



The kinetic properties of the glutamate dehydrogenase of *Teladorsagia circumcincta* and their significance for the lifestyle of the parasite

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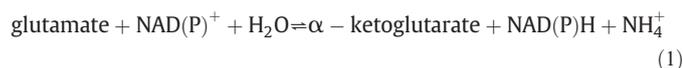
ABSTRACT

Like other nematodes, both L₃ and adult *Teladorsagia circumcincta* secrete or excrete NH₃/NH₄⁺, but the reactions involved in the production are unclear. Glutamate dehydrogenase is a significant source NH₃/NH₄⁺ in some species, but previous reports indicate that the enzyme is absent from L₃ *Haemonchus contortus*. We show that glutamate dehydrogenase was active in both L₃ and adult *T. circumcincta*. The apparent K_ms of the L₃ enzyme differed from those of the adult enzyme, the most significant of these being the increase in the K_m for NH₄⁺ from 18 mM in L₃ to 49 mM in adults. The apparent V_{max} of the oxidative deamination reaction was greater than that of the reductive reaction in L₃, but this was reversed in adults. The activity of the oxidative reaction of the L₃ enzyme was not affected by adenine nucleotides, but that of the reductive reaction was stimulated significantly by either ADP or ATP. The L₃ enzyme was more active with NAD⁺ than it was with NADP⁺, although the activities supported by NADH and NADPH were similar at saturating concentrations. While the activity of the oxidative reaction was sufficient to account for the NH₃/NH₄⁺ efflux we have previously reported, the reductive amination reaction was likely to be more active.

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1. Introduction

Both L₃ and adult *T. circumcincta* secrete or excrete NH₃/NH₄⁺ (Muhamad et al. 2004; Simpson et al. 2009), but the metabolic sources have not been identified. About sixty enzymes catalyse reactions involving NH₃/NH₄⁺ (Moss 2009) and only some of these are found in multicellular eukaryotes (for example, nitrogenase is restricted to bacteria). Of the latter, an important example is glutamate dehydrogenase (E.C. 1.4.1.3) which catalyses the reversible oxidative deamination of glutamate according to the reaction



where the cofactor can be NAD⁺ or NADP⁺ with differing reaction efficiency. The activity of the enzyme is allosterically controlled by adenine nucleotides in mammals (Bailey et al. 1982; Hornby et al. 1984), but not in bacteria or fungi (Hudson and Daniel, 1993; Smith and Stanley, 2008). Despite considerable research, the mechanism of

the allosteric control of glutamate dehydrogenase is yet to be elucidated (Smith and Stanley, 2008; Laskowski et al. 2009).

In most species glutamate dehydrogenase provides both a means for incorporating NH₃/NH₄⁺ into amino acids and of generating α-ketoglutarate and NH₃/NH₄⁺ depending on the circumstances (Marzluf 1981; Magasanik 2003). However, Skuce et al. (1999) were able to detect neither glutamate dehydrogenase mRNA nor the protein in L₃ *H. contortus*, although they were able to detect both in adult nematodes and the enzyme is present in adult *H. contortus* (Rhodes and Ferguson, 1973; Kapur et al. 1984). The absence of the protein from L₃ *H. contortus* is unusual because of the importance of glutamate dehydrogenase in most species. Moreover, if the enzyme were also absent from L₃ *T. circumcincta* then it could not be a source of the secreted or excreted NH₃/NH₄⁺ we have observed (Muhamad et al. 2004; Simpson et al. 2009). Therefore, we investigated the presence of the enzyme activity in L₃ and adult *T. circumcincta* in order to determine whether the enzyme could be involved in the generation of the NH₃/NH₄⁺ that is secreted or excreted by the nematode (Muhamad et al. 2004; Simpson et al. 2009). We have characterised the kinetics of the enzyme in both L₃ and adult *T. circumcincta* to assess this possibility, but also to (i) provide a comparison with the enzyme of other gut flora and the sheep and (ii) to develop insight into the parasitic lifestyle of *T. circumcincta*.

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