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The mechanism of Trypanolysin mediated lysis of  
*Trypanosoma brucei brucei*  
(Kinetoplastida: Trypanomastidae)

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## ABSTRACT

Previous studies have identified trypanolysin as a bloodmeal induced protein from the midgut of tsetse fly (*Glossina spp.*), with a non-denaturing (NATIVE) molecular weight of ~669 kDa and two subunit molecular weights of 55 kDa and 29 kDa under denaturing conditions. Trypanolysin was shown to lyse only bloodstream-form but not procyclic-form trypanosomes, *Leishmania major* and the cellular stage of *Plasmodium falciparum*. The mechanism of trypanolysin-mediated lysis of trypanosomes was elucidated in this study by assessing its binding properties on bloodstream-form *T. b. brucei*. The lytic activity of trypanolysin was inhibited by specific chemical modifiers; N-acetylimidazole and Diethylpyrocarbonate suggesting that histidine and tyrosine residues may be essential for its biological activity.

Furthermore, purified trypanolysin was resolved on a non-denaturing PAGE, transferred onto nitrocellulose paper and its ability to bind to biotinylated *T. b. brucei* Variant surface glycoprotein (VSG) assessed by staining with streptavidin horseradish peroxidase. Binding of trypanolysin to VSG was ascertained further using gel retardation assays on NATIVE Polyacrylamide Gel Electrophoresis (PAGE). The ability of trypanolysin to bind to VSG in both cases suggests that the VSG is the recognition site for trypanolysin and this may be the vital initial step in the lysis of bloodstream-form trypanosomes.