Nitrogen Metabolism in *Haemonchus contortus* and *Teladorsagia circumcincta*

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Abstract

This is the first study to characterise proline, arginine and lysine metabolism in homogenates of L3 and adult *Haemonchus contortus* and *Teladorsagia circumcincta*. The properties of glutamate dehydrogenase (GDH), glutamate synthase and the GABA shunt were also compared in the two species. The kinetic properties of 26 enzymes were determined. The gene encoding *T. circumcincta* GDH was sequenced and recombinant TcGDH expressed and biochemically characterised.

The ornithine-glutamate-proline pathway was fully functional. The mammalian α-AAA (saccharopine) and pipecolate pathways of lysine catabolism, but not the bacterial enzymes lysine dehydrogenase and decarboxylase, were present in adult worms. The pipecolate pathway was incomplete in L3 of both species, as Pip2CR activity was undetectable. Unusually, lysine ketoglutarate reductase and saccharopine dehydrogenase, Δ^1-pyrroline-5-carboxylate synthase and reductase were able to use both co-factors. The glutamine synthetase-glutamate synthase pathway of ammonia incorporation into glutamate was present, except in L3 *H. contortus*. *T. circumcincta* GDH was cloned, purified and characterised and the predicted protein sequence was very similar to *H. contortus* GDH. *T. circumcincta* recombinant and *H. contortus* homogenate GDH were both dual co-factor specific, although the latter had 50% greater activity with NAD^+/H as co-factor. GDH activity was inhibited by GTP and stimulated by ADP whereas ATP either inhibited or stimulated depending on the concentration and direction of the reaction. The GABA shunt enzymes glutamate decarboxylase and succinic semialdehyde dehydrogenase was not detected in homogenates of whole L3 or adult *H. contortus* or *T. circumcincta*.

Neither parasite had a full functional ornithine urea cycle, nor appeared to use bacterial pathways to covert arginine to ornithine. NOS

were demonstrated histochemically in nerves of adult *H. contortus*, but was undetectable in homogenates of both species. There was species variation in polyamine metabolism: *T. circumcincta* used arginase to form ornithine, followed by decarboxylation by ODC, while in *H. contortus* there was the additional pathway of first decarboxylation by ADC to form agmatine, then hydrolysis by agmatinase to putrescine. The present study helped in the better understanding of nitrogen metabolism and these enzymes can be useful targets if they differ antigenically from the host, provided the enzyme is accessible to blockage by immune effectors.

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A. aegypti	*Aedes aegypti*
A. caninum	*Ancylostoma caninum*
A. galli	*Ascaridia galli*
A. lumbricoides	*Ascaris lumbricoides*
A. suum	*Ascaris suum*
ADC	arginine decarboxylase
ADP	adenosine diphosphate
ADI	arginine deiminase
AGAT	arginine:glycine amidinotransferase
AK	arginine kinase
ASL	argininosuccinate lyase
ASS	argininosuccinate synthetase
ATP	adenosine triphosphate
BH_4	tetrahydrobiopterin
B. malayi	*Brugia malayi*
B. mori	*Bombyx mori*
B. patei	*Brigia patei*
bp	base pair
cDNA	complementary deoxyribonucleic acid
C. briggsae	*Caenorhabditis briggsae*
C. elegans	*Caenorhabditis elegans*
CK	creatine kinase
cNOS	constitutive nitric oxide synthase
CP	carbamoyl phosphate
CPS	carbamoyl phosphate synthetase
D. immitis	*Dirofilaria immitis*
D. melanogaster	*Drosophila melanogaster*
DFMO	difluoromethylornithine
E. coli	*Escherichia coli*
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide

F. gigantica	*Fasciola gigantica*
F. hepatica	*Fasciola hepatica*
g	gram
g	gravitational force
GABA	gamma aminobutyric acid
GABA-T	gamma aminobutyrate transferase
GAD	glutamate decerboxylase
GDH	glutamate dehydrogenase
G. intestinalis	*Giardia intestanalis*
GOGAT	glutamate synthase
GS	glutamate synthetase
GST	glutathione S-transferases
GTP	guanosine triphosphate
h	hour
H. contortus	*Haemonchus contortus*
H. diminuta	*Hymenolepis diminuta*
H. polygyrus	*Heligmosomoides polygyrus*
H. pylori	*Helicobacter pylori*
iNOS	inducible nitric oxide synthetase
IPTG	isopropyl-1-thio-β-D-galactopyranoside
kDa	kilodalton
LDC	lysine decarboxylase
LDH	lysine dehydrogenase
LKR	lysine ketoglutarate reductase
LO	lysine oxidase
L3	third stage larva
L4	fourth stage larva
M	molar
M. expansa	*Moniezia expansa*
mg	milligram
mGDH	mitochondrial glutamate dehydrogenase
min	minute
ml	millilitre
mM	millimolar
mRNA	messenger ribonucleic acid

n	number
N. brasiliensis	*Nippostrongylus brasiliensis*
NAD^+	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NADPH-d	reduced nicotinamide adenine dinucleotide phosphate diaphroase
nmole	nanomole
NOS	nitric oxide synthase
nNOS	neuronal nitric oxide synthase
O. volvulus	*Onchocerca volvulus*
OAA	oxaloacetic acid
OAT	ornithine aminotransferase
ODC	ornithine decarboxylase
OTC	ornithine transcarbamylase
OUC	ornithine urea cycle
PAGE	polyacrelamide gel electrophrosis
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PheH	phenylalanine hydroxylase
PipO	piperideine oxidase
PLP	pyridoxal 5'-phosphate
PO	proline oxidase
P. redivivus	*Panagrellus redivivus*
P5C	pyrroline-5-carboxylate
P5CDH	pyrroline-5-carboxylate dehydrogenase
P5CR	pyrroline-5-carboxylate reductase
P5CS	pyrroline-5-carboxylate synthase
RNA	ribonucleic acid
RO	reverse osmosis
SDH	saccharopine dehydrogenase
SDS	sodium duodecyl sulphate

S. mansoni	*Schistosoma mansoni*
SOX	sarcosine oxidase
SSA	succinic semialdehyde
SSADH	succinic semialdehyde dehydrogenase
T. circumcincta	*Teladorsagia circumcincta*
TLC	thin layer chromatography
μCi	microcurie
μg	microgram
μl	microlitre
2-OG	2-oxoglutarate