Role of Amino Acids in Nitrogen Fixation

during the *R. leguminosarum*/Pea Symbiosis

The University of Reading, School of A.M.S.

Alexandre Bourdès, James White, Emma Lodwig and Philip Poole

INTRODUCTION

The possible cycling of amino acids between plant and bacteroid has been proposed to be essential for nitrogen assimilation (Lodwig et al, (2003) Nature 422:722-726). Amino acid uptake by *R. leguminosarum* is dominated by two ABC transporters, the general amino acid permease (aap) and the branched amino acid permease (bra). An aap/bra double mutant is $Fix^{reduced}$ as measured by Nitrogenase activity. However, the nodules are pink compared to the white nodules of a true Fix^- or red nodules of the wild type. The plants are nitrogen starved as observed by plant dry weight and total nitrogen content analysis.

To explain this we proposed a model, whereby amino acids are required to cycle between the bacteroid and the plant. In this model an amino acid such as glutamate as well as a C_4-dicarboxylic acid (e.g. L-malate), is taken up by the bacteroid to drive secretion of amino acids such as aspartate or alanine.

Fig 1: effect of aap/bra mutant on plants

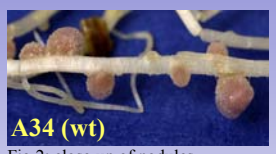

Fig 2: close-up of nodules

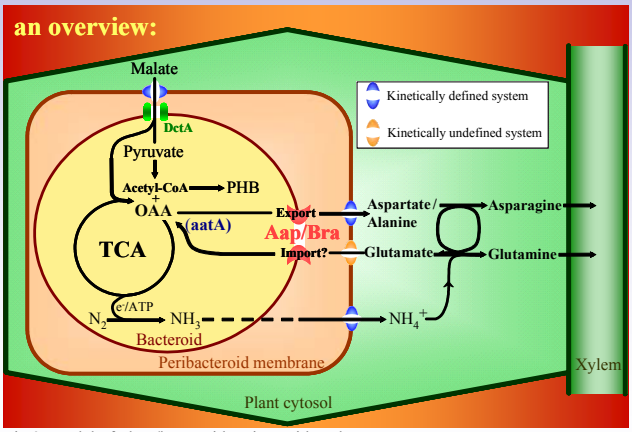

Fig 3: model of plant/bacteroid amino acid cycle

Role of the Aminotransferases

Since aminotransferases link the TCA cycle and amino acid synthesis, we expected they would have a major effect on nitrogen fixation. A mutant in the enzyme aspartate aminotransferase (aatA) formed defective nodules that are not able to fix nitrogen. The role of aatA would be the transamination of oxaloacetate by glutamate to form aspartate (named AAT activity). Furthermore, we have found that *R. leguminosarum*, and possibly all bacteria, lack the classical alanine aminotransferase activity (widely distributed in eukaryotes) which links glutamate and alanine (named GPT activity).

Equally remarkably, we have discovered that aatA catalyses a second transamination reaction in which aspartate will transaminate pyruvate to form alanine (named APT activity) . Thus, the glutamate, aspartate and alanine pools in *R. leguminosarum* are inter-connected by the one enzyme (aatA). This has profound consequences for the regulation of bacteroid metabolism and amino acid shuttling.

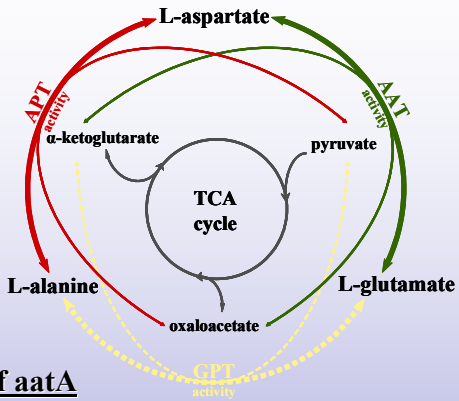

Fig 4: effect of aatA mutant and complemented mutant on plants

Strain	Growth conditions	AAT activity (μmol.min^{-1}.mg prot.$^{-1}$)	APT activity (μmol.min^{-1}.mg prot.$^{-1}$)
A34	Glucose/NH4	0.299 ± 0.125	0.075 ± 0.004
A34	Succinate/NH4	0.451 ± 0.157	0.194 ± 0.056
RU1640 (aatA mutant)	Succinate/NH4	0.020 ± 0.005	0.013 ± 0.002
RU1640 pJP2 aatA (complemented)	Succinate/NH4	1.600 ± 0.835	0.365 ± 0.158
RU1357 (aap/bra mutant)	Succinate/NH4	0.514 ± 0.219	0.121 ± 0.076
Bacteroids	4 week plants	0.173 ± 0.015	0.049 ± 0.035

Table 1: summing up of AAT and APT activities

Purification and Kinetics of aatA

AatA has been cloned into pET101/D. The plasmid transformed and expressed into BL21 Star™(DE3). The enzyme is his-tagged, allowing a simple two step purification with a His•Bind® Resin loaded column and a Sephacryl™ S-200.

	AAT activity (μmol.min^{-1}.mg of prot.$^{-1}$)	Total AAT activity (μmol.min^{-1})
Crude Extract	3.00 ± 0.34	1103 ± 125
Pure aatA	163 ± 13.4	284 ± 23.5

Table 2: yield of aatA purification

Fig 5: aatA purification

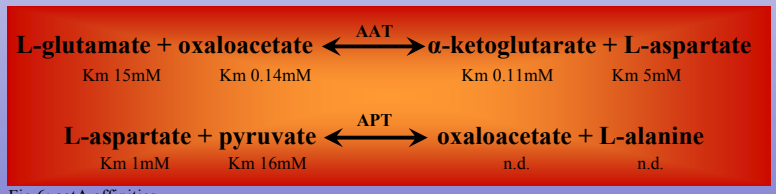

Fig 6: aatA affinities

Conclusions

- AatA is essential for nitrogen fixation.
- AatA has two activities that allow the conversion of glutamate to aspartate and aspartate to alanine.
- This provides a powerful explanation the origins of the aspartate and of the alanine secreted in various ratios by bacteroids.

Contacts:
p.s.poole@reading.ac.uk
a.bourdes@reading.ac.uk