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Characterization of *Trypanozoon* stocks by isoelectric focusing and isoenzyme analysis

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Summary

Isoelectric focusing on ultrathin polyacrylamide foils, coupled with isoenzymatic analysis using five different enzymes, was used to characterize bloodforms of *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* and procyclic forms of *T. b. brucei* and *T. b. rhodesiense*. The protein concentrations in the lysates were quantified. Qualitative as well as quantitative differences were found.

Key words: isoelectric focusing; isoenzyme analysis; *Trypanosoma b. brucei*, *T. b. rhodesiense*, *T. b. gambiense*.

Introduction

Trypanosomes can be characterized by serotyping, DNA-analysis, and/or by their isoenzymatic patterns. Zymodemes have usually been determined by using starch gel- or cellulose-acetate-electrophoresis (Gibson et al., 1980; Kreutzer and Sousa, 1981): the advantage of cellulose acetate- over starch gel electrophoresis for *T. cruzi* has been shown by Lanham et al. (1981). Ebert (1982) introduced isoelectric focusing in thin layer polyacrylamide and agarose gels to characterize *T. cruzi* stocks. Lysates from twelve stocks were compared using seven different enzymes.

This paper extends the use of isoenzymatic analysis by isoelectric focusing to African *Trypanozoon* stocks.

Material and Methods

T. b. brucei (Clone STIB 247-F) derived from a primary isolate STIB 247, isolated from a hartebeest in the Serengeti National Park, Tanzania in 1971. The clone was selected from an ICR white mouse after 24 successive cyclical transmissions of STIB 247 through *Glossina m. morsitans*.

T. b. rhodesiense (Clone STIB 389-F) derived from a primary isolate STIB 389, isolated from man in Northern Tanzania in 1980. The clone was selected from an ICR white mouse after five successive cyclical transmissions of STIB 389 through *G. m. morsitans*.

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T. b. gambiense (STIB 387-A) was originally isolated by Dr. D. Mehlitz (Hamburg) from man at the Ivory Coast in 1978 as TH3/78E (031). The trypanosomes were harvested from *Microtus montanus* after one cyclical transmission through *G. p. gambiensis*.

Procyclic forms of *T. b. brucei* and *T. b. rhodesiense* were grown in medium SDM-79 (Brun and Schönenberger, 1979).

The bloodstream trypanosomes were separated from blood on a DEAE column (Lanham and Godfrey, 1970). The parasites were counted in a Neubauer chamber and finally pelleted at 1400 *g* for 10 min at 2–4° C. They were then lysed by adding an equal amount (v/v) of an aqueous solution of 2 mM dithiothreitol and 2 mM EDTA by three cycles of freezing on dry ice and thawing. The lysates were spun at 10,000 *g* for 30 min. The supernatant volumes were measured with a Hamilton syringe and the protein concentration estimated by a modified Lowry method (Peterson, 1977). The lysates were immediately frozen in aliquots as beads in liquid nitrogen.

Isoelectric focusing (= IEF) was carried out using Servalyt Precotes pH 3–10 (Serva, Heidelberg). The foils were handled as described in the manual. They were placed on an LKB-Multiphor cooled to 8° C. The samples were loaded as 5 µl quantities, diluted to the appropriate protein concentrations (see results) into the applicator slits. Focusing was done with a PS 10 A Multistab (W. Meyer, Luzern) with a limited voltage of 1000 V. The current was restricted to 5 mA per foil and the focusing was stopped after 3½ hours. The foil was then cut into two pieces, and one was immediately overlayered with agarose and enzyme substrates and incubated at 37° C. The rest of the foil was used to determine the pH-gradient with a surface electrode (Ingold, Zürich).

The incubation mixtures for the different enzyme reactions were as described by Gibson et al. (1978). Five enzymes were used to characterize the trypanosomes: phosphoglucomutase (PGM) (E. C. 2.7.5.1), isocitrate dehydrogenase (ICD) (E. C. 1.1.1.42), aspartate aminotransferase (ASAT) (E. C. 2.6.1.1), alanine aminotransferase (ALAT) (E. C. 2.6.1.2) and aminopeptidase B (E. C. 3.4.11.1).

Results

To obtain a quantitative comparison between the lysates, the total number of trypanosomes was correlated with the protein concentration in each case. The protein concentrations had to be adjusted for the determination of each enzyme. Protein concentrations were adjusted to 1 mg/ml for ALAT, and to 5 mg/ml for ASAT. 10 mg/ml were needed for adequate staining of PGM, ICD and PEP. The lysates from procyclic forms had always to be used at half the above concentrations. These dilutions were necessary to avoid the overloading of the gel. The number of trypanosomes needed to get reproducible isoenzyme patterns ranged from $0.7–2.5 \times 10^7$ trypanosomes, depending on the type of trypanosome populations as well as the enzyme.

We were able to discriminate between *T. b. brucei* and *T. b. gambiense* by the pattern obtained with PGM (Fig. 1a Nr. 1 and 3). *T. b. gambiense* shows a band focused at a pI of 5.7, whereas *T. b. brucei* shows two strong bands at pI 5.6 and 5.5. With ICD, *T. b. gambiense* (Fig. 1b Nr. 3) has a band at a pI 5.5, which is not present in *T. b. brucei*, which shows, besides minor bands, a relatively strong one at pI 5.7. *T. b. rhodesiense* showed the same pattern as *T. b. brucei*, but quantitative differences were found; in all assays performed so far *T. b. rhodesiense* showed less enzyme activity than *T. b. brucei*, even when the same amount of protein was applied (see Fig. 1). The patterns for ASAT and PEP were not strikingly different, but there may be quantitative differences between *T. b. brucei* and *T. b. gambiense*.

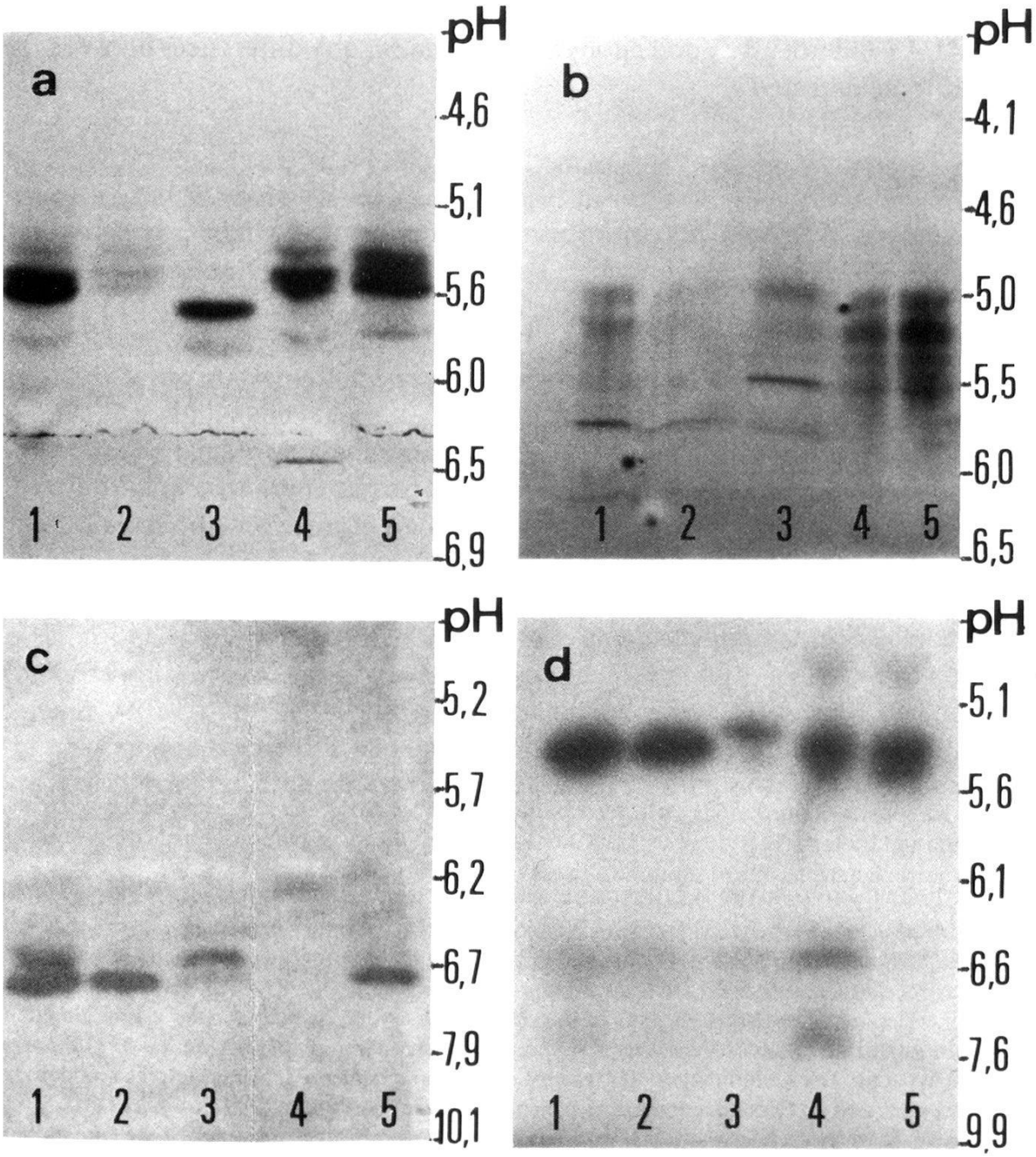

Fig. 1. Isoenzyme patterns of *Trypanosoma b. brucei* (1), *T. b. rhodesiense* (2), *T. b. gambiense* (3) bloodforms and the procyclic forms of *T. b. brucei* (4) and *T. b. rhodesiense* (5). All samples were focused on Servalyt precotes pH 3–10. a) PGM, b) ICD, c) ASAT, d) PEP.

With all enzyme patterns tested the bloodform lysates can be distinguished from the procyclic forms of *T. b. brucei* and *T. b. rhodesiense*. With PGM a third strong band appears at pI 5.4 (Fig. 1a Nr. 4/5). With ICD-staining, four more strong anodic bands appear, in contrast to the single band at pI 5.7 from the bloodforms. *T. b. brucei* has only a weak band at pI 6.3 with ASAT (Fig. 1c Nr. 4). A band in addition to those found with the bloodforms is found with PEP at a pI 4.9.

ALAT did not give good enough resolution for any differences between the lysates to be detected.

Discussion

Analysis of enzyme polymorphism requires a system which is sensitive and gives reproducible and easily-characterized results. Starch gel and cellulose-acetate electrophoresis are helpful techniques for the identification of trypanosomes (Gibson et al., 1980), but they have some inherent disadvantages, since no internal markers are included, which makes the comparison of different results impossible. Using isoelectric focusing the enzyme bands are characterized by their isoelectric point (pI) and the amount of protein applied can be accurately determined, so that the isoenzymes can be compared quantitatively.

Using the qualitative and quantitative differences we were able to discriminate between the *Trypanozoon* stocks studied, using only four enzymes. We are now investigating the general applicability of the patterns described using a variety of different African stocks.

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