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Characterisation of a major enzyme of bovine nitrogen metabolism

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INTRODUCTION

Efforts are underway to produce transgenic pigs as source of organ transplants, transgenic fish for food, and transgenic livestock that are resistant to many animal diseases and can survive harsh environmental conditions. Nguni breed of cattle can survive under conditions that other bulk grazers, such as the European cattle breeds, find extremely testing. It is with this background, that our attention was drawn to the physiological and biochemical pathways of nitrogen metabolism in bovines (Bester, et al., 2003).

Glutamine is one of the most abundant free amino acids, making about 20% of the total amino acid content in the body of most mammalian species, with the bulk being present in skeletal muscle, where it plays a role in the maintenance of cellular protein metabolism (Curthoys & Watford, 1995; Meister, 1974). Glutamine functions as a major inter-organ transport form of nitrogen, carbon and serves as a source of energy between tissues such as brain, liver, kidney and even muscles, in the mammalian body. Glutamine catabolism in the liver provides substrates for gluconeogenesis and urea synthesis. Although the exact pathway of precursor formation is not fully understood, the only known reaction yielding glutamine is that catalysed by glutamine synthetase, which converts glutamate and ammonia to glutamine, with the hydrolysis of ATP (Bester, et al., 2003).

L-glutamate + NH_4^+ + ATP → L-glutamine + ADP + Pi

GLUTAMINE SYNTHETASE (GS) – AN OVERVIEW

In mammalian tissues, GS is not uniformly distributed. Most GS activity is found in liver, brain, and kidney, while minor activities occur in muscle, spleen, testes and heart (Van Straaten *et al*., 2006). The high activities found in liver, brain and kidney has prompted studies around those organs of Bos taurus.

In most mammalian species, there is marginal or no net absorption of dietary glutamine into circulation. Thus, the GS pool is essentially synthesized de novo (Watford & Reeds, 1995). The only enzyme capable of producing glutamine is GS, which is highly expressed in tissues such as liver perivenous cells, where it is proposed to function to remove excess ammonia. In tissues such as skeletal muscles, its role is to provide glutamine for the rest of the mammalian body (Van Straaten, *et al*., 2006).

METHODS AND RESULTS

Purification of GS was carried out as follows:

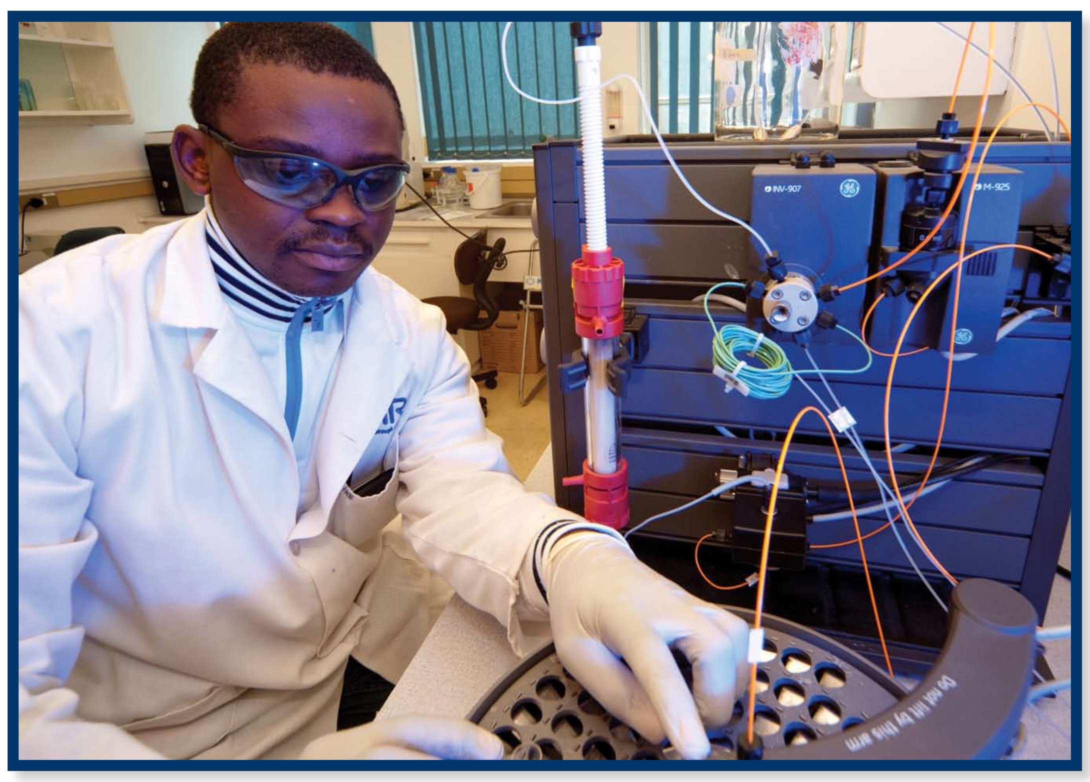

Figure 1: The AKTA, protein purification laboratory

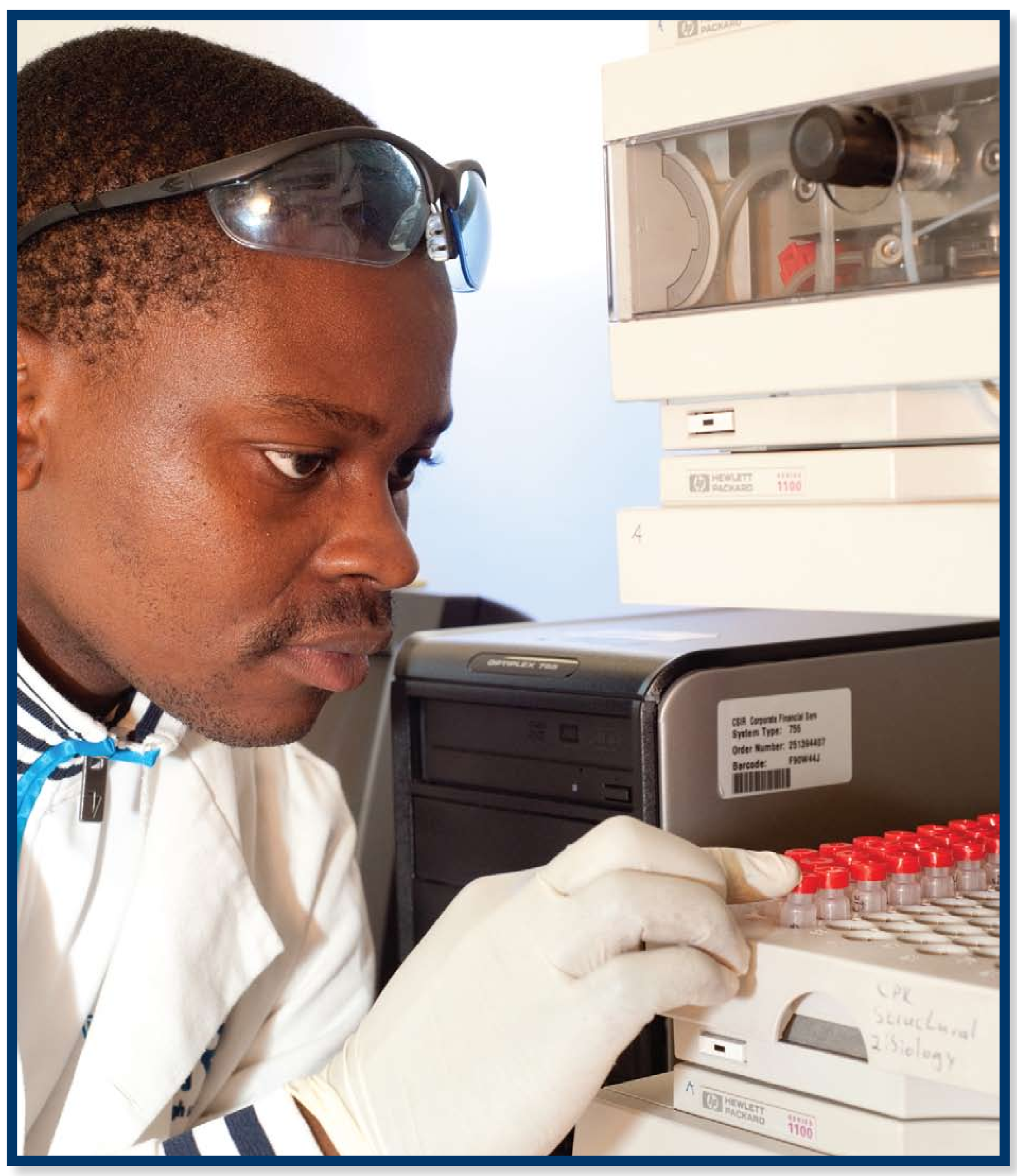

Figure 2: HPLC, protein assay laboratory

Tissues were homogenised and sonicated to obtain cell free extracts, which were then respectively subjected to precipitation with streptomycin sulphate and ammonium sulphate, followed by desalting and Adenosine Monophosphate (AMP) Agarose column chromatography. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was then used to identify band sizes of various proteins (**Figure 3**), after which identification of the desired protein was analysed by Matrix Assisted Laser Desorption/Ionisation (MALDI) Mass spectrophotometry.

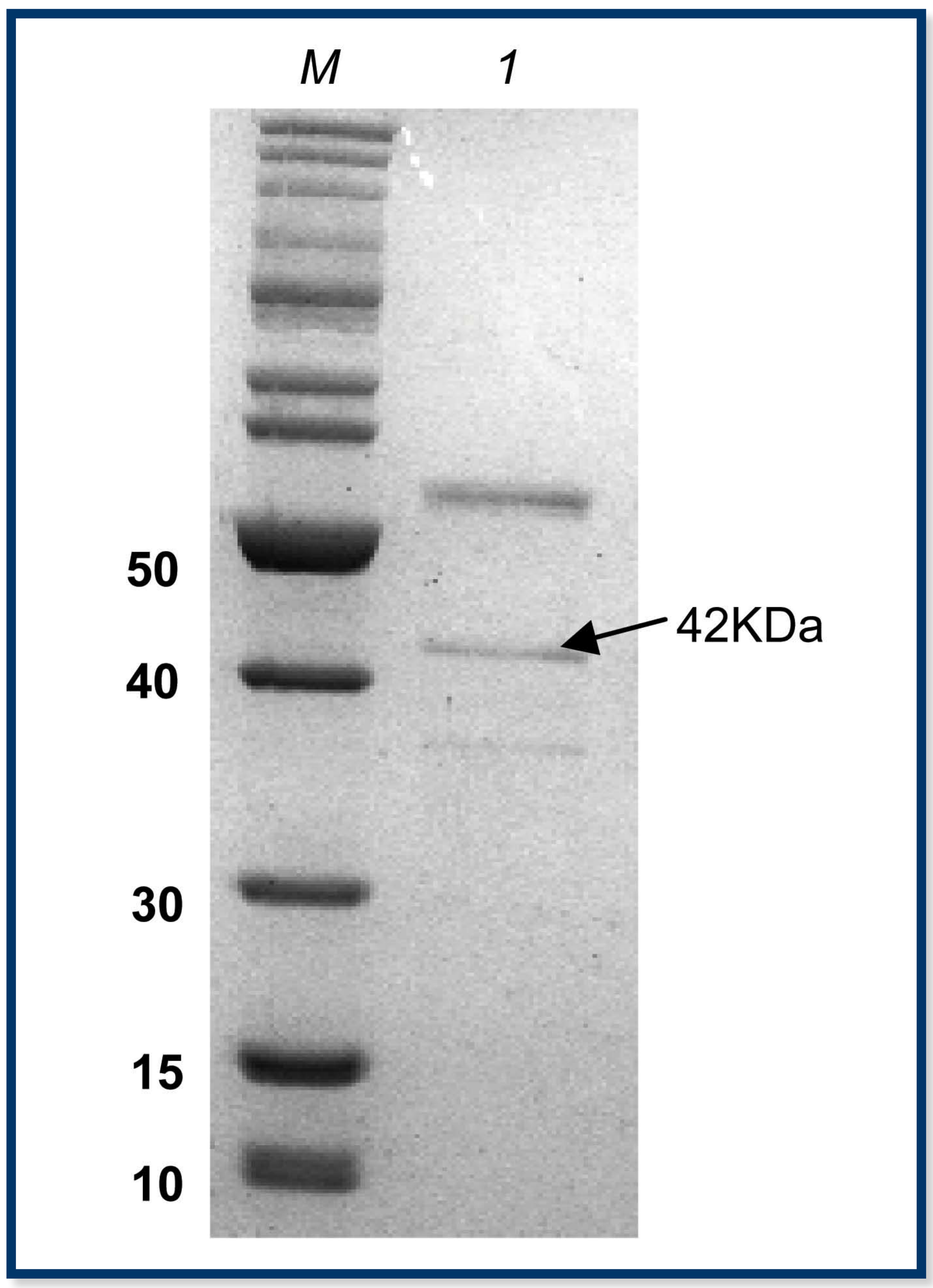

Figure 3: SDS PAGE pattern of bovine GS (second lane) purified on AMP Agarose Column. The first lane is a mixture of standard proteins, with increasing molecular weights in Kilo-Daltons (KDa)

Enzyme activity was generally assayed by gamma glutamyltransferase assay before specific activity was measured. High Performance Liquid Chromatography (HPLC) was used to determine the concentration of ADP, which was correlated to the amount of ATP hydrolysed by bovine glutamine synthetase, thereby giving a measure of specific activity of GS. Protein concentration was performed by the Quibit protein concentration kit method.

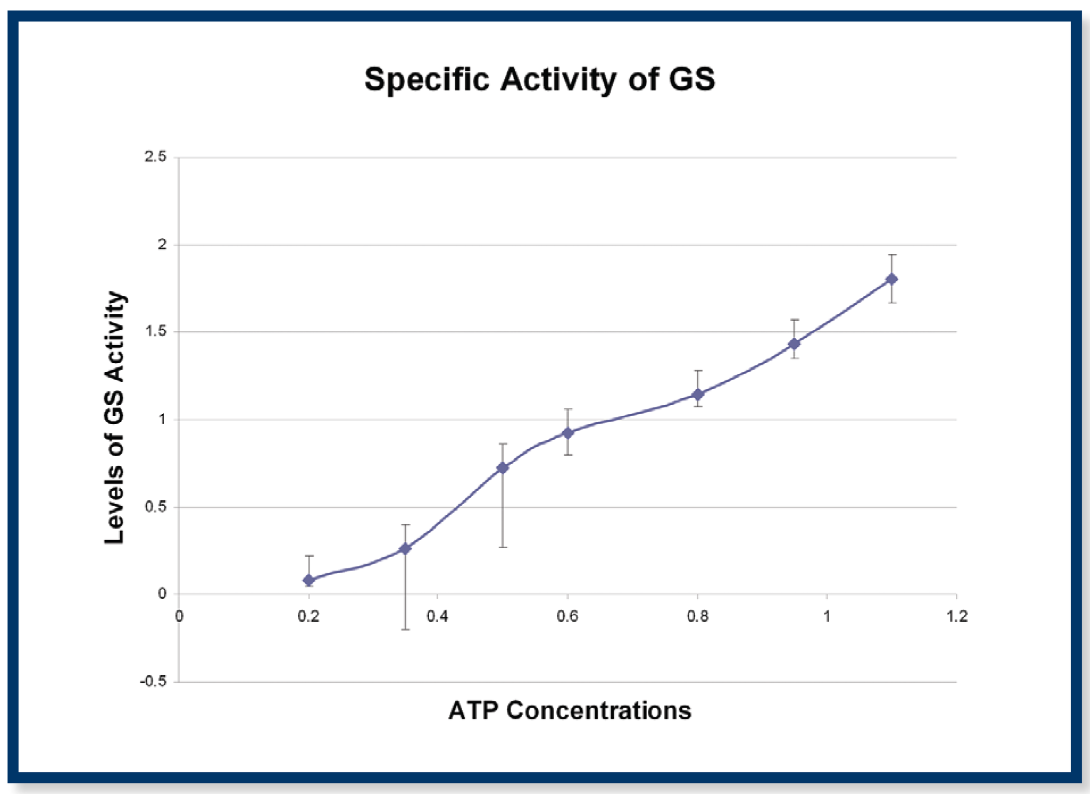

Figure 4: Results obtained with the High Performance Liquid Chromatography Assay. When the concentration of ATP was varied, L-glutamate was kept constant at 40mM. Ammonium chloride was held constant at 68mM

DISCUSSION

The purified bovine GS preparation migrated as double bands, 42 KDa band when subjected to 10% polyacrylamide gels at ph 7.12. This suggested glycolsylation of the desired protein, as was apparent in other purifications of bovine GS. Protein concentration that was done by the Quibit protein concentration determination method at the time of HPLC assay was 0.118mg and a significantly high for determination of high specific activity of GS. With specific activity of GS being 1.8u/min, at the highest concentration of ATP, there is accordingly correlation with bovine enzyme hydrolysing ATP, specifically bovine GS. The curve indicates thus increasing specific activity of bovine GS acting on substrate L-glutamate with varying ATP concentrations.

Glutamate-to-glutamine conversion by glutamine synthetase (GS) provides the nitrogen donor for the first step in the biosynthesis of many amino acids, purines, pyrimidines, and amino sugars.

CONCLUSIONS & RECOMMENDATION

The double band obtained from the purified preparation of bovine GS, suggests that glycosylation has taken place, or that the methodology used needs to include DEAE column and hydroxylapitite column purification, in order to obtain a single band. To increase the reliability of the specific activity results, varied concentrations of L-glutamate and the ammonium chloride should also be assayed. These assays should give more or less the same results with that of the varied concentrations of ATP. More work still needs to be done, in order to fully characterise bovine GS, including Magnetic Resonance Spectroscopy and structural molecular modelling applications to the data.

Functional and structural characterisation of all the GS activity at the end of this research project, will be used in a proteomics research programme, specifically to establish the degree of efficiency of GS in liver and other organs of interest, as well as to identifying possible target genes and for genetic selection of enzyme polymorphism, that confer resistance to adverse conditions in Nguni animals.

Figure 5: The Nguni bull and the Hereford bull exhibiting their different phenotypic characteristics

ACKNOWLEDGMENTS

Many thanks to Innovation Fund.

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