BIOCHEMICAL STRAIN CHARACTERIZATION OF *TRYPANOSOMA CRUZI* BY RESTRICTION ENDONUCLEASE CLEAVAGE OF KINETOPLAST–DNA

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1. Introduction

An important problem in the study of *Trypanosoma cruzi*, the agent of Chagas' disease, is the characterization of the different strains isolated from patients or vectors. Differences between *T. cruzi* strains have already been shown in relation to criteria such as antigenic constitution [1], isoenzyme patterns [2], drug sensitivity [3], parasitemia curve in experimentally infected animals [4], proportion of trypomastigotes to epimastigotes in culture [5] and dependence on temperature of intracellular differentiation [6]. These differences suggest the occurrence of subspecies in *T. cruzi* and demand sensitive methods for routine strain characterization. In this paper we show that restriction endonuclease [7] digestion of isolated kinetoplast-DNA provides a powerful tool for the molecular typing of *T. cruzi* at the genotype level.

Abbreviations: K-DNA kinetoplast-DNA, the mitochondrial DNA of hemoflagellates; SSC 0.15 M NaCl, 0.015 M sodium citrate

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2. Materials and methods

2.1. *Cells*

T. cruzi strains CL and FL were isolated from naturally infected *Triatoma infestans* [8]; the Gilmar and the Y strains were isolated from acute cases of Chagas' disease [6,9]. All strains were supplied by Dr Zigman Brener (Federal University of Minas Gerais, Belo Horizonte, Brazil). They were grown in LIT (liver infusion–tryptose) liquid medium [10]. *Herpetomonas samuelpessoai* (ATCC 30252, [11]) was grown in a complex medium described previously [12].

2.2. *Isolation of K-DNA networks*

K-DNA networks were isolated by the hot sarkosyl–pronase method [13] without the RNAase and deproteinization final steps; further purification was carried out by ethidium bromide–CsCl equilibrium gradient centrifugation [13]. The fractions containing the K-DNA were extracted with isoamylalcohol to remove the dye, dialysed against SSC and stored at 4°C.

2.3. *Digestion by restriction endonucleases*

DNA, 5–15 μg, was digested with 1–2 μl of the nuclease preparation in a final volume of 20–30 μl. The buffer conditions for each nuclease were adjusted by addition of 10 X buffer. After incubation (2–3 h) 5 μl of 50% sucrose, 0.1 M EDTA, pH 7.0, 0.05% bromophenol blue were added to each sample to

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prepare them for electrophoresis. Control experiments showed that digestion was complete under these conditions.

2.4. *Polyacrylamide gel electrophoresis of DNA fragments*

Electrophoresis was at room temperature on 3.5% acrylamide–0.09% bisacrylamide slab-gels in 0.04 M triethanolamine, pH 7.8, 0.02 M sodium acetate, 0.002 M EDTA. After heating for 2 min at 60°C, the samples were loaded and electrophoresed at 10–30 mA. After staining with 0.5–1.0 μg/ml ethidium bromide the gels were photographed under ultraviolet light on Tri-X film using an orange filter.

2.5. *Materials*

Restriction endonucleases were the generous gift from New England Biolabs, Beverly, USA (for the nomenclature of these enzymes see [14]). SV-40 DNA was from Bethesda Research Laboratories, Rockville, USA. λ-DNA was prepared from a strain of *Escherichia coli* carrying the prophage λc I857S7 kindly provided by Dr B. J. McCarthy, University of San Francisco, California, USA. Liver infusion was from Difco. Tryptose and cesium chloride were from Merck. Ethidium bromide, sarkosyl (*N*-lauroyl sarcosine) and pronase were from Sigma. All other reagents were analytical grade.

3. Results and discussion

Figure 1 shows the analysis of the restriction fragments generated by the *Hpa* II treatment of K-DNA isolated from 4 *T. cruzi* strains and *H. samuelpessoai.* The molecular weights of the main bands were determined by comparing their electrophoretic mobilities with those of the *Hin*dIII fragments of λ-[15] or SV-40-[7,16] DNAs (fig.2). It is clear that strains Y and Gilmar (slots 5 and 4) have a fragment pattern that differs from that common to FL and CL strains (slot 2 and 3). Bands C, D and E are completely absent from the latter digests. On the other hand, the *Herpetomonas* fragments are different from those of the *T. cruzi* strains.

Bands A and B are common to all four *T. cruzi*

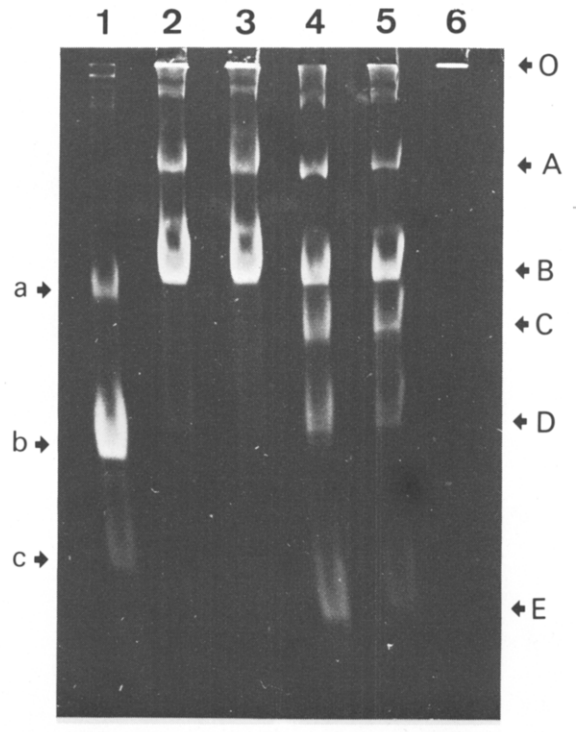


Fig.1. Polyacrylamide gel electrophoresis of *Hpa* II fragments of K-DNA from *H. samuelpessoai* (slot 1) and *T. cruzi* strains FL (slot 2), CL (slot 3), Gilmar (slot 4) and Y (slot 5). Slot 6 is undigested K-DNA control from Y strain. 15 μg of K-DNA from each sample was digested as explained in Materials and methods. Electrophoresis was at 20 mA for 8 h. O origin, A–E bands from *T. cruzi,* a–c bands from *H. samuelpessoai.*

strains tested. Band B corresponds to a DNA of about 1470 base pairs, close to the value of 1442 ± 91 base pairs reported for the *T. cruzi* kinetoplast minicircle [17]. Band B should then originate from the linearization of minicircles possessing only one *Hpa* II restriction site. The origin of band A is less certain; it could be constituted either by covalently closed minicircles which migrate more slowly than the linearized ones [18] or by oligomer-, catenane- or 'figure-8'-[17,19] derived structures (A. Simpson, personal communication).

Figure 3 and fig.4 show the results of similar experiments with *Hae* III and *Eco*RI nucleases. Strains that could not be distinguished on the basis of K-DNA cleavage with *Hpa* II alone could be differentiated by the use of another enzyme. The Y and Gilmar strains, which have identical pattern with *Hpa* II, are different

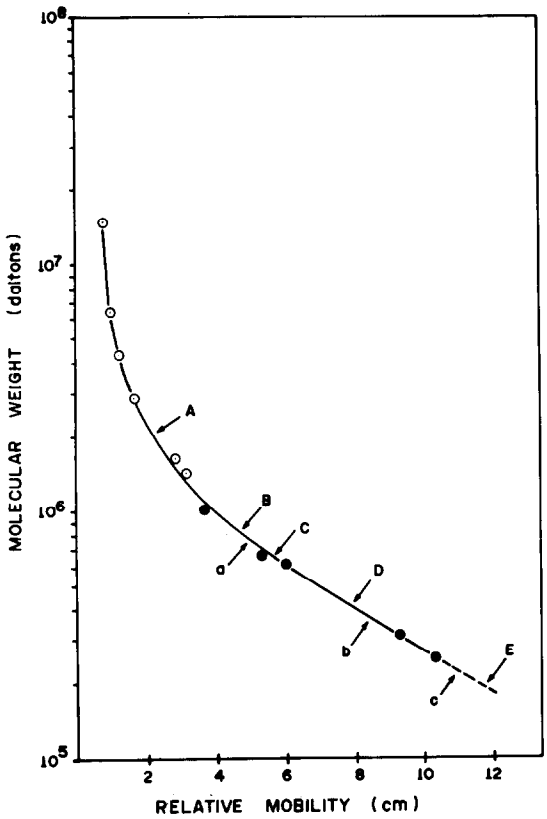


Fig.2. Molecular weight determination for the main *Hpa* II fragments of the experiment shown in fig.1. The standard curve was obtained with the λ- (○) or SV-40- (●) fragments produced by *Hin*dIII. A–E bands from *T. cruzi*, a–c bands from *H. samuelpessoai*.

when *Hae* III is used (slots 4 and 5, fig.3). With *Hae* III the fragments of *Herpetomonas* K-DNA differ again from those of *T. cruzi*. In the case of the *Eco*RI experiment (fig.4) the differences between the *T. cruzi* strains are only quantitative. In each case only bands A and B appear, albeit in different amounts. Digestion is most pronounced for the Gilmar strain, being practically non-existent in the Y strain. The strains we used seem to be different from that studied by Riou and Yot, as these authors reported that 5 fragments are generated from *T. cruzi* K-DNA by *Eco*RI [18].

Our results confirm previous findings that there is a heterogeneity in the minicircle population of a given kinetoplast network [18,20]. The sum of the molecular weights of the fragments in some digests is much greater than that of the minicircle itself (e.g., the *Hpa* II digests of Y and Gilmar strains). In addition, some enzymes such as *Eco*RI, while leaving most of the DNA intact, cut and liberate some minicircles from the network, again pointing to minicircle heterogeneity (comparable results were obtained with *Hin*dIII, *Hin*cII and *Hpa* I, not shown).

The experiment shown in fig.5 indicates a hitherto undetected type of sequence organization in K-DNA. In this experiment K-DNA from the Gilmar strain was digested with *Hpa* I, *Hpa* II and *Hae* III. The fragments obtained with *Hpa* II and *Hae* III (slots 2 and 3) are identical even for the low molecular weight bands C, D and E. As these 2 enzymes cleave different sequences, we suggest that there is some reiteration of DNA sequences inside K-DNA of this strain. However, we do not have data to corroborate such a hypothesis or to

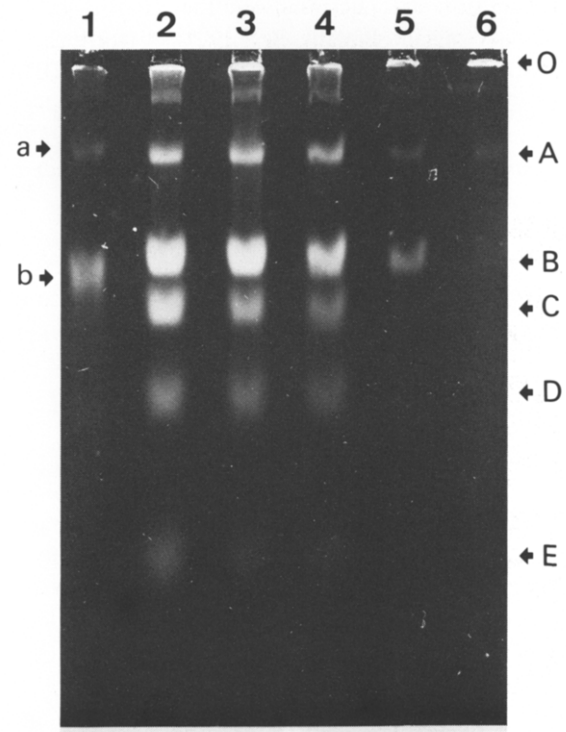


Fig.3. Polyacrylamide gel electrophoresis of *Hae* III fragments of K-DNA from *H. samuelpessoai* (slot 1), and *T. cruzi* strains FL (slot 2), CL (slot 3), Gilmar (slot 4) and Y (slot 5). Slot 6 is undigested K-DNA control from Y strain. 12 μg of K-DNA from each sample were digested as explained in Materials and methods. Electrophoresis was at 12.5 mA for 12 h. O origin, A–E, bands from *T. cruzi*, a and b bands from *H. samuelpessoai*.

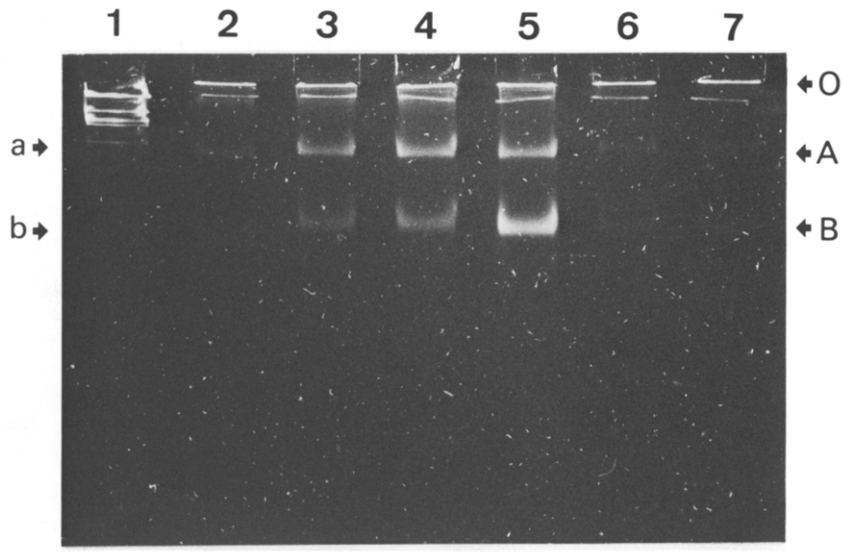
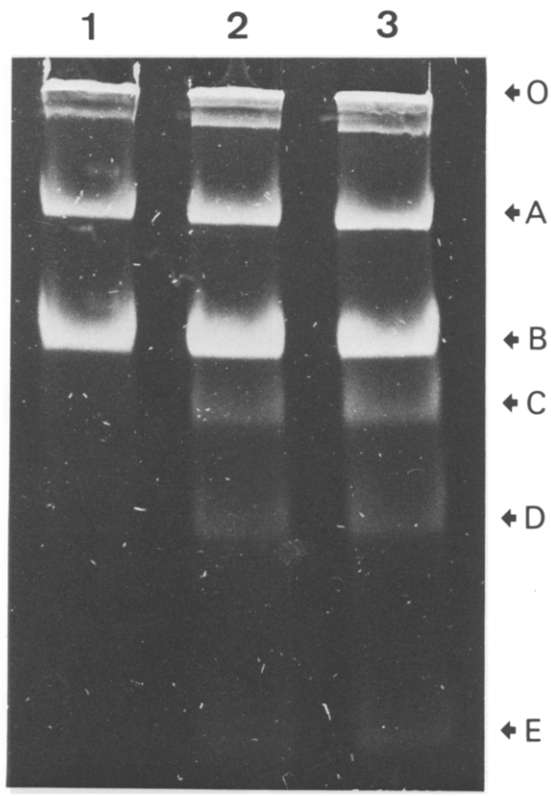


Fig.4. Polyacrylamide gel electrophoresis of *Eco*RI fragments of K-DNA from *H. samuelpessoai* (slot 2) and *T. cruzi* strains FL (slot 3), CL (slot 4), Gilmar (slot 5) and Y (slot 6). Slot 1 is *Eco*RI-digested λ-DNA and slot 7 is undigested K-DNA control from Y strain. 12 μg from each K-DNA sample and 2.5 μg of λ-DNA were digested as explained in Materials and methods. Electrophoresis was at 10 mA for 17 h. O origin, A and B bands from *T. cruzi*, a and b bands from *H. samuelpessoai*.



decide in which DNA component of the network such reiteration is localized.

Our main conclusion is that restriction endonuclease digestion of K-DNA can be used for the characterization of kinetoplastid flagellates, even at the strain level. The differentiation between closely related organisms by restriction analysis of specific DNA molecules has already been applied in a number of cases [7,21]. This method of K-DNA fingerprinting by restriction endonuclease digestion has several features which recommend it for the routine use in kinetoplastid taxonomy, in addition to classical methods such as isoenzyme analysis [2,22]. It characterizes at the genotype level, requires low amounts of material and the electrophoretic techniques are simple and inexpensive.

Fig.5. Polyacrylamide gel electrophoresis of K-DNA fragments from the Gilmar strain of *T. cruzi* generated by endonucleases *Hpa* I (slot I), *Hpa* II (slot 2) and *Hae* III (slot 3). For each digestion 22 μg of DNA were used. Electrophoresis was at 10 mA for 17 h. O origin, A–E main bands.

Acknowledgements

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