A SYNTHETIC OLIGONUCLEOTIDE PROBE THAT DISCRIMINATES BETWEEN THE SUBGENERA Schizotrypanum and Megatrypanum

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Summary:
The article reports the use of synthetic oligonucleotides as an additional tool for discriminating between the subgenera Schizotrypanum and Megatrypanum of the genus Trypanosoma. Three oligonucleotides were synthesized based on conserved sequence blocks in the minirepeat of the kinetoplast DNA minicircles (kDNA) from T. cruzi and used as probes in dot-blot hybridization experiments against kDNA from trypanosomatids belonging to different genera. Under specific hybridization and washing conditions the oligoprobes were found to hybridize distinctly with protozoa from the subgenera Schizotrypanum and Megatrypanum and with two monogenic insect trypanosomatids. One of the oligonucleotides showed to be specific for the subgenus Schizotrypanum. No cross-hybridization was detected with T. rangeli, which, in fact, belongs to another subgenus [Tejeraia, Añez, 1982], confirming the specificity of the probe. This promising result reinforces the use of oligoprobes for typing of trypanosomes.

KEY WORDS: oligonucleotides, kDNA, minicircles, hybridization, Trypanosoma.

The genus Trypanosoma comprises parasites of a heteroxenous life cycle with great number of mammalian host and some species are pathogenic to man and domestic animals (Vickerman, 1976). The genus has been subdivided into subgenera according to Hoare (1964). In the subgenus Schizotrypanum the type-species T. cruzi, which is transmitted by triatomine bugs, is associated with Chagas' disease in Central and South America (Brener, 1977). Triatomine insects are also vectors of T. rangeli which belongs to a different subgenus, Tejeraia (Añez, 1982). The possibility of mixed infection in humans in endemic areas where both parasites are transmitted can be a factor confounding the diagnosis of Chagas disease. However, recently several molecular techniques have been reported which distinguish between these parasites (Houguin et al., 1987; Murthy et al., 1992; Souto & Zingales, 1993; Steindel et al., 1994). Besides these two subgenera triatomin bugs can be host to at least one species of the subgenus Megatrypanum, T. conorhini (Deane et al., 1986). This latter subgenus has been reviewed by Wells (1976) and is considered of veterinary importance with some species being responsible for infections in Cervidae and Bovidae (Dirie et al., 1990). Both Schizotrypanum and Megatrypanum are found in opossums which are the wild mammals most frequently examined in epidemiological investigations of Chagas' disease. Thus, the distinction between them is of importance in field studies.

In this short report we describe a simple method, using a synthetic oligonucleotide as a probe, to differentiate between the subgenera Schizotrypanum and Megatrypanum in dot-blot hybridization experiments. Different heteroxenous and monoxenous trypanosomatids of the genera Trypanosoma, Leishmania, Endotrypanum, Phytomonas, Herpetomonas, Cribidida and Blastocrithidia were tested in molecular hybridization experiments using synthetic oligoprobes. Three oligonucleotides were synthesized based on T. cruzi mini-
Fig. 1. - Autoradiography showing dot-blot hybridizations with the synthetic oligoprobes TC4 (I), TC2 (II) and TC7 (III) against 20 ng of purified total kDNA from different trypanosomatids.

A1) T(S) cruzi (Y strain), B1) T(S) cruzi (CL strain), C1) T(S) cruzi (opossum strain 234LI), D1) T(S) cruzi (human isolate), E1) T(S) cruzi-like (bat), F1) T(S) cruzi-like (bat, Hastatus strain MJ7), G1) T(S) dionisii, H1) T(S) rangeli (San Agostin strain), A2) T(S) rangeli (San Agostin strain), B2) T(S) freitasi (IOC G219), C2) Endotrypanum schaudinni (IM217), D2) Herpetomonas samuelpessoai (ATCC 30252), E2) Crithidia fasciculata (ATCC 11745), F2) C. deanei (ATCC 30255), G2) C. luciliae (ATCC 14765), H2) C. oncopelti (ATCC 12982), A3) Bastocrithidia culicis (ATCC 30268), B3) Phytomonas davidii, C3) Leishmania (L) enriettii (I.V90), D3) Sauroleishmania tarentolae (UCLA strain), E3) L. (L) chagasi (PP75), F3) L. (V) guyanensis (M4147), G3) L. (L) amazonensis (M2269), H3) L. (L) major (LRC L137).

circle repeat sequences (Degrave et al., 1988), encompassing the conserved blocks CSB1 (TC2 and TC4) and CSB2 (TC7) described by Ray (1989) with respective positions in the multiple alignment from Degrave et al., (1988): TC2 (26-mer, positions 31-56) TGGTTTTGGGAGGGG(C/G)(C/G)(T/G)TCAA(A/C)TTT; TC4 (22-mer, positions 31-52) TGGTTTTGGGAGGGGC-TCAA; and TC7 (26-mer, positions 69-94) TCATGCATCTCCCCCGTACATTATTT. Probes were labelled with 32P gamma ATP using polynucleotide kinase and hybridized in dot-blot experiments with purified total kDNA from different genera spotted onto nylon membranes. Techniques for kDNA extraction and dot-blot hybridization have been previously described (Pacheco et al., 1986, 1990). Hybridizations were carried out at 42 °C overnight. The membranes were washed at the same temperature in 2X standard saline citrate (SSC)/0.5 % sodium dodecyl sulphate (SDS) and exposed to X-ray films with intensifying screen at −70 °C overnight.

The probes TC2 and TC4, the latter being 4 nucleotides shorter and lacking the redundancy in TC2, showed a very similar hybridization specificity and were found to hybridize with kDNA from parasites belonging to the subgenera Schizotrypanum and Mega-trypanum. The probe TC2 was found to hybridize only with the genus Trypanosoma while TC4 showed an additional, albeit weak, signal with two monogenetic insect trypanosomatids. However, the probe TC7 was found to be specific to the subgenus Schizotrypanum, showing no cross-hybridization with the kDNA from T. rangeli or any other tested genera (Fig. 1). This result is not unexpected, as the CSB2 motif (cCCCGTNC) is shorter than the CSB1 motif (AgGGGGGTTT) (Ray, 1989), and suggests the use of the TC7 oligoprobe as a tool for typing the subgenus Schizotrypanum. In addition, further applications for this probe can be found in preliminary screens of sylvatic animals captured in epidemiological surveys, in the quality control of strain banks and in the investigation of mixed infections.

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REFERENCES


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