Experimental Parasitology 127 (2011) 9–13

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Glutamate synthase, but not GABA shunt enzymes, contributes to nitrogen metabolism of the sheep abomasal nematode parasites *Haemonchus contortus* and *Teladorsagia circumcincta*

S. Umair [a], R.J. Bland [b], H.V. Simpson [a,*]

[a] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11-222, Palmerston North 4442, New Zealand
[b] AgResearch Ltd., Private Bag 11-008, Palmerston North, New Zealand

ARTICLE INFO

Article history:
Received 11 March 2010
Received in revised form 22 April 2010
Accepted 25 May 2010
Available online 1 June 2010

Keywords:
Haemonchus contortus
Teladorsagia (Ostertagia) circumcincta
Glutamate synthase (E.C. 1.4.1.14)
Glutamate decarboxylase (E.C. 4.1.1.15)
Succinic semialdehyde dehydrogenase (E.C. 1.2.1.24)
Ammonia assimilation

ABSTRACT

Glutamate synthase (E.C. 1.4.1.14) (GOGAT) activity was not detectable in L3 *Haemonchus contortus*, but was present in L3 *Teladorsagia circumcincta* and adult worms of both species. GOGAT activity was inhibited by 80% by azaserine. Activity (nmol min^{-1} mg^{-1} protein) was 33–59 in adult *H. contortus*, 51–91 in adult *T. circumcincta* and 24–41 in L3 *T. circumcincta*, probably depending on exposure to ammonia, as incubation with 1 mM NH_4Cl doubled GOGAT activity. The pH optimum was 7.5 in both species. Either NAD or NADP acted as co-factor. The mean apparent K_m for 2-oxoglutarate was 0.7 (0.5–0.9) mM and for glutamine was 1.0 (0.5–1.7) mM for different homogenates. There was no detectable activity in whole parasite homogenates of glutamate decarboxylase (E.C. 4.1.1.15) or succinic semialdehyde dehydrogenase (E.C. 1.2.1.24), the first and third enzymes of the GABA shunt, respectively, suggesting that the GABA shunt is not important in general metabolism in these species.

1. Introduction

Parasitic nematode larvae grow rapidly and adult worms lay large numbers of eggs, both requiring very active nitrogen and energy metabolism. Worm enzymes, particularly those not present in the host, are therefore potential targets for controlling the parasites through new therapies, either chemical or immunological. As glutamate plays a central role in nitrogen metabolism, being converted to other amino acids and in both in excretion of ammonia and its incorporation in lower organisms, some enzymes associated with glutamate metabolism are of particular interest. Two aspects which could provide opportunities for the control of parasitic nematodes are their use of the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway to synthesise amino acids from ammonium and possible use of the GABA shunt to bypass a part of the tricarboxylic acid cycle (TCA) which has low activity in anaerobic conditions.

In a recent study of nitrogen excretion by *Teladorsagia circumcincta* in vitro, there appeared to be re-uptake of excreted ammonia during several hours of incubation (Simpson et al., 2009). It was suggested that the disappearance of the ammonia from the medium was caused by its incorporation into glutamate by the GS-GOGAT pathway. Whereas GS is universally present, GOGAT is usually absent in animals, but is expressed in some nematodes and insects, particularly the silkworms *Bombyx mori* (Hirayama et al., 1998) and *Samia cynthia ricini* (Osanai et al., 2000), the mosquito *Aedes aegypti* (Scaraffia et al., 2005) and in the *Spodoptera frugiperda* Sf9 insect cell line (Doverskog et al., 2000). GOGAT activity has been demonstrated in homogenates of *T. circumcincta* by Muhamad et al. (2005) and gene sequences for GOGAT from *Caenorhabditis elegans* (Vanoni and Curti, 1999), *Caenorhabditis remanei*, *Caenorhabditis briggsae* and *Brugia malayi* have been deposited in databases.

The GS-GOGAT pathway is important for ammonia assimilation in bacteria and plants (Miflin and Habash, 2002; Magasanik, 2003) when the ammonia concentration is low and ATP is available (Helling, 1994, 2002), whereas there is a low affinity for ammonium for the alternative enzyme glutamate dehydrogenase (GDH), including the *T. circumcincta* enzyme with a K_m of 18 mM (Muhamad et al., 2004). There are three forms of GOGAT according to the co-factor requirements: NADH-GOGAT and NADPH-GOGAT are present in plants and bacteria, whilst Fd-GOGAT is the form in photosynthetic organisms (Vanoni and Curti, 1999). In the present experiments, the activity and properties of GOGAT have been investigated in *Haemonchus contortus* and *T. circumcincta*, the two important abomasal nematode parasites of small ruminants.

* Corresponding author. Fax: +64 6 350 5636.
E-mail address: H.V.Simpson@massey.ac.nz (H.V. Simpson).

0014-4894/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2010.05.023

The GABA (gamma-amino butyric acid) shunt has been reported to play a role in nitrogen metabolism in addition to the role of GABA as neurotransmitter in nematodes (Schuske et al., 2004). The GABA shunt is a three enzyme pathway that forms succinate from glutamate via GABA, bypassing the part of the TCA cycle (Balazs et al., 1970) in which α-ketoglutarate is oxidatively decarboxylated to succinate by α-ketoglutarate dehydrogenase. First, L-glutamate is decarboxylated irreversibly to GABA by glutamate decarboxylase (GAD), then GABA is converted reversibly to succinic semialdehyde (SSA) by 4-aminobutyrate transaminase (GABA-T) and finally succinic semialdehyde dehydrogenase (SSADH) irreversibly converts SSA to succinate. The GABA shunt operates in plants (Shelp et al., 1999) and in bacteria during anaerobic conditions (Dunn, 1998) and may be important in the larger parasitic helminths, especially those in the more anaerobic environments.

GAD and GABA-T activities have been observed in *Ascaris lumbricoides*, *Ascaridia galli*, *Taenia solium*, *Macracanthrynchus hyrudinaceus* and *Moniezia expansa* (Monteoliva et al., 1965; Rasero et al., 1968). GAD has been located in the *A. lumbricoides* reproductive tract and intestine (Monteoliva et al., 1965) and the *A. galli* cuticle (Singh et al., 1983). GABA-T has been partially purified and characterised in *Nippostrongylus brasiliensis* (Watts and Atkins, 1983, 1984) and activity reported in this species by Walker and Barrett (1991). Evidence for a metabolic role for this pathway in helminths is conflicting, being supported by Monteoliva et al. (1965) and Rasero et al. (1968) for three species of intestinal parasites and in *Onchocerca volvulus* and *Brugia pahangi* by MacKenzie et al. (1989), but not by Cornish and Bryant (1975) for *M. expansa*. As there appear to be no studies in ruminant parasitic nematodes, the activities of GABA shunt enzymes and a possible metabolic function has been investigated in these nematodes.

2. Materials and methods

All chemicals were purchased from Sigma Chemical Co. (MO, USA) unless stated otherwise.

2.1. Parasites

L3 *T. circumcincta* or *H. contortus* were cultured from the faeces of sheep infected with a pure strain of parasite and stored in reverse osmosis (RO) water at 4 °C for the former and at 10 °C for the latter species. Fresh larvae were used or prior to each experiment, L3 were baermannised in RO water to remove inactive worms and re-suspended in buffer. Adult worms were recovered from the abomasa of infected sheep using the technique of Simpson et al. (1999). Briefly, abomasal contents were mixed 2:1 with 3% agar and, after solidification, the agar blocks were incubated at 37 °C in a saline bath. Clumps of parasites were removed from the saline soon after emergence and placed in buffer.

2.2. Preparation of homogenates

About 50,000 L3 were centrifuged at 600*g* for 5 min, washed twice by re-suspending in assay buffer followed by centrifugation, then finally re-suspended in 1 ml buffer. The concentrated L3 suspension was transferred to a chilled mortar and frozen at −20 °C for at least 15 min. The frozen pellet was then disrupted using a chilled pestle and the homogenate was examined microscopically to ensure complete disruption of the parasites. Homogenates were similarly prepared using approximately 5 mg of clumped adult worms. Finely chopped sheep brain was homogenised in ice-cold 320 mM sucrose and 4.5 mM mercaptoethanol, according to the method of Hao and Schmit (1991). When homogenates were to be used for more than one assay, aliquots were kept in Eppendorff tubes on ice until required. The protein concentrations of homogenates were determined by the Bradford method (1976).

2.3. Enzyme assays

2.3.1. Glutamate synthase (GOGAT) (E.C. 1.4.1.14)

GOGAT activity was determined at 30 °C from the rate of conversion of NADH to NAD^+ during the reaction of glutamine, NADH and 2-oxoglutarate to form 2 glutamate and NAD^+. The reaction was monitored spectrophotometrically at 340 nm using an Ultraspec III (Pharmacia LKB) equipped with a temperature control unit.

(1) The presence of enzyme activity was determined in homogenates of L3 ($n = 2$) and adult ($n = 2$) *T. circumcincta* and L3 ($n = 3$) and adult ($n = 2$) *H. contortus*. The reaction mixture (total volume of 1 ml) contained 2.5 mM glutamine,10 mM 2-oxoglutarate and 50 μg homogenate protein in 100 mM phosphate buffer, pH 7.5; the reaction was initiated by the addition 0.2 mM NADH.
(2) The apparent K_m for glutamine was determined in homogenates of *T. circumcincta* L3 and adult worms and adult *H. contortus* ($n = 2$). The substrate concentrations were 10 mM 2-oxoglutarate, 0.2 mM NADH and glutamine varying from 0 to 30 mM.
(3) The apparent K_m for 2-oxoglutarate was determined in homogenates of L3 and adult *T. circumcincta* and adult *H. contortus* ($n = 2$). The substrate concentrations were 10 mM glutamine, 0.2 mM NADH and 2-oxoglutarate varying from 0 to 30 mM.
(4) Activity with NADPH as co-factor was determined in homogenates of L3 and adult *T. circumcincta* and *H. contortus* ($n = 2$). The substrate concentrations were 10 mM glutamine, 10 mM 2-oxoglutarate and 0.2 mM NADPH.
(5) The effect of azaserine was determined in homogenates of *T. circumcincta* L3 and adult worms and adult *H. contortus* ($n = 2$). Two millimolar azaserine was included in the reaction mixture with 10 mM glutamine and 10 mM 2-oxoglutarate; the reaction was initiated by the addition 0.2 mM NADH.
(6) The effect of pre-incubation of L3 with NH_4Cl was determined in L3 *T. circumcincta* and *H. contortus* ($n = 3$). Approximately 50,000 L3 were suspended in 10–15 ml 50 mM phosphate buffer, pH 7.5, containing either 0, 0.5 or 1 mM NH_4Cl. The tubes were incubated at 37 °C overnight. Homogenates were made and GOGAT activity assayed with substrate concentrations of 10 mM glutamine, 10 mM 2-oxoglutarate and 0.2 mM NADH.

2.3.2. Glutamate decarboxylase (GAD) (E.C. 4.1.1.15)

GAD activity was assayed at in homogenates of L3 ($n = 3$) and adult ($n = 2$) *T. circumcincta*, L3 ($n = 3$) and adult ($n = 2$) *H. contortus* and in sheep brain ($n = 2$) as a positive control. Enzyme activity was determined by measuring the amount of $^{14}CO_2$ released from L-[1-14C]glutamic acid (Hao and Schmit, 1991). The reaction mixture (total volume 200 μl) was prepared in 50 mM Tris–HCl, pH 5.5, containing 0.9 mM PLP, 0.9 mM EDTA, 0.9 mM 2-mercaptoethanol, 45 mM sodium piperazine-*N*,*N*′-bis (2-ethanosulphonic acid) and 50 μg homogenate, in 2 ml disposable plastic tubes with an airtight lid. A small plastic tube containing filter paper dipped in 250 μM KOH was placed in the larger tube for absorption of $^{14}CO_2$. The reaction was started by the addition of 30 mM glutamic acid and 0.055 μCi of L-[1-14C]glutamic acid (GE Healthcare Amersham, UK). After 60 min incubation in a shaking bath at 37 °C, the reaction was stopped by the addition of 200 μl of 2 N H_2SO_4. The CO_2 was allowed to be absorbed for 2 h, mixed with cocktail (Optiphase super mix, Wallac Scintillation Products, Loughborough, UK) and