NOTE

Adaptations in the Glucose Metabolism of Procyclic Trypanosoma brucei Isolates from Tsetse Flies and during Differentiation of Bloodstream Forms

Koen W. A. van Grinsven,† Jan Van Den Abbeele, Peter Van den Bossche, and Aloysius G. M. Tielens

Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, 3584 CM Utrecht, The Netherlands; Department of Parasitology, Unit of Entomology, Institute of Tropical Medicine Antwerp, B-2000 Antwerp, Belgium; Department of Animal Health, Institute of Tropical Medicine Antwerp, B-2000 Antwerp, Belgium; Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Onderstepoort, South Africa; and Department of Medical Microbiology and Infectious Diseases, Erasmus MC University Medical Center, 3015 GD Rotterdam, The Netherlands

Received 20 March 2009/Accepted 4 June 2009

Procyclic forms of Trypanosoma brucei isolated from the midguts of infected tsetse flies, or freshly transformed from a strain that is close to field isolates, do not use a complete Krebs cycle. Furthermore, short stumpy bloodstream forms produce acetate and are apparently metabolically preadapted to adequate functioning in the tsetse fly. African trypanosomatids comprise various pleomorphic trypanosome species that proliferate in the bloodstream of their mammalian hosts as long slender bloodstream form (BSF) trypanosomes, and at the peak of parasitemia they differentiate into nondividing short stumpy form trypanosomes (1). After being ingested during a bloodmeal by a tsetse fly (Glossina sp.), short stumpy form trypanosomes differentiate into procyclic form (PCF) trypanosomes, which actively multiply and colonize the midgut of the fly. Subsequently, PCF Trypanosoma brucei migrates to the salivary glands while undergoing a complex differentiation (22). Here, attached epimastigote forms start multiplying, after which nondividing metacyclic trypanomastigotes develop. The life cycle of T. brucei is completed when these metacyclic trypanomastigotes are injected into a mammal through the bite of an infected fly, after which they transform into long slender BSF trypanosomes. During this life cycle, trypanosomes encounter different environments to which they have adapted, resulting in distinct stages, characterized by morphological as well as metabolic changes. Long slender BSF trypanosomes degrade glucose by glycolysis and excrete pyruvate as the sole metabolic end product (12, 13, 23). On the other hand, PCF trypanosomes do not excrete pyruvate but degrade glucose to acetate and succinate as main end products (25). Krebs cycle activity was thought previously to be present in trypanosomatids, at least in insect stages of some African trypanosomatids (3, 9, 10, 12, 21). However, this presumed flux through the Krebs cycle is supported only poorly by direct experimental evidence and was based mainly on the presence of certain enzyme activities. Although genes for all enzymes of this cycle are indeed present in the genome and expressed in the insect stages, recent studies revealed that at least in T. brucei, the cycle is not used for the complete oxidation of acetyl-coenzyme A (CoA) to carbon dioxide (2, 26). Instead, parts of the cycle are most likely used in anabolic pathways, such as gluconeogenesis and fatty acid formation, and also for the final steps in the degradation of amino acids (6). It is possible that the reported discrepancies on the presence or absence of full-circle Krebs cycle activity are caused by differences in the number of passages through mice after the isolation of the strain from the field. Such passages may have been ongoing for many years, during which the parasites were continuously propagated as BSF trypanosomes. Furthermore, most insect form trypanosomes that were investigated up to now have been propagated for many years as PCF trypanosomes in rich culture media. Hence, the reported discrepancies could be due to differences between freshly differentiated PCF trypanosomes and those well adapted to in vitro culture, and the absence of an active Krebs cycle in PCF trypanosomes could be the result of an adaptation caused by the prolonged in vitro culturing. To investigate these possibilities, we analyzed the glucose metabolism of PCF T. brucei directly after isolation from the midguts of tsetse flies. We also studied freshly differentiated PCF trypanosomes from the AntAR 1 strain, a T. brucei strain that has had a minor history of animal passaging since its field isolation (15, 17). To investigate the cause of the conflicting reports on Krebs cycle activity in PCF trypanosomes, we first analyzed the effect
of environmental factors by comparing the carbohydrate metabolism of PCF trypanosomes well adapted to in vitro culturing and PCF trypanosomes isolated from their natural environment, the midguts of tsetse flies. These experiments were performed with PCF TREU 927 *T. brucei*, a pleomorphic strain that has been thoroughly characterized and is still able to infect *Glossina morsitans*, performing a complete physiological life cycle (2). For the infection of tsetse flies, male *G. morsitans* flies originating from the colony maintained at the Institute of Tropical Medicine in Antwerp, Belgium, were infected with procyclic TREU 927 *T. brucei* by in vitro membrane feeding and subsequently maintained for 10 days by feeding on rabbit blood (15). Then, flies were dissected on a sterile glass slide and the infected midguts were isolated and incubated for at least 30 min at 28°C in SDM-79 medium that was gently rotated. After sedimentation of the midguts by gravity, insect gut debris was removed by centrifugation at 300 \( \times g \) for 5 min. PCF trypanosomes were then isolated from the collected supernatant by centrifugation at 1,500 \( \times g \) for 10 min. Since PCF trypanosomes could not be isolated from the midgut without minor amounts of contaminating insect gut material, such as gut cells and debris, we also investigated the glucose metabolism of this fraction. Analysis of metabolic end products produced from [6-\(^{14}\)C]glucose in this control incubation of insect gut debris, which also contained minor amounts of trypanosome cells, showed the formation of \(^{14}\)C-labeled pyruvate, CO\(_2\), acetate, and lactate (Fig. 1A). Minor amounts of lactate were also produced in the incubations with PCF trypanosomes isolated from the midgut, which also contained minor amounts of insect gut debris. Since lactate is not an excreted end product of *T. brucei*, this labeled lactate is indicative for the glucose degradation activity of insect gut debris. Therefore, end product formation in the incubations with PCF trypanosomes isolated from the midgut was corrected for end products produced by the contaminating insect gut debris by subtracting all produced lactate and the calculated accompanying amounts of other end products produced in the insect gut debris incubation. The metabolic incubations with PCF trypanosomes directly after isolation from the tsetse midgut showed that these trypanosomes degrade glucose to the same metabolic end products, acetate, succinate, and pyruvate, as the in vitro culture-adapted PCF trypanosomes (Fig. 1A). Furthermore, the ratio of acetate and succinate produced by PCF trypanosomes isolated from the midgut were similar to that of in vitro-cultured PCF trypanosomes (Fig. 1A). On the other hand, a major difference was observed in the amount of glucose consumed since the PCF trypanosomes isolated from the midguts of tsetse flies consumed 16-fold less glucose than PCF trypanosomes that were derived from in vitro cultures. This difference in glucose consumption can probably be explained by our observation that both motility and especially growth of PCF trypanosomes isolated from the midgut were significantly reduced compared to the in vitro culture-derived PCF trypanosomes. Apparently, the environmental conditions in the midgut of the fly did affect the PCF trypanosomes, but they did not significantly alter the metabolic pathways used for energy metabolism. However, PCF trypanosomes isolated from the midgut of the fly excreted more pyruvate (Fig. 1A), which suggests that pyruvate is a more important metabolic end product for PCF trypanosomes under physiological conditions than ac-
(94% long slender versus 6% short stumpy), whereas at day 7 after infection, predominantly short stumpy BSF trypanosomes were isolated (92% short stumpy versus 8% long slender). Analysis of glucose-derived metabolic end products from incubations with BSF AntAR 1 trypanosomes isolated at day 4 or at day 7 after infection showed that short stumpy BSF trypanosomes indeed produce significant amounts of acetate as an end product of glucose metabolism (Fig. 1B). In the incubations with predominantly long slender BSF AntAR 1 T. brucei cells, some acetate was also produced, but this relatively small amount of acetate formation can be explained by the presence of a certain amount of short stumpy cells. Although the incubations were started with nearly 95% long slender BSF cells, BSF cells from the AntAR 1 strain are highly pleomorphic and rapidly differentiate to short stumpy forms during in vitro culture conditions. Therefore, increasing amounts of short stumpy form T. brucei were formed during our incubations (up to 40 to 50% at the end of incubation), which accounts for the amount of acetate formed during these incubations.

Since acetate production in Trypanosomatidae is catalyzed by the mitochondrial enzyme acetate:succinate CoA transferase (ASCT), which was previously shown not to be expressed in in
vitro-cultured BSF *T. brucei* (20), we examined the ASCT enzyme activity in lysates derived from cultures containing predominantly long slender BSF trypanosomes (BSF LS; 94%), or exclusively short stumpy BSF trypanosomes (BSF SS; 92%), or exclusively PCF trypanosomes (PCF). Shown are the means ± standard deviations of three experiments.

In conclusion, the data described in this paper demonstrate the absence of a functional Krebs cycle in the mitochondria of PCF *T. brucei*, isolated from the tsetse midgut or freshly differentiated from BSF trypanosomes. Furthermore, we show that short stumpy BSF *T. brucei* cells produce large amounts of acetate. Therefore, the mitochondria of short stumpy trypanosomes are metabolically divergent from the mitochondria in long slender BSF *T. brucei* cells. These results are consistent with prior work (4, 5, 8, 11). The functional changes might be a preadaptation that allows short stumpy BSF *T. brucei* to function in the intestines of infected tsetse flies and enables them to differentiate further into PCF trypanosomes.

REFERENCES


