Ann. Parasitol. Hum. Comp., 1991, *66 :* n° 6, 256-258.

Mémoire.

Key-words: Trypanosoma. Haemoflagellates. Kinetoplast DNA. Maxicircles. Minicircles.

Mots-clés : Trypanosoma. Hémoflagellés. ADN kinétoplastique. Maxicercles. Minicercles.

THE KINETOPLAST DNA STRUCTURE OF *TRYPANOSOMA cf. CARASSII*

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SUMMARY

The structure of the kinetoplast DNA (kDNA) network of fish trypanosomes is described for the first time. The kDNA was isolated from lysed cells of trypanosomes grown in culture by differential centrifugation combined with chloroform-isoamyl alcohol extraction. Electron microscopy of spread DNA networks revealed the existence of two kinds of mutually interlocked circular molecules. The maxicircle size was 27.5 ± 2.5 kbp (kilobase pairs) and that of the predominant minicircles 1.9 ± 0.1 kbp.

RÉSUMÉ : **L'ADN kinétoplastique chez *Trypanosoma cf. carassii.***

La structure de l'ADN kinétoplastique des trypanosomes de poissons est décrite pour la première fois. L'ADN kinétoplastique a été isolé des cellules de trypanosomes de culture lysées par centrifugation différentielle combinée avec une extraction par chloroforme-isoamyl alcohol. L'étude en microscopie électronique du réseau kinétoplastique étalé a révélé l'existence de deux populations de molécules circulaires d'ADN en réseau concaténaire. La taille des maxicercles était de 27,5 ± 2,5 kb (kilobases) et celles des minicercles prédominants de 1,9 ± 0,1 kb.

INTRODUCTION

The presence of the kinetoplast, the fibrous structure within a single mitochondrion (reviewed in Kallinikova, 1977), represents a characteristic feature of kinetoplastid flagellates. However, the function of this organelle is still not fully understood. Kinetoplast DNA typically consists of two groups of mutually interlocked circular DNA molecules — the so called maxicircles and minicircles. There are a few dozens of sequentially homogeneous maxicircles per cell coding for several mitochondrial rRNAs and certain subunits of respiratory chain enzymes. The size of the maxicircles was estimated to be 22.2 kilobase pairs (kbp) in *Trypanosoma brucei* (Stuart, 1979) and 24.3 kbp in *Trypanosoma mega* (converted from the molecular mass published by Borst *et al.*, 1977). Minicircles occur in thousands of copies per cell and thus represent a considerably higher proportion of the kinetoplast DNA mass. Recently it was shown that some guide RNAs used in the RNA editing process were transcribed from minicircles but even so the role of the minicircles in the cell is not completely elucidated so far (Weiner and Maizels, 1990). The minicircles usually display sequence heterogeneity, but are nearly homogeneous in size in most species. The size of *Leishmania aethiopica* kinetoplast minicircles is about 0.9 kbp (converted from contour length published by Barker *et al.*, 1982) and that of *Leishmania mexicana* 0.7 kbp (Rogers and Wirth, 1988), while *Trypanosoma brucei* possess minicircles of 1.1 kbp (Donelson *et al.*, 1979) and *T. cruzi* of 1.4 kbp (Macina *et al.*, 1986). The smallest minicircles among all flagellates of the order Kinetoplastida were found in *T. vivax* and *T. congolense* — 0.5 kbp (Borst *et al.*, 1985), whereas the opposite extremes are represented by the minicircles of *Phytomonas* sp. (suborder Trypanosomatina) with a size approaching 2.9 kbp (Riou *et al.*, 1987), and those of *Bodo caudatus* (suborder Bodonina) with a size of 10 kbp and 12 kbp (Hadjuk *et al.*, 1986). The kinetoplast DNA of fish trypanosomes parasitizing a large spectrum of fish species and transmitted by leeches has not been studied in detail so far. The aim of this study focused on the most common fish-infecting species *Trypanosoma cf. carassii* was to examine the general structure of the kDNA and the size of its basic components.

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Accepté le : 23 décembre 1991.

MATERIALS AND METHODS

The trypanosomes (*Trypanosoma cf. carassii,* strain Ts-Ab-SR) were isolated from infected bream *(Abramis brama)* captured in Staňkovský pond in South Bohemia (Czechoslovakia) and were cultivated in L4NHS medium (Baker *et al.*, 1976) as described previously (Zajíček and Pecková, 1990).

The kDNA was isolated from trypanosome cells washed twice (1,000 *g*/10 min) in NET-100 buffer (100 mM NaCl, 100 mM EDTA, 20 mM Tris; pH 8.0) and resuspended in this buffer to a concentration of 10^6 cells/ml. The cells were lysed by SDS (Sodium dodecylsulphate, Sigma) at the final concentration 0.5 % (w/v) for 5 minutes at 60° C. The lysate viscosity was reduced by repeated suction into a 10 ml glass pipette. After lysis, pronase (Calbiochem) was added to a final concentration of 1 mg/ml and the cell lysate was digested at 60° C for 2.5 hours. The cell debris were then removed by centrifugation at 3,000 *g*/10 min. The kDNA fraction was sedimented by centrifugation (50,000 *g*/10 min) and resuspended in 15 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). The kDNA was then purified by two successive centrifugations at 80,000 *g* for 50 min and resuspended in 2 ml of TE buffer. After addition of NaCl to the final concentration 1 M, the kDNA fraction was deproteinized by chloroform-isoamyl alcohol mixture (24:1) and ethanol-precipitated.

The samples of kDNA were prepared for electron microscopy by a cytochrome c spread technique (Fergusson and Davis, 1978). The kDNA samples were mounted onto Pro-celloidin (Fluka) coated copper grids, stained with uranyl acetate and rotary shadowed with Pt:Pd (80:20) alloy at an angle of 5° in the high vacuum. The grids were examined in a JEOL JEM 100B electron microscope operating at 60 kV. The contour lengths of the mini- and maxicircles were measured from prints using a HIPAD digitizing tablet (Houston Instruments) connected to the computer (PV9900-EDAX System). The precise magnification was determined by replica grating (Balzers). The sizes of mini- and maxicircles in kbp were determined by using plasmid pBR322 (Sutcliffe, 1979) as an external standard.

RESULTS AND DISCUSSION

Electron microscopic examination of spread extracted kDNA of *Trypanosoma cf. carassii* revealed the classical network structure typical for kDNA of trypanosomes *(Fig. 1)*. The kinetoplast DNA consisted of two populations of circular DNA molecules — the minicircles and the maxicircles *(Fig. 2 A, B)*. Their contour length were found to be 0.60 ± 0.04 μm (n = 32) and 8.74 ± 0.79 μm (n = 10) which corresponds to the sizes 1.9 ± 0.1 kbp and 27.5 ± 2.5 kbp (n = 10) for the mini- and the maxicircles, respectively. These sizes slightly differ from those found in other trypanosomatid species but still fall into the published value range for kinetoplastids.

Trypanosoma cf. carassii is one of the most commonly found species of freshwater fish trypanosomes which represent a well-defined group within the family Trypanosomatidae. The data presented here show that the basic organization of its kDNA is similar to that of other trypanosome species hitherto studied.

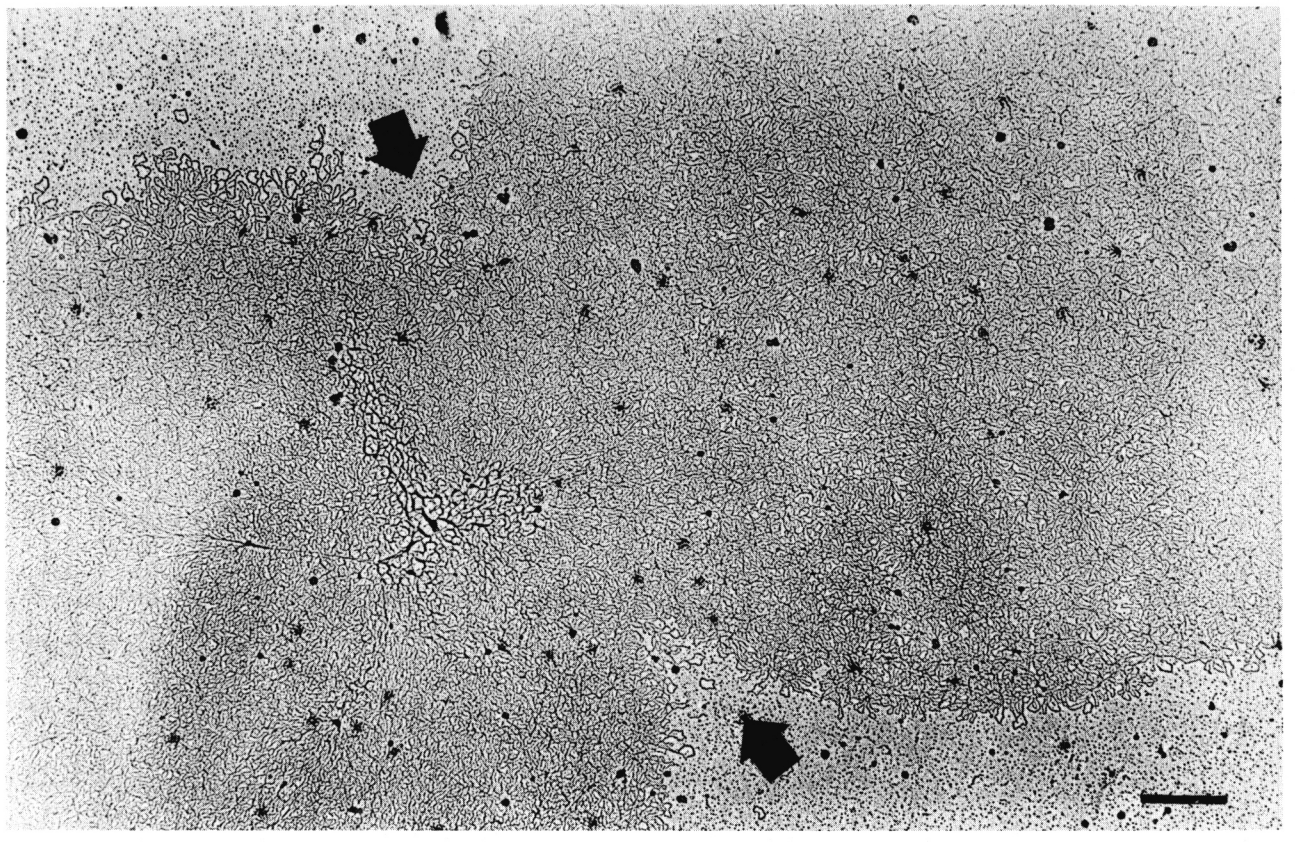

FIG. 1. — A part of kDNA of the C-type (after Hoeijmakers and Weijers, 1980).
The arrows show the central constriction of the kDNA network. The bar represents 1 μm.

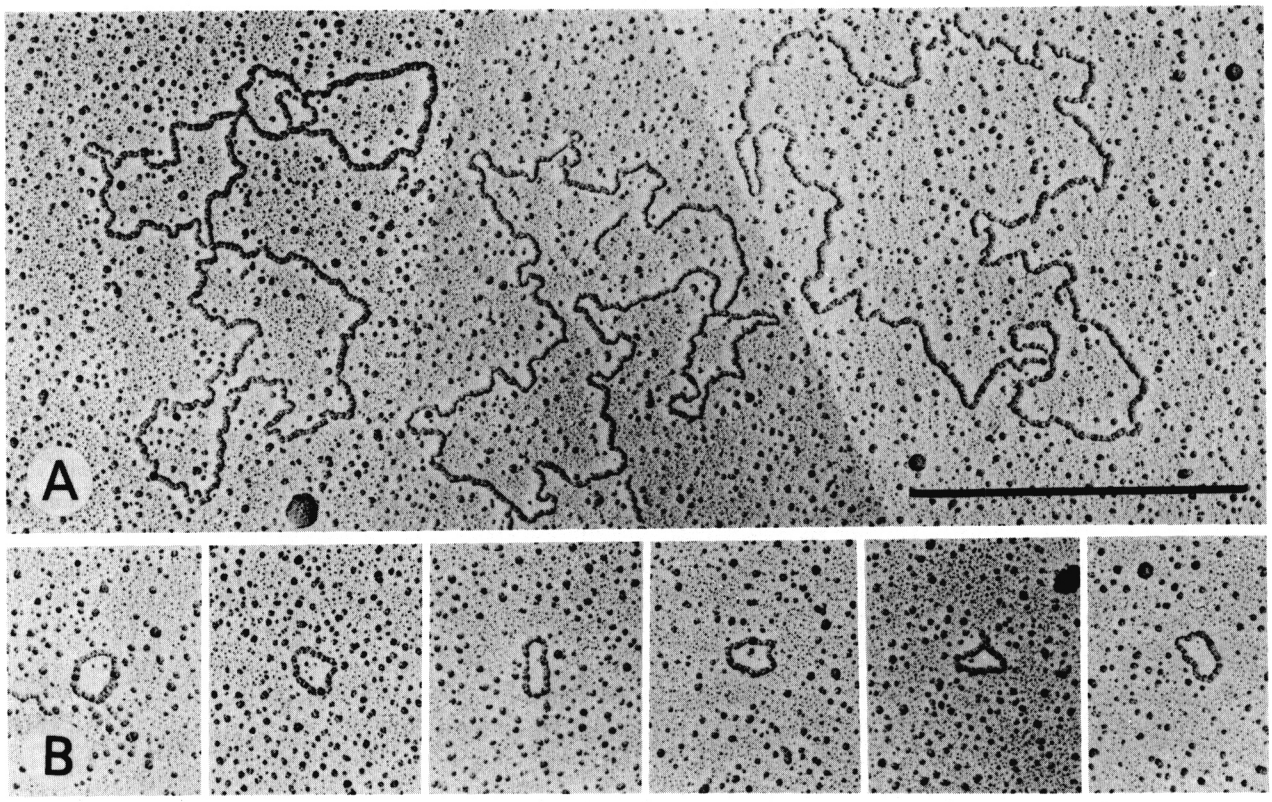

FIG. 2. — A) The maxicircles. B) A gallery of the minicircles. The bar represents 1 μm.

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