" PATHWAYS OF GLYCEROL METABOLISM IN THE BLOODSTREAM AND CULTURED INSECT FORMS OF TRYPANOSOMA ~~BRUCEI~~ BRUCEI"

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Summary of thesis

The in vitro propagated midgut forms, proventricular forms, midgut-like forms, metacyclic forms derived from tsetse salivary glands and the blood stream forms of Trypanosoma brucei brucei had high activities of the glycerol kinase and other enzymes for glycerol metabolism. These enzyme activities may account for the ability of these trypanosomes to use glycerol as substrate under aerobic condi tions. Of these trypanosome stages, only the blood stream and the in vitro propagated metacyclic forms derived from tsetse salivary glands produce glycerol from glucose under anaerobic conditions (Njogu and Nyindo, 1981), although all the stages had significant glycerol kinase activity.

The production of pyruvate, glycerol, and glycerol3-phosphate by digitonin-permeabilized bloodstream forms of T.b.brucei was studied with glucose or glycolytic intermediates as substrates.
Under aerobic conditions hexose phosphates gave maximum glycolysis in the presence of 40-60 micrograms of digitonin/10^8 trypanosomes while the triosephosphates gave it at 20-30 micrograms of digitonin/10^8 trypanosomes. In the presence of salicylhydroxamic acid (SHAM) and glycolytic intermediates, permeabilized trypanosomes produced equimolar amounts of pyruvate

and Gro3P but glucose catabolism under the same conditions produced equimolar amounts of pyruvate and glycerol plus Gro3P It is proposed that glycerol production from glucose in intact trypanosomes is regulated by ATP/ADP ratios and that ATP and ADP have a carrier at the glycosomal membrane. Glycerol kinase in digitonin permeabilized **T.b.brucei** catalysed ATP production at fairly high concentrations of Gro3P and ADP. The $S_{0.5}$ for Gro3P in permeabilized trypanosomes was 33.2 $\pm$ 5.8 mM. Pyruvate production from glucose in the presence of SHAM was significantly inhibited by more than 10 mM Gro3P indicating that high concentrations of Gro3P may also inhibit some glycolytic reaction(s). It was observed that high concentrations of Gro3P inhibited the Gro3P dehydrogenase. The apparent Ki for unpurified Gro3P dehydrogenase in the presence of GrnP was 29.5 mM. It was therefore concluded that the concentrations of Gro3P that can effectively transphosphrylate ADP also inhibit the reoxidation of NADH, thus inhibiting glycolysis. Aerobically, intact bloodstream trypanosomes catabolised glucose, fructose, mannose and glycerone to pyruvate. The rates of pyruvate production in micromoles/h/10^8 trypanosomes were: glucose, 6.0., fructose, 6.8., mannose, 5.0., and glycerone, 1.3. In the presence of SHAM, pyruvate production was reduced by

half, and equimolar amounts of pyruvate and glycerol were produced from glucose and mannose. SHAM caused about 85% and 100% decrease of pyruvate production from fructose and glycerone respectively, although equimolar amounts of pyruvate and glycerol were produced from fructose. In addition, it caused 50-70% decrease of glycolytic intermediates and 40-50% decrease of ATP concentration from glucose catabolism, whereas it caused 70-90% decrease of ATP production from fructose catabolism. It also caused 80% decrease of triosephosphates level and 81% decrease of the already low level of ATP from glycerone catabolism. There was a 2 to 2.5, 4 to 5.5 and 2.5 to 4 fold increase in the level of ADP, AMP and Gro3P respectively from glucose catabolism after the addition of SHAM. The level of Gro3P from fructose and mannose catabolism was in the same range as from glucose. Gro3P production from glycerone in the presence of SHAM was about half of that from glucose. Gro3P concentration decreased rapidly in trypanosomes pre-incubated with fructose for 20-30 min or with glycerone for 5 min after the addition of glucose or mannose but not D-galactose or 2-deoxy D-glucose. There was a slight decrease in Gro3P level in trypanosome pre-incubated with SHAM and glycerone for 5 minutes after the addition of fructose. It is proposed that Gro3P transphosphory-

lates with a hexose under the catalysis of the novel enzyme, Gro3P : glucose phosphotransferase. Pyruvate production under aerobic conditions from glucose, fructose and mannose was inhibited 80-90% by 10 micrograms oligomycin/10^8 trypanosomes but was not affected from glycerol catabolism. This inhibition lead to a rapid initial accumulation of Glc6P, Fru6P, Fru1,$6P_2$, GrnP, Gra3P, Gro3P, ADP and AMP but a decrease in Gri2P, PEPyr and ATP from glucose but not from glycerol catabolism.

Exogenous ATP or ADP did not alleviate oligomycin inhibited glycolysis. In addition oligomycin did not inhibit pyruvate production from glycolytic intermediates in digitonin-permeabilized trypanosomes. Increase in some glycolytic intermediates after the addition of oligomycin could be also obtained by addition of a combination of SHAM, glycerol, and ATP to the trypanosomes. These results are discussed.