digestion by *Fnu4HI*. To determine whether this applied to other serovars of *U. urealyticum*, we obtained additional strains from D. Birch, Nephrology Unit, Royal Melbourne Hospital, and cells were grown as described above and extracted for DNA. As shown in Fig. 2, DNAs from serovars

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DNAs of these serovars where overlapping sites occur, e.g., for the first group may be due to GCNGC modification, masking some of the potential digestion patterns observed previously (16). The evidence for the distribution of the serovars into two distinct groups as previously based on electrophoresis of cell proteins (5, 13, 20, 24) and specifically urease (4), DNA-DNA hybridization (2), chromosome cleavage patterns (16), manganese resistance (18) and serological cross-reactions (8). Among other procaryotes, strains of the same species often possess different restriction modification systems, and it has been suggested that these systems act as an “index of relatedness” between strains (25). The evidence for the Uur960I restriction system in one group of the ureaplasmas extends the relatedness within this group. However, these serovars may have other restriction endonucleases which could vary between them. No restriction modification pattern has been defined in serovars I, III and VI and DNA from

FIG. 1. Sequences at the end of fragments of Uur960I-digested pMC1403 DNA cloned into the Smal site of M13mp9. DNA fragments in 20 μl of the digestion mixture were end filled by 10 min of further incubation following the addition of 5 U of the Klenow fragment of DNA polymerase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) plus deoxyadenosine triphosphate (dATP), dCTP, dGTP, and TTP each at 50 μM. After phenol-chloroform extraction and ethanol precipitation (9), the DNA was ligated into the Smal (Boehringer Mannheim) site of M13mp9 and used for transformation of Escherichia coli JM101 and selection of recombinant M13mp9 clones for sequencing of the inserted DNA by the dideoxy method of Sanger (19). Bases are numbered according to their position relative to the EcoRI site in pMC1403, and cloned portions of the Uur960I recognition sequence are underlined.

I, III, and VI were digested by Fnu4HI, but DNAs from serovars II, IV, V, VII, VIII, and IX were reproducibly not digested. DNAs from all serovars were digested well by several other enzymes (e.g., HaeIII [Fig. 2]). Figure 3 shows that Fnu4HI was still active against pBR322 DNA during incubation with ureaplasma DNA preparations from serovars V and VIII, which are not digested. Digestion of serovar VI DNA was again quite apparent. Fnu4HI was also active against pBR322 when incubated with the other ureaplasma DNAs resistant to its action (data not shown). The lack of digestion of DNAs from serovars II, IV, V, VII, VIII, and IX by Fnu4HI suggests that their DNA is modified at 5'-GCNGC-3' sites and that the Uur960I activity would be common to them.

The grouping of the serovars according to the susceptibility of their DNA to Fnu4HI parallels the groupings made according to overall cleavage patterns observed previously with several restriction endonucleases (16). One of the differences observed by Razin et al. (16) in their comparison of restriction endonuclease digest is that DNAs from the ureaplasma serovars II, IV, V, VII, VIII, and IX are less susceptible to PstI digestion (6 bands) than DNAs from serovars I, III, and VI (16 bands). The lower susceptibility for the first group may be due to GCNGC modification, masking some of the potential PstI (CTGCAG) sites in the DNAs of these serovars where overlapping sites occur, e.g., since inhibition of PstI sites by modification at GCNGC has been observed previously (14).

The present taxonomic classification of human ureaplasmas as one species has been open to debate (6). The observations presented here document and extend the evidence for the division of the serovars into two distinct groups as previously based on electrophoresis of cell proteins (5, 13, 20, 24) and specifically urease (4), DNA-DNA hybridization (2), chromosome cleavage patterns (16), manganese resistance (18) and serological cross-reactions (8). Among other procaryotes, strains of the same species often possess different restriction modification systems, and it has been suggested that these systems act as an “index of relatedness” between strains (25). The evidence for the Uur960I restriction system in one group of the ureaplasmas extends the relatedness within this group. However, these serovars may have other restriction endonucleases which could vary between them. No restriction modification pattern has been defined in serovars I, III and VI and DNA from

FIG. 2. Sensitivity of DNAs from various U. urealyticum serovars to digestion by Fnu4HI and HaeIII restriction endonucleases. DNAs from serovars I, II, III, IV, VI, VII, VIII, and IX (ATCC 27813, 27814, 27815, 27816, 27818, 27819, 27618, and 33175, respectively) were prepared as described previously (9). DNA from serovar V (ATCC 27817) was prepared as described by Marmur (10). U. urealyticum chromosomal DNA (0.2 to 1 μg) was incubated with 2.5 to 10 U of Fnu4HI or HaeIII or without enzyme in a total volume of 15 μl according to the manufacturer’s instructions. The products from all digestes were analyzed by electrophoresis in 0.8% agarose as described before (9) and stained with 0.5 μg of ethidium bromide per ml for 40 min before visualization with a 300-nm transilluminator. Gels were photographed on Polaroid 667 film. Lanes contained serovar V DNA (1 μg) digested with XbaI, PstI, HindIII, NcoI, and EcoRI; lanes contained serovar V DNA (1 μg) digested with XbaI, PstI, HindIII, NcoI, and EcoRI; lanes contained serovar V DNA (1 μg) digested with XbaI, PstI, HindIII, NcoI, and EcoRI; lanes contained serovar V DNA (1 μg) digested with XbaI, PstI, HindIII, NcoI, and EcoRI; lanes contained serovar V DNA (1 μg) digested with XbaI, PstI, HindIII, NcoI, and EcoRI; lanes contained serovar V DNA (1 μg) digested with XbaI, PstI, HindIII, NcoI, and EcoRI.
serotype III was digested by all of 16 restriction enzymes tested.

The observations in this paper suggest that digestion of chromosomal DNA with Fnu4HI provides a single test on which other ureaplasma isolates might be assessed for classification into one of the existing two groups, either by lack of digestion for the Urr9601 group or by identity of restriction pattern with that of digested DNA from the other group.

This work was carried out with support from the Australian Research Grants Scheme and during the tenure of a Commonwealth Postgraduate Research Award by B.C. The c6A DNA was kindly supplied by I. B. Powell.

LITERATURE CITED


