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RESTRICTION CLEAVAGE MAP OF KINETOPLAST DNA MINICIRCLES FROM TRYPANOSOMA EQUIPERDUM

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Summary. The cleavage of the kDNA minicircles of Trypanosoma equiperdum by the restriction endonucleases Hinf I, Bgl II, Mbo I, Taq I and Mbo II revealed that this kDNA is homogeneous in base sequence. This is in contrast with the kDNA of minicircles of the other species of trypanosomes so far studied. The 10 cleavage sites, obtained with these endonucleases, were ordered and a restriction cleavage map of the minicircles was thus drawn.

We have previously characterized the components of the kinetoplast DNA (kDNA) network of Trypanosoma equiperdum (5) (7). The kDNA represents about 6 % of the total cellular DNA and is composed of about 3000 supercoiled minicircles of 1 kilobase (1 kb) and of about 50 circular supercoiled molecules of 23 kb, topologically interlocked. These maxicircles are homogeneous in size and are selectively cleaved by several restriction endonucleases which do not cleave the minicircles (7). This property permits to isolate the minicircles under the form of core kDNA network form I and to fractionate them from the maxicircles (7). Preliminary experiments have shown that the T. equiperdum minicircles are homogeneous in base sequence (7) in contrast with the minicircles of T. cruzi (8) and of other species of trypanosomes (Borst et al.)(1).

In this paper we present the results which permitted us to draw a restriction cleavage map of the kDNA minicircles of T. equiperdum. These results also contribute to the analysis of the phylogenetic relationships between the different species of trypanosomes.

MATERIALS AND METHODS

Strain. The kinetoplastic strain of T. equiperdum was obtained from the Institut Pasteur. The trypanosomes were fractionated from blood rat as previously described (6).

Preparation of kDNA minicircles. Total DNA was extracted from the trypanosomes and kDNA form I fractionated in propidium diiodide (PDI)-CsCl gradients under conditions already described (9). The PDI was extracted with isopropanol (3). The core kDNA network form I, exclusively composed of minicircles was obtained after Bam HI cleavage of maxicircles, as previously described (7).

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Digestion by restriction endonucleases. The restriction endonucleases were obtained from New England Biolabs and used under the standard digestion conditions recommended for each enzyme.

Gel electrophoresis. The endonuclease digests of kDNA minicircles were analysed after electrophoresis in 2 % agarose slab gels, as previously described (9). The sizes of the fragments were estimated by comparing their mobilities with those of the Hae III fragments of the bacteriophage Ø X 174 RF, whose genome is composed of 5375 base pairs (Sanger et al. 10).

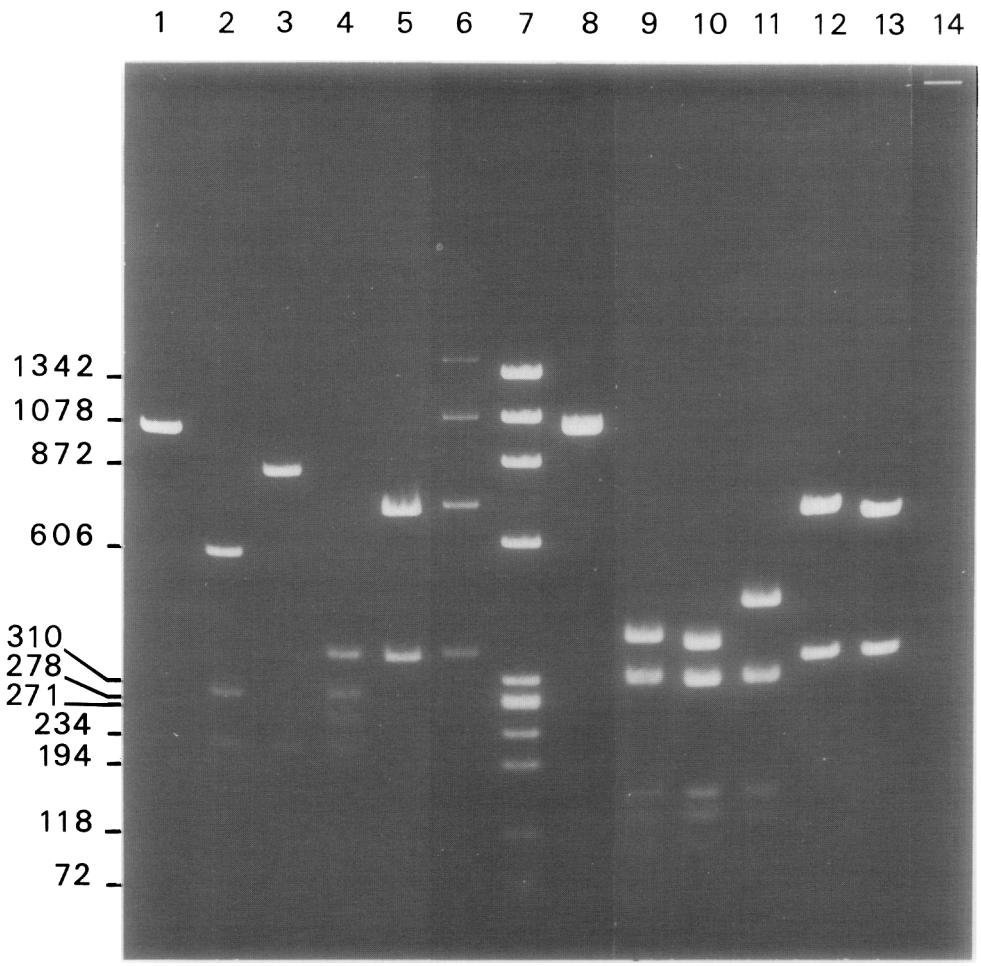

Fig. 1. Electrophoresis in 2 % agarose gel of the fragments generated by cleavage of kDNA minicircles of T. equiperdum by restriction endonucleases : (1) Bgl II, (2) Taq I + Bgl II, (3) Taq I, (4) Taq I + Mbo I, (5) Mbo I, (6) T. cruzi kDNA fragments obtained by Hae III cleavage, (7) Ø X 174 RF DNA fragments obtained by Hae III cleavage and used as standard for size, (8) Hinf I, (9) Mbo I + Mbo II, (10) Hinf I + Mbo II, (11) Bgl II + Mbo II, (12) Bgl II + Mbo I, (13) Hinf I + Mbo I, (14) unreacted core kDNA minicircles. The numbers indicate the molecular weight in base pairs of the Hae III digest of Ø X 174 DNA.

Table I. Molecular weights (expressed in kb) of the fragments of T. equiperdum kDNA minicircles generated through cleavage by different restriction endonucleases.

Fragments	Restriction endonucleases						
	Hinf I	Blg II	Mbo I	Taq I	Mbo II	Hinf I Blg II	Hinf I Mbo I
1	1.04	1.04	0.71	0.85	0.47	0.69	0.69
2			0.34	0.20	0.31	0.36	0.35
3					0.15		
4					0.12		
			1.05	1.05	1.05	1.05	1.04

	Hinf I Mbo II	Hinf I Taq I	Bgl II Taq I	Bgl II Mbo I	Bgl II Mbo II	Mbo I Mbo II	Taq I Mbo I
1	0.38	0.63	0.57	0.70	0.47	0.39	0.34
2	0.31	0.22	0.28	0.35	0.31	0.31	0.27
3	0.15	0.20	0.20		0.15	0.15	0.24
4	0.12				0.12	0.12	0.20
5	0.10					0.09	
	1.06	1.05	1.05	1.05	1.05	1.06	1.05

RESULTS

We have used 21 different restriction endonucleases to cleave the kDNA minicircles (Alu I, Ava II, Bam HI, Bgl I, Bgl II, Eco RI, Hae II, Hae III, Hha I, Hind II, Hind III, Hinf I, Hpa II, Kpn I, Mbo I, Mbo II, Pst I, Sal I, Tac I, Taq I and Xba I). Only 5 enzymes, Bgl II, Hinf I, Taq I, Mbo I and Mbo II, cleave the minicircles in respectively 1, 1, 2, 2 and 4 fragments ; the other endonucleases do not cleave the kDNA minicircles significantly. The sizes of the fragments were determined by gel electrophoresis and the results are shown in figure 1 and Table 1. Since each enzyme has a limited number of cleavage sites per DNA molecule it is relatively straightforward to draw a map of this molecule (figure 2). The unique cleavage site of Hinf I is arbitrarily taken as origin, and the unique cleavage site of Bgl II is used as secondary reference. The sizes of the DNA fragments obtained by double digestion are listed in Table 1. The cleavage sites have been ordered on the genome as presented in figure 2. The third fragment found in the double digest Hinf I/Mbo I cannot be observed by gel electrophoresis and is estimated to consist of about 10 base pairs. This is confirmed by the results observed in the double digests Bgl II/Mbo I and Hinf I/Bgl II, the Bgl II site (4) being included in the Mbo I site (2).

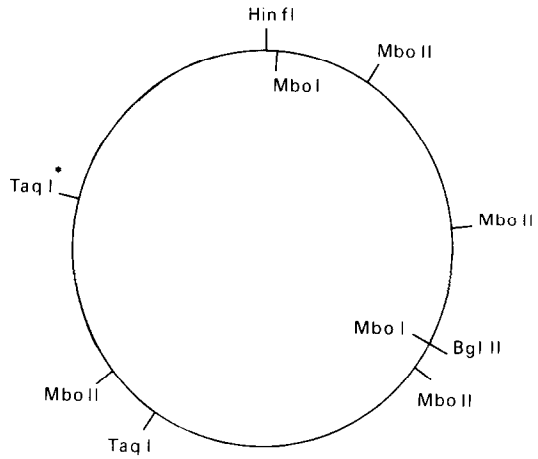

Fig. 2. Restriction cleavage map of kDNA minicircle of T. equiperdum. The Taq I* site is cleaved by incubation at 60°C but not at 37°C (11).

DISCUSSION

The kDNA minicircles of the Trypanosomatidae so far studied were found to be heterogeneous in base sequence , as demonstrated by restriction enzyme cleavage (7) (1). The complex reassociation kinetics obtained with the T. cruzi kDNA minicircles also indicate a sequence heterogeneity of these molecules (8). In contrast, T. equiperdum kDNA minicircles are homogeneous in base sequence as demonstrated by cleavage with restriction enzymes, and the kinetics of reassociation also indicate the presence of a single class of nucleotide sequence (7). The ten sites of cleavage of T. equiperdum minicircles, obtained with Hinf I, Bgl II, Mbo I, Taq I and Mbo II can be ordered unambiguously and a physical map can thus be drawn.

The biological role of kDNA has not yet been elucidated, only maxicircles having been found to hybridize with messenger RNA (12). We do not know whether the minicircles are transcribed,nor whether they possess the genetic signals necessary to initiate the synthesis of messenger RNAs. An approach to this problem might be the determination of the DNA sequence of minicircles. The unique position of the restriction endonucleases cleavage sites on the T. equiperdum minicircles makes this approach feasible.

The restriction map could be also useful in the determination of the sequential evolution of the kDNA minicircles and thus help in the taxonomic classification of trypanosomes.

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