Nitrogen Metabolism in *Ostertagia (Teladorsagia) circumcincta*

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Chapter 4 Metabolism of Alanine and Aspartate in *Ostertagia circumcincta*

Abstract

The aim of the experiments was to investigate some key areas of nitrogen metabolism in adult and third-stage larval *Ostertagia (Teladorsagia) circumcincta*, to seek enzymes either not present in mammals or with distinctive kinetic properties, which clearly differentiated the nematode and host metabolic systems. The study encompassed excretion and uptake in intact worms and determining the kinetic properties of eleven enzymes involved in the metabolism of arginine, urea, alanine aspartate and glutamate.

The metabolism of *O. circumcincta* was different from that in mammals and more like that of microorganisms and plants. Ammonia was the main excretory product, with a little urea, both apparently crossing the cuticle through specific permeases. The excretion rate increased with temperature, but decreased as the external ammonia concentration increased, suggesting that ammonia may be a source of nitrogen additional to amino acids, which were taken up by adult worms. Ammonia could be incorporated directly into glutamate and other amino acids through the glutamine synthetase-glutamate synthase pathway, which was more active in adult worms. Glutamate dehydrogenase was able to use either NADH or NADP in the deaminating direction, which would be the predominant direction because of the low affinity of GDH for ammonia. In the aminating direction, there was greater activity with NADH than NADPH.

Creatinase and arginase were probably the sources of excreted urea. There was no urease activity to convert urea to ammonia. No role could be assigned to creatinase other than to degrade host creatine, perhaps to supply sarcosine for metabolism. The unusual feature of aspartate metabolism was aspartase activity in addition to aspartate aminotransferase, which, in larvae, had the highest activity of all enzymes studied. In adult worms, which are

believed to have a more anaerobic metabolism than larvae, aspartase would allow aspartate to be formed directly from fumarate in association with only a partial TCA cycle.

Perhaps the most important finding was the identification in the parasites of three enzymes, creatinase, aspartase and glutamate synthase, which are not believed to be expressed in the sheep host or other mammals, making them possible candidates for developing novel anthelmintic therapies.

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"The man who removes a mountain begins by carrying away small stones"

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2D	two dimensional
A. aegypti	*Aedes aegypti*
A. galli	*Ascaridia galli*
A. lumbricoides	*Ascaris lumbricoides*
A. marina	*Arenicola marina*
A. suum	*Ascaris suum*
aa	amino acids
ADC	arginine decarboxylase
AGAT	arginine-glycine amidinotransferase
AK	arginine kinase
AlaAT	alanine aminotransferase
AMP	adenosine monophosphate
APC	acid-polyamine-choline
AS	asparagine synthetase
AspAT	aspartate aminotransferase
ATF1	amino acid transporter superfamily 1
B. malayi	*Brugia malayi*
B. mori	*Bombyx mori*
B. pahangi	*Brugia pahangi*
BCAT	branched chain aminotransferases
C. briggsae	*Caenorhabditis briggsae*
C. elegans	*Caenorhabditis elegans*
C. emasculans	*Cercaria emasculans*
C. lingua	*Cryptocotyle lingua*
C. oncophera	*Cooperia oncophera*
cAlaAT	cytosolic alanine aminotransferase

cAspAT	cytosolic aspartate aminotransferase
CCBL	cysteine *S*-conjugate β-lyase
cGDH	cytosolic glutamate dehydrogenase
Ci	curie
CK	creatine kinase
cNOS	constitutive nitric oxide synthase
CoA	Coenzyme A
CPS	carbamoyl phosphate synthetase
D. immitis	*Dirofilaria immitis*
D. melanogaster	*Drosophila melanogaster*
D. polymorpha	*Dreissena polymorpha*
DFMO	difluoromethylornithine
DNA	deoxyribonucleic acid
dpm	disintegrations per minute
E. coli	*Escherichia coli*
EDTA	Ethylene diamine tetra acetic acid
e.p.g.	eggs per gram
ES	excretory/secretory
Expt	experiment
F. hepatica	*Fasciola hepatica*
Fd	ferrodoxin
g	gram
g	gravitational force
G. intestinalis	*Giardia intestinalis*
G. lamblia	*Giardia lamblia*
GABA	γ-aminobutyric acid
GABA-T	4-aminobutyrate:2-oxoglutarate aminotransferase
GAMT	*S*-adenosyl-L-methionine:N-guanidinoacetate methyltransferase

GDH	glutamate dehydrogenase
GOGAT	glutamate synthase
GS	glutamine synthetase
GSH	glutathione
GST	glutathione *S*-transferase
GTP	guanosine triphosphate
h	hour
H. alvei	*Hafnia alvei*
H. citelli	*Hymenolepis citelli*
H. contortus	*Haemonchus contortus*
H. diminuta	*Hymenolepis diminuta*
H. nana	*Hymenolepis nana*
H. polygyrus	*Heligmosomoides polygyrus*
H. pylori	*Helicobacter pylori*
IMP	inosine monophosphate
iNOS	inducible nitric oxide synthase
kg	kilogram
L. carinii	*Litomosoides carinii*
L3	third stage larva
L4	fourth stage larva
LASPO	L-aspartate oxidase
M	molar
M. expansa	*Moniezia expansa*
M. similis	*Microphallus similis*
mAlaAT	mitochondrial alanine aminotransferase
mAspAT	mitochondrial aspartate aminotransferase
mCi	millicurie
MDH	mitochondrial malate dehydrogenase

MFS	major facilitator superfamily
mg	milligram
mGDH	mitochondrial glutamate dehydrogenase
min	minute
ml	millilitre
mM	millimollar
MW	molecular weight
n	number
N. americanus	*Necator americanus*
N. brasiliensis	*Nippostrongylus brasiliensis*
NAD^+	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NAG	N-acetylglutamate
nl	nanolitre
nm	nanometre
nmole	nanomole
NOS	nitric oxide synthase
O. circumcincta	*Ostertagia circumcincta*
O. cuniculi	*Obeliscoides cuniculi*
O. volvulus	*Onchocerca volvulus*
OAA	oxaloacetate
OAT	ornithine aminotransferase
ODC	ornithine decarboxylase
OTCase	ornithine transcarbamylase
OUC	Ornithine-Urea Cycle
p	probability

P. crassipalpis	*Parasarcophaga crassipalpis*
P. freudenreichii	*Propionibacterium freudenreichii*
P. islandicum	*Pyrobaculum islandicum*
P. pacifica	*Pista pacifica*
P. putida	*Pseudomonas putida.*
P. redivivus	*Panagrellus redivivus*
P5C	Δ^1-pyrroline-5-carboxylic acid
P5CDH	pyrroline-5-carboxylate dehydrogenase
P5CR	pyrroline-5-carboxylate reductase
P5CS	pyrroline-5-carboxylate synthase
RO	reverse osmosis
PBS	phosphate buffer saline
PC	pyruvate carboxylase
PEP	Phosphoenolpyruvate
PEPCK	phosphoenolpyruvate carboxykinase
PLP	pyridoxal 5'-phosphate
PMP	pyridoxamine 5'-phosphate
POT	proton oligopeptide transporter
RNA	ribonucleic acid
S. sclerotiorum	*Sclerotinia* sclerotiorum
S. bibionis	*Steinernema bibionis*
S. cerevisiae	*Saccharomyces cerevisiae*
S. cynthia ricini	*Samia cynthia ricini*
S. frugiperda	*Spodoptera frugiperda*
S. japonicum	*Schistosoma japonicum*
S. mansoni	*Schistosoma mansoni*
S. solida	*Semele solida*
S. typhimurium	*Salmonella typhimurium*

SAMdc	*S*-adenosyl methionine decarboxylase
SDS	sodium-dicarboxylate symporters
SEM	standard error of the mean
SL	sheathed larva
T. colubriformis	*Trichostrongylus colubriformis*
T. cruzi	*Trypanosoma cruzi*
T. spiralis	*Trichinella spiralis*
TCA	tricarboxylic acid
µCi	microcurie
µg	microgram
µl	microlitre