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Lipidomics analysis of acetyl-CoA-depleted *Trypanosoma brucei*

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ABSTRACT

T. brucei is a kinetoplastid parasite that causes sleeping sickness ("nagana") in cattle. Both the mammalian-host bloodstream (BF) and insect-host procyclic (PF) forms of *T. brucei* utilize complex fatty acid elongation mechanism. BF *T. brucei* uses this elongation mechanism to generate multiple long-chained fatty acid species, including myristate (C14:0), which is the primary fatty acid for GPI anchoring of the antigenic molecule variable surface glycoprotein (VSG). This fatty acid elongation mechanism requires a two-carbon donor primer, which is supplied by the carboxylation of acetyl-CoA by Acetyl-CoA Carboxylase (ACC). Our lab generated a knockdown cell line that reduces ACC through RNA interference (RNAi). This cell line has no phenotypic difference *in vitro* compared to the parental 90-13 strain, suggesting that the ACC RNAi cell line have a mechanism to compensate for the lack of fatty acid elongation. However, ACC RNAi cell line shows reduced virulence in mouse model. To further examine how *T. brucei* responds to the loss of ACC, we are performing global lipidomics and proteomics analyses. Our preliminary data suggested that there was no change in the free fatty acid level for myristate. However, the data suggested changes in other lipid species. Further investigation in both lipidomics and proteomics will give better understanding the lipid homeostasis in bloodstream *T. brucei*.

BACKGROUND

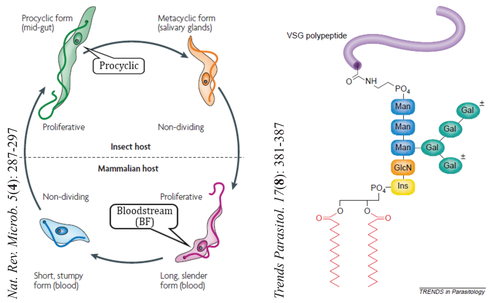


Figure 1. Left: diagram of T. brucei's life cycle. Right: diagram of BF *T. brucei*'s variant surface glycoprotein (VSG) with GPI anchoring.

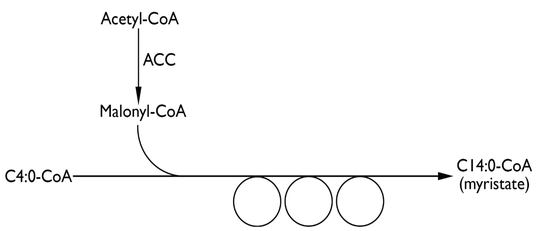


Figure 2. Simplified diagram of *de novo* myristate synthesis through the elongation (ELO) pathway in BF *T. brucei*.

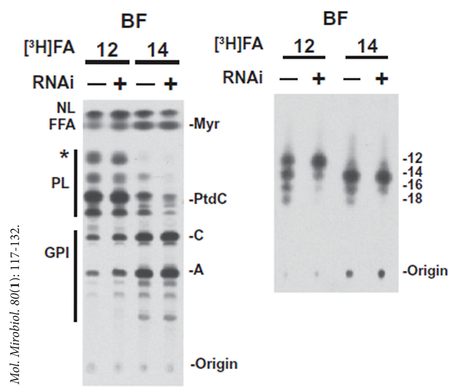
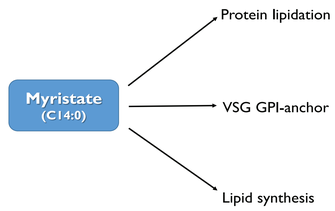


Figure 3. Thin Layer Chromatography (TLC) of bulk fatty acid and lipidated species in BF *T. brucei*. Picture is modified from the original.

OBJECTIVE / MAIN QUESTION

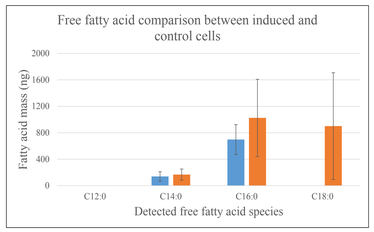
What is the effect of ACC reduction on:

- VSG GPI anchoring
- Protein lipidation (besides GPI anchoring)
- Free myristate (C14:0) pool
- Other lipid species' pool



CURRENT WORK / RESULTS

1. Myristate (C14:0) pool did NOT significantly change under ACC RNAi condition.



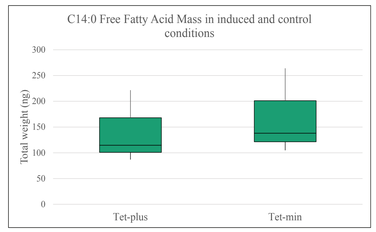


Figure 4. Lipidomics of medium-chain fatty acids of ACC RNAi cells and control cells. Cells were induced for 10 days with tetracycline. 6 x 10^6 cells were chemically lyzed after induction. Free fatty acids were converted into methyl esters, extracted using organic-phase extraction, and and analyzed with Gas Chromatography - Flame Ionization Detector (GC-FID) analysis. n = 3. Left: overall C12:0 - 18:0 free fatty acid pool. Right: box-and-whisker plot for C14:0, the primary FA utilized in BF *T. brucei*. Tet-plus: tetracycline-induced cells; tet-min: control cells.

2. ACC RNAi changed the lipidome of *T. brucei*

Lipid Species	%Up/ Down	StdDev
Cer(d20:1/16:0)	23.03	18.29
Co(Q8)	20.11	6.70
DG(18:0/20:4)	-42.94	13.30
dMePE(19:0/18:1)	-14.84	7.80
LPC(20:4)	-50.20	36.65
LPC(36:3)	11.70	6.64
PE(16:0p/18:2)	-14.98	8.02
PE(18:0/18:1)	-24.99	12.15
PE(18:0p/18:2)	-16.19	0.56
TG(14:0/14:0/20:4)	-60.52	8.15
TG(14:0/14:0/22:6)	-50.85	18.09
TG(14:0/18:2/20:4)	-56.48	10.68
TG(18:2/18:2/20:4)	-57.15	3.81
TG(22:4/18:2/22:6)	-40.07	11.20
TG(22:5/18:2/22:6)	-53.53	9.23
TG(22:5/20:4/22:6)	-59.27	10.12

Table 1. The lipid species that were significantly regulated under ACC RNAi condition ($p < 0.05$). ACC RNAi cells were induced for 10 days. 1 x 10^8 cells were harvested, then mechanically lyzed. The cellular lipids were extracted using organic-phase extraction. The organic phase was analyzed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Data was analyzed using ThermoScientific LipidSearch software. n = 2. Up: upregulated from control cells. Down: donwregulated from control cells. StdDev: standard deviation from %Up/Down.

Some abbreviations for the lipid species on Table 1 (and within the poster):

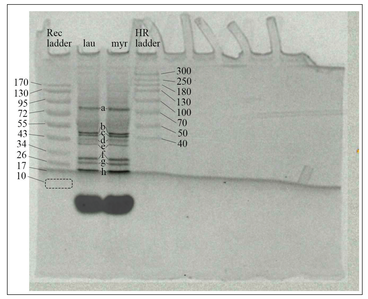
ACC: Acetyl-CoA Carboxylase
Cer: Ceramide
Co: Coenzyme
DG: Diglyceride
dMePE: Dimethylphosphatidylethanolamine
GPI: Glycosylphosphatidylinositol
LPC: Lysophosphatidylcholine
PE: Phosphatidylethanolamine
TG: Triglyceride

DISCUSSIONS

- ACC RNAi cells could support myristate levels.
- Significant ($p < .05$) neutral lipids are downregulated.
- ACC RNAi somehow affect sphingolipid synthesis.

FUTURE WORK

1. Perform initial proteomics using autoradiography labeling.
2. Perform proteomics for myristoylated proteins using CuAAC click chemistry.



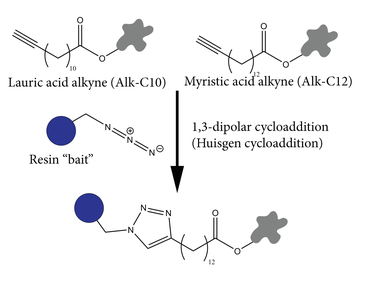


Figure 6. Left: radiolabeled gel example. BF Lister 427 were incubated with radiolabeled [^{14}C]-C12:0 (lau) and [^{14}C]-C14:0 for 36 hours. Cells were harvested and lyzed chemically. Whole cell lysate for both lau and myr were loaded into 10% SDS-acrylamide gel. Gel were exposed using autoradiography film for 10 days. Ladder were overlayed with autoradiography film. Right: proposed scheme for proteomics identification.

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