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THE EFFECT OF 5-THIOGLUCOSE ON THE ENERGY METABOLISM OF *SCHISTOSOMA MANSONI IN VITRO*

ALOYSIUS G. M. TIELENS, MARTIN HOUWELING and SIMON G. VAN DEN BERGH
Laboratory of Veterinary Biochemistry, State University of Utrecht, P.O. Box 80177, 3508 TD Utrecht, The Netherlands

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Abstract—5-Thioglucose (5-TG) had a marked effect on the energy metabolism of *Schistosoma mansoni in vitro*: the conversion of external glucose into lactate by intact worms was severely inhibited. This inhibition of glycolysis was instantaneous, independent of the oxygen concentration and competitive with respect to glucose. Degradation of 0.5 mM external (^{14}C-labelled) glucose was inhibited for 80% in the presence of 20 mM 5-TG. On the other hand the degradation of endogeneous glycogen to lactate was uninhibited. This shows that the inhibition of glucose breakdown occurred at the entrance of glucose into the cell and/or at the hexokinase reaction. It was demonstrated that 5-TG inhibited both the uptake of glucose and the activity of hexokinase. However, it was concluded that in the intact worm 5-TG blocked glycolysis by its competitive inhibition of hexokinase. In intact *S. mansoni* worms hexokinase is probably the rate-limiting enzyme of glycolysis. Krebs-cycle activity and lactate production do not occur at a fixed ratio: at lower rates of pyruvate formation Krebs-cycle activity was favoured.

5-Thio-D-glucose (5-TG),* an analogue of natural D-glucose containing a sulphur atom in the ring instead of oxygen, was first prepared by Feather and Whistler in 1962 [1]. This compound which is essentially non-toxic to animals [2] has intriguing properties: it has a specific toxicity towards hypoxic cells [3] and it completely inhibits spermatogenesis and fertility in male mice [4].

Schistosoma mansoni infection (schistosomiasis or Bilharziasis) is a major parasitic disease which still afflicts many people, mainly in developing countries. The disease is caused by paired worms which live in the mesenteric veins. These worms use large quantities of glucose which they, like all other parasitic helminths, do not completely oxidize to carbon dioxide and water. The main end product of glucose catabolism in *S. mansoni* is lactate [5]. The parasite is strongly dependent on glucose, a characteristic it shares with tumour cells [6], hypoxic cells [3] and testes [4] which are affected by 5-TG. Therefore, we investigated the effect of 5-TG on the energy metabolism of *S. mansoni*.

The results showed that the degradation of exogeneous glucose by *S. mansoni* is strongly inhibited by 5-TG, whereas the degradation of endogeneous glycogen is not impaired. This phenomenon was shown to be the result of a competitive inhibition of hexokinase by 5-TG.

MATERIALS AND METHODS

Chemicals. 5-TG was purchased from the Sigma Chemical Co. (St. Louis, MO) and radioactive glucose was from New England Nuclear (Boston, MA). All enzymes were from Boehringer, Mannheim (F.R.G.).

Parasites. Seven-week-old *Schistosoma mansoni* worms were isolated from ether-anaesthetized hamsters by perfusion via the aorta at 37° with a medium (pH 7.4) containing: 20 mM HEPES, 117 mM NaCl, 5.4 mM KCl, 0.7 mM Na_2HPO_4, 1 mM $MgSO_4$, 1.5 mM $CaCl_2$ and 5 mM glucose.

Incubations of 3 or 4 worm pairs were carried out for 1.5 hr in closed 25-ml Erlenmeyer flasks in a gyrotory shaker at 37° in 2 ml of the isolation medium in which 25 mM NaCl was replaced by 25 mM $NaHCO_3$. The glucose concentration was 0.5 mM unless stated otherwise and 2 μCi D-[6-^{14}C]glucose was added per incubation. In aerobic incubations the gas phase was air containing 5% CO_2, while anaerobic incubations were performed under N_2 with 5% CO_2 in the presence of ascorbate and ascorbate oxidase as described earlier [7]. The incubations were terminated by adding through the rubber stopper 100 μl 6 M KOH to a removable centre well and 150 μl 70% $HClO_4$ to the medium.

Glucose uptake was studied after a 5-min preincubation of the worms in the incubation medium containing 0.5 mM glucose whereupon they were transferred on a sieve to 2 ml incubation medium with or without 20 mM 5-TG containing 0.5 mM D-[U-^{14}C]glucose (30 μCi). After various incubation periods they were rapidly rinsed by immersing them 4 times in incubation buffer which also contained 0.5 mM glucose. The worms were then transferred in ethanol to a glass scintillation vial and after evaporation they were dissolved in 0.5 M KOH. An aliquot was used for the protein determination and to the remainder scintillation cocktail was added.

Homogenates were prepared with a Potter-Elvehjem homogenizer and after removal of the

* Abbreviations used: 5-TG, 5-thio-D-glucopyranose; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid.

debris by centrifugation (2 min, 1400 *g*), the supernatant was incubated in our incubation medium fortified, as described by Shapiro and Talalay [8], with 5 mM ATP, 5 mM NAD^+, 5 mM NaH_2PO_4 and 8 mM $MgCl_2$.

Assays. All the carbon dioxide was trapped in the centre well by shaking another 2 hr after the termination of the incubation. The radioactivity in this fraction was counted in Tritosol, modified according to Pande [9], which was also the scintillation cocktail for all other radioactive compounds in these experiments.

Lactate was separated from the neutralized incubation medium on an acid-washed alumina column (2 g, $h = 3.5$ cm) which was eluted with H_2O (50 ml) to isolate glucose, whereupon the Al_2O_3 was dispersed in 1.5 ml of a quaternary ammonium hydroxide ("Lumasolve", Lumac systems AG, Basel, Switzerland). After the addition of scintillation cocktail the radioactivity in this mixture was counted. The total of labelled and unlabelled lactate was determined in the neutralized incubation medium with a standard enzymatic method [10]. Lactate produced from unlabelled substrates was calculated by subtracting the radioactive from the total amount.

Glycogen was determined by comparison of the amount of glucose present before and after acid hydrolysis in aliquots of a homogenate of the worms in 0.5 ml 0.5 M KOH.

Protein was determined with the Lowry method, using bovine serum albumin as standard.

Glucose was assayed enzymatically with the GOD-Perid test combination (Boehringer, Mannheim, F.R.G.).

Hexokinase (EC 2.7.1.1.) activity was measured spectrophotometrically on an Aminco DW-2a at 25° using a 30-min, 8000-*g* supernatant of a worm homogenate in 250 mM sucrose/5 mM EDTA. The assay (1.1 ml, pH 7.6) contained 20 mM triethanolamine, 7 mM $MgCl_2$, 0.8 mM NADP, 0.5 mM ATP and 14 U glucose-6-phosphate dehydrogenase (EC 1.1.1.49.). It was checked that under the assay conditions 20 mM 5-TG did not significantly inhibit the dehydrogenase reaction, neither directly nor after a possible conversion to 5-TG-6-phosphate by *S. mansoni* hexokinase.

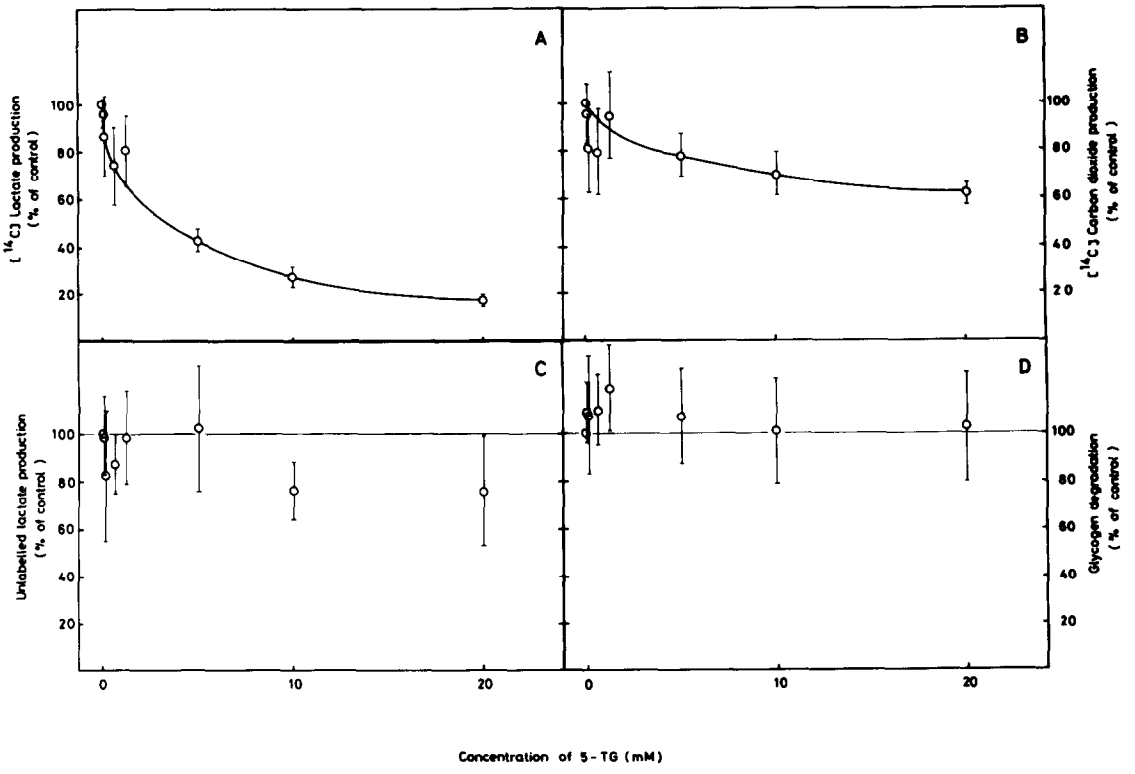

Fig. 1. Effect of 5-TG on the carbohydrate metabolism of *Schistosoma mansoni*. Intact worm pairs were incubated for 1.5 hr in the presence of various concentrations of 5-TG and a fixed [6-^{14}C]glucose concentration of 0.5 mM. (A) Excreted amounts of radioactive lactate as a percentage of the control (100% = 715 ± 117 nmoles/hr per mg protein). (B) Excreted amounts of radioactive carbon dioxide as a percentage of the control (100% = 72 ± 18 nmoles/hr per mg protein). (C) Excreted amounts of unlabelled lactate as a percentage of the control (100% = 1078 ± 209 nmoles/hr per mg protein). These values were calculated by subtracting the radioactive from the total amount. (D) Degradation of endogeneous glycogen as a percentage of the control (100% = 594 ± 63 nmoles glucose units/hr per mg protein). These values were calculated by comparing the amount of glycogen present at the end of the incubations with the amount of glycogen present at the beginning (determined on separate samples). Each point in A–D represents the mean ± S.D. of 6 experiments.

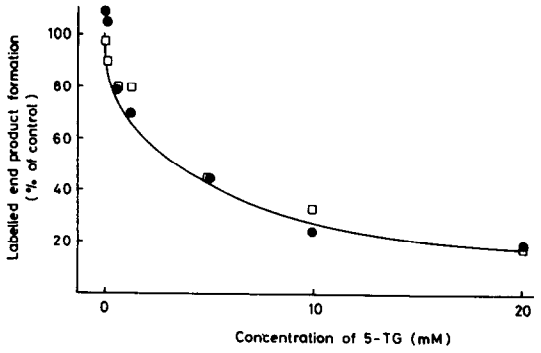

Fig. 2. Effect of anaerobiosis (□) on the inhibition of radioactive lactate excretion by intact worm pairs and the inhibition of radioactive end-product formation (●) by 5-TG of a homogenate of *Schistosoma mansoni*. The solid curve represents the inhibition of radioactive lactate excretion by intact worm pairs under aerobic conditions (Fig. 1A). All values are expressed as a percentage of their control.

RESULTS

Effect of 5-TG on the metabolism of intact worms

Initial experiments showed that 5-TG strongly inhibited the glucose catabolism of *S. mansoni*. These incubations were performed after a 2-hr preincubation of the worms in 5-TG. Subsequent experiments with preincubation times varying from 0 to 2 hr showed that the inhibition was independent of the preincubation time. Apparently, 5-TG acts instantaneously and, therefore, all further studies were performed without preincubation.

The effect of increasing 5-TG concentrations (0, 0.05, 0.1, 0.5, 1, 5, 10 and 20 mM) on glucose metabolism of *S. mansoni* in the presence of a constant (0.5 mM) glucose concentration is shown in Fig. 1. The degradation of external (labelled) glucose to lactate was strongly inhibited by 5-TG (Fig. 1A). With increasing 5-TG concentrations this inhibition increased and reached a maximum of 80% at 20 mM 5-TG. The production of $^{14}CO_2$ was also inhibited in the presence of 5-TG (Fig. 1B) but to a smaller extent (max. 40%). The production of unlabelled lactate (Fig. 1C) and the degradation of glycogen (Fig. 1D) were not affected by the presence of 5-TG. The same inhibition pattern was observed under aerobic and anaerobic conditions (Fig. 2). In the absence of oxygen or in the presence of the higher concentrations of 5-TG the worms were not as motile as in control incubations.

Effect of 5-TG on worm homogenates

The formation of the (unidentified) radioactive end product from [6-^{14}C]glucose by fortified worm homogenates was inhibited to the same extent by 5-TG as the production of radioactive lactate was in intact worms (Fig. 2). These homogenates did not produce $^{14}CO_2$.

Effect of 5-TG on the hexokinase activity

The effects of 2 and 20 mM 5-TG on the hexokinase activity in a homogenate of *S. mansoni* were investigated. A Lineweaver–Burk plot (Fig. 3) shows that 5-TG was a competitive inhibitor of this enzyme. The K_m of glucose was 0.081 mM, and the K_i of 5-TG was about 1 mM.

Effect of 5-TG on the uptake of glucose

To study whether 5-TG has any effect on the uptake of glucose under the conditions that showed inhibition of glucose degradation, worms were pre-

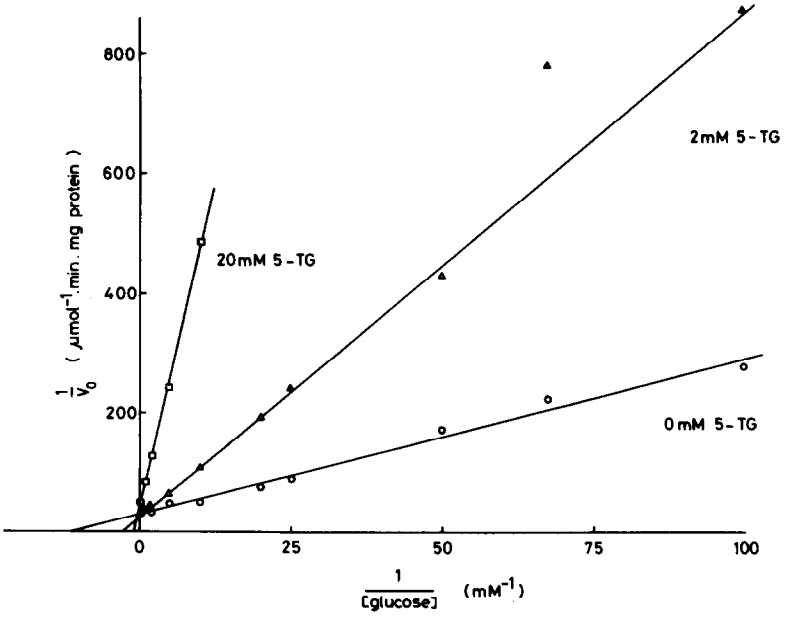

Fig. 3. Lineweaver–Burk plot of the inhibition of *Schistosoma mansoni* hexokinase by 5-TG in a worm homogenate.

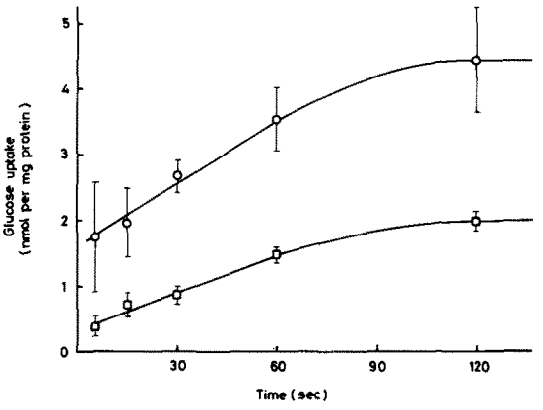

Fig. 4. Time course of [U-^{14}C]glucose uptake by intact *Schistosoma mansoni* worm pairs in the absence (○) and presence (□) of 20 mM 5-TG. The incubations contained 0.5 mM glucose. Each point represents the mean ± S.D. of 5 incubations.

incubated for 5 min in 0.5 mM unlabelled glucose whereupon the uptake of radioactive glucose was measured for various periods in the absence and presence of 20 mM 5-TG. Figure 4 shows that under these conditions 5-TG inhibited the uptake of glucose by *S. mansoni* worms. Both the rapid uptake in the first few seconds as well as the more slowly second phase of glucose uptake were inhibited by 5-TG.

DISCUSSION

5-TG is known to affect certain cell types that are strongly dependent on glycolysis [3, 6] and, therefore, its effect was studied on *Schistosoma mansoni* which has a very high rate of glycolysis and produces almost exclusively lactate.

It could indeed be shown that glycolysis of intact *S. mansoni* worms is markedly inhibited by 5-TG and that this inhibition was competitive with respect to glucose. In order to obtain more information on the mode of action of 5-TG, all further experiments were performed with a low glucose concentration (0.5 mM), although the mesenteric veins—the habitat of *S. mansoni*—have a tenfold higher glucose concentration.

The results presented in Fig. 1A show that glycolysis in intact *Schistosoma mansoni* worms was inhibited by 5-TG. This inhibition was instantaneous, which can be concluded from the observation that it made no difference whether or not the worms were preincubated in the presence of 5-TG.

It has been reported that lung cells [3], tumor cells [6] and multicell spheroids [11, 12] are only affected by 5-TG when cultivated under hypoxic conditions. In contrast, with *S. mansoni* the inhibition of glycolysis was independent of the concentration of oxygen: the inhibition curves under both aerobic and fully anaerobic conditions were identical (Fig. 2). Probably, in the reported cell types anoxia results in an increase in the rate of glycolysis, which is then more susceptible to inhibition. On the other hand, in *S. mansoni* anoxia will hardly increase the rate of glycolysis because even in the presence of oxygen *S. mansoni* is functioning almost completely anaerobic *in vitro*.

The results presented in Fig. 1 show that although under the conditions studied the degradation of external (labelled) glucose was strongly inhibited, the degradation of endogenous carbohydrate was not inhibited by 5-TG. The absolute amounts of glycogen degraded and unlabelled lactate produced closely matched (Figs 1C and 1D), which is expected as glycogen is the main endogeneous substrate of *S. mansoni* and lactate the main end product. The presence of 5-TG did not alter the amount of glycogen broken down during the incubation, nor did it change the amount of lactate produced from unlabelled substrates. Therefore, if it is assumed that the degradation of glucose and glycogen occurred in the same compartment (cytosol) and largely via the same pathway, the inhibition of glucose catabolism by 5-TG must have happened before its degradative pathway coincides with that of glycogen at the glucosephosphate isomerase (EC 5.3.1.9.) step. This would imply that the entrance of glucose into the cell and/or the hexokinase reaction were inhibited.

Hexokinase of *S. mansoni* was strongly inhibited by 5-TG (Fig. 3). This inhibition was competitive with a K_i for 5-TG of about 1 mM. This K_i is substantially lower than the reported K_i (20 mM) of 5-TG for yeast hexokinase [13]. This competitive effect of glucose and 5-TG was also observed when intact worms were incubated with glucose concentrations varying from 0.05 to 20 mM in the presence of a fixed concentration of 5 mM 5-TG (not shown). The K_m of hexokinase for glucose was 81 μM, which is in reasonable agreement with the K_m of about 70 μM reported in the literature for *S. mansoni* [8].

Since the degradation of labelled glucose by homogenates was inhibited by 5-TG to the same extent as that by intact worms (Fig. 2) and, furthermore, since in the presence of 0.5 mM glucose and 20 mM 5-TG hexokinase was inhibited to almost the same extent (72%) as glycolysis in intact worms (80%), we conclude that the effect of 5-TG on hexokinase was responsible for the decrease in the rate of glycolysis in intact worms. Therefore, although glucose uptake is also inhibited by 5-TG (Fig. 4), the inhibition of a transport mechanism is probably not the cause of the observed decrease in radioactive lactate production. Inhibition of glucose-transport systems by 5-TG has already been reported [14].

Lactate dehydrogenase (EC 1.1.1.27) of *S. mansoni* was not inhibited by 5-TG (not shown). Therefore, the remarkable difference in the extent of the inhibition by 5-TG of the production of radioactive lactate and carbon dioxide (Figs. 1A and 1B), indicates that Krebs-cycle activity and lactate formation do not occur at a fixed ratio: at lower rates of pyruvate supply Krebs-cycle activity was favoured. On the other hand, from the inhibition of the $^{14}CO_2$ production (40%) it may not be concluded that the Krebs-cycle activity of *S. mansoni* is dependent on the flux through the glycolytic pathway. From the present data it can not be excluded that 5-TG has an inhibitory effect on the Krebs-cycle itself.

As explained above in intact *S. mansoni* worms

hexokinase was the only enzyme of glycolysis inhibited by 5-TG. The percentage inhibition of glycolysis was about equal to that of hexokinase. Together, these data indicate that hexokinase is the rate-limiting enzyme of glycolysis in intact worms, and not only of glycolysis in fortified homogenates, as was already shown by Shapiro and Talalay [8].

Although 5-TG is a powerful competitive inhibitor of hexokinase, the rate-limiting enzyme of glycolysis in *S. mansoni*, it is probably not a very satisfactory schistosomicide, due to the inevitably high glucose concentration in the natural environment of the worm. However, 5-TG in combination therapy or structural analogs of it could be of value in future drug research, because it is essentially non-toxic to animals and—at least under certain conditions—it can severely inhibit the main energy-yielding process of *S. mansoni*.

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