EXTRA COMPONENTS IN KINETOPLAST DNA PREPARATIONS FROM CRITHIDIA FASCICULATA

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SUMMARY

Kinetoplast DNA from the order Kinetoplastidae (trypanosomatids) exists as large associations (molecular weight 4×10^{10}), made up of about 10^4 small, probably circular, molecules, commonly known as 'minicircles'. These minicircles were originally thought to be identical in base composition, suggesting that the coding capacity of kinetoplast DNA is very restricted. However, linear molecules have also been observed in preparations of kinetoplast DNA, which, if they contain unique sequences, could represent additional genetic information. This linear DNA has been assumed to be derived from the kinetoplast, but the possibility of it being nuclear contamination has not been definitely ruled out. Work presented in this paper demonstrates that nuclear DNA contamination may indeed be present in kinetoplast DNA prepared by a commonly used method.

Introduction

Isolation of a satellite DNA from several trypanosomatids has been achieved and has been shown to correspond to DNA localized in the kinetoplast. Electron microscopy of kinetoplast DNA (K-DNA*) isolated from several species showed the presence of a heterogenous series of molecular types of small molecular weight, the predominant species being a small circular molecule varying in size from 0.29 μm to 0.79 μm in contour length, depending upon the species of trypanosome (1,2,3,4). This small circular molecule is now known as the minicircle. Large associations whose molecular structure is difficult to interpret are also present.

One of the most important unanswered questions at the present time concerns the nature of longer molecules which have also been observed in

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Abbreviations ** K-DNA: kinetoplast DNA
T_m: the temperature at which 50% of the total increase in absorbance has occurred when DNA is heat denatured.
SSC: 0.15 M NaCl, 0.015 M sodium citrate

K-DNA preparations from several species of trypanosome including Trypanosoma cruzi (1), Leishmania tarentolae (2), T. mega (5), T. gambiense (6), T. lewisi (3) and Crithidia acanthocephali (4). The proportion of these longer molecules (frequently referred to as 'linear' DNA) varies from 1% in T.cruzi to 33% in L.tarentolae. If these molecules contain non-reiterated sequences, the K-DNA complex would have a greater coding capacity than could apparently be represented by the minicircle alone. There are two opposing lines of evidence concerning the 'linear' DNA, some workers believing it to consist of a tandem arrangement of minicircles possibly representing a replication intermediate (7,8). Others have presented evidence that it may contain unique sequences (9,10,11). These two opposing hypotheses could perhaps be reconciled by the presence of two kinds of 'linear' DNA in K-DNA associations.

However, no evidence has been presented that these linear molecules are true components of K-DNA and not nuclear DNA contamination. In this paper we demonstrate extra components, probably representing nuclear DNA contamination, in many preparations of K-DNA from C.fasciculata. Properties of a preparation containing these extra components are compared with those of a 'normal' preparation, isolated by the same method. These results suggest that caution should be exercised in the interpretation of studies of isolated K-DNA.

Materials and Methods

Crithidia fasciculata (Anopheles strain: ATCC 11745) was grown at 25°C in an undefined medium of composition: 20 g Difco proteose peptone, 2g Difco liver infusion, 2 g Difco yeast extract, 50 mg adenine, 5 mg folic acid, 10 g glucose, 1 mg haemin per litre of glass distilled water, pH 8.0. Cells were harvested in mid-log phase of growth ($3-4 \times 10^7$ cells/ml).

K-DNA was prepared by the method of Laurent et al (1971). Analytical CsCl centrifugation was performed in a Beckman Model E ultracentrifuge at 44,000 rev./min, 20°C for 16-18 h (12). Ultraviolet absorption photographs of the DNA distribution were scanned using a Joyce-Loebl recording densitometer. Buoyant densities were calculated using Micrococcus luteus DNA (1.731 g/ml) as marker (13). Thermal denaturation of DNA was followed by its hyperchromicity at 260 nm (14), using a Pye Unicam SP8000 recording spectrophotometer and SP876 Temperature Programme Controller, coupled to a dual-pen

recorder. The temperature of samples, in 1.2 ml stoppered cuvettes was increased at a rate of 0.5°C/min. Absorbance measurements were corrected for the thermal expansion of water. (G+C) contents were calculated from T_ms using the formulae of ref. 14.

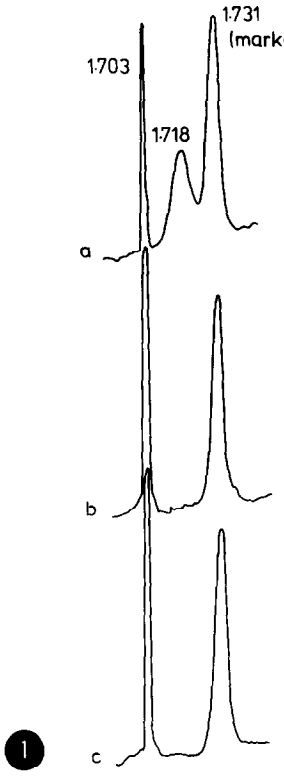
Electron microscopy. DNA molecules were visualized by the microversion (15) of the protein monolayer technique (16). The DNA was picked up on copper grids and shadowed with fused 90% platinum, 10% iridium wire on a double tungsten filament. The grids were examined with a Phillips EM300.

Results

In 1971 a procedure was developed by Laurent et al (17) for the isolation of K-DNA from C.luciliae, and this method, or variations of it, has been widely used for the isolation of K-DNA from many species of trypanosome. This method was followed precisely in the present work. The DNA at each stage of the purification was analysed. The results obtained for a preparation containing the usual single component in purified K-DNA, are given in Fig. 1. The buoyant densities of K-DNA (ρ=1.703 g/ml) and nuclear DNA (ρ=1.718 g/ml) agree with the values obtained by Hill & Bonilla (18) and Simpson & Simpson (19). The preparative CsCl gradient used in the final purification step is illustrated in Fig 2(a). In electron micrographs of rotary shadowed preparations the K-DNA was shown to be in the form of large associations, surrounded with a fringe of small loops. There was a ring of highly condensed material near the periphery. In addition to the associations, there were free minicircles having a contour length of 0.79 μm.

Analyses of the DNA at each stage of the purification of K-DNA which contained extra components are given in Fig. 3. Extra components were observed in 6 preparations from a total of 16. The general pattern was similar. As shown by the analysis of DNA in whole cell lysates, the extra components were not evident prior to phenol extraction. In addition one of the extra components was fast bonding (normally considered to be an exclusive characteristic of K-DNA (20)).

The extra component could be due to aggregation of nuclear and K-DNA during the extraction procedure. That nuclear DNA may



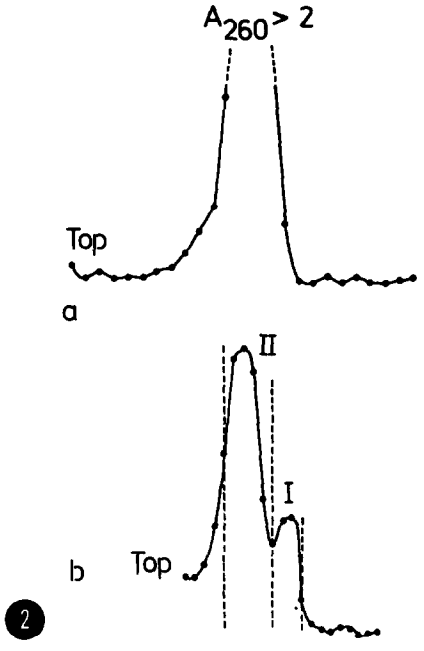


Figure 1. Densitometer tracing of U.V. film of analytical CsCl equilibrium centrifugation (44,000 rpm, 20°C) of C.fasciculata DNA at stages during the purification of K-DNA. Micrococcus luteus DNA (1.731 g/ml) was used as a density marker

a. Total cell extract
b. K-DNA after sedimentation through 20% sucrose
c. Final purified K-DNA after preparative equilibrium centrifugation in CsCl (see Fig. 2a)

Figure 2. Preparative CsCl equilibrium density gradient centrifugation of K-DNA

a. 'Normal K-DNA
b. K-DNA containing extra components. The fractions within the dotted lines were pooled to give components I and II

indeed be involved was indicated by two observations. Two components were observed in the final preparative CsCl step (Fig. 2b): these were examined with the electron microscope and by analytical CsCl centrifugation. Component II had an identical

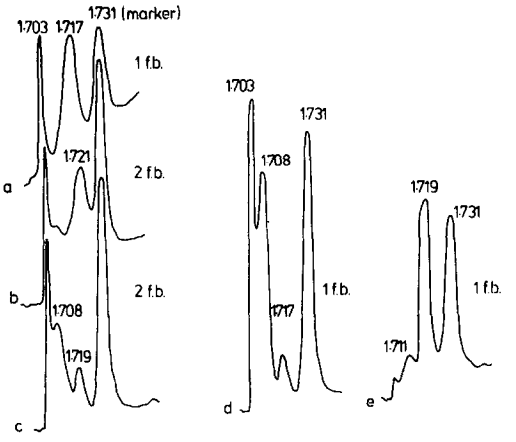


Figure 3. Analytical CsCl equilibrium centrifugation at stages of a K-DNA preparation containing extra components

(f.b. = peak present after 2 hour centrifugation)

a. Total cell lysate
b. Total DNA after phenol extraction
c. 'K-DNA' after sedimentation through 20% sucrose
d. Component II from the preparative CsCl gradient (Fig. 2b)
e. Component I from the preparative CsCl gradient (Fig. 2b)

Table 1. MELTING CHARACTERISTICS OF DNA FROM C.FASCICULATA : (G+C) CONTENTS

DNA	NATURE OF MELTING CURVE	(G+C) CONTENT (FROM T_m)		
Nuclear	Biphasic	49.3	64.0	
Component I	Biphasic	47.8	59.1	
'Normal' K-DNA	Triphasic	38.9	46.8	57.3
Component II	Triphasic	38.0	47.8	60.1

The DNA samples were extensively dialysed against either SSC or 0.1 x SSC before thermal denaturation was carried out.

structure to the 'normal' K-DNA preparations, containing associations. Component I, however, contained, in addition to some associations, 'linear' DNA. In analytical CsCl gradients both components I and II contained multiple DNA species (Fig. 3d,e).

'Normal' K-DNA from C. fasciculata exhibited a triphasic melting curve (cf. ref. 21), nuclear DNA a biphasic curve. The (G + C) contents calculated from the T_ms are given in Table 1. When the melting characteristics of components I and II are compared with those of 'normal' K-DNA and nuclear DNA it was noted that component I had a similar melting pattern to nuclear DNA, whereas component II was comparable with K-DNA.

Discussion

Aggregation of nuclear DNA, forming high molecular weight complexes which co-purify with K-DNA, may occur during K-DNA purification. Whether nuclear and kinetoplast components are subsequently separated could depend on the resolution of the preparative CsCl gradients. Even so, the K-DNA may remain heterogeneous. These results suggest that caution should be exercised in the interpretation of results from structural studies on 'K-DNA' prepared by the method of Laurent et al (17), particularly with regard to the presence of minor DNA components which could arise from nuclear contamination.

Similar results have been found in this laboratory for other species of trypanosome (Dr. B. A. Newton, Miss M. Foulkes, personal communication) and also for C. luciliae (Dr. M. Steinert, personal communication).

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