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Distinct physiological roles for the two L-asparaginase isozymes of *Escherichia coli*

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ABSTRACT

Escherichia coli expresses two L-asparaginase (EC 3.5.1.1) isozymes: L-asparaginse I, which is a low affinity, cytoplasmic enzyme that is expressed constitutively, and L-asparaginase II, a high affinity periplasmic enzyme that is under complex co-transcriptional regulation by both Fnr and Crp. The distinct localisation and regulation of these enzymes suggest different roles. To define these roles, a set of isogenic mutants was constructed that lacked either or both enzymes. Evidence is provided that L-asparaginase II, in contrast to L-asparaginase I, can be used in the provision of an anaerobic electron acceptor when using a non-fermentable carbon source in the presence of excess nitrogen.

1. Introduction

L-Asparaginases are widely distributed in the three domains of life and play a central role in amino acid metabolism and utilisation. L-Asparaginase enzymes catalyse the hydrolysis of L-asparagine (EC 3.5.1.1) generating L-aspartate and ammonia (NH_3). Based on studies with *Escherichia coli* and *Salmonella enterica*, bacterial L-asparaginases are of two types: a high affinity enzyme located in the periplasm (L-asparaginase II encoded by *ansB*) and a cytoplasmic low affinity enzyme (L-asparaginase I encoded by *ansA*). *E. coli* and a few other bacteria have been shown to possess both type I and type II isozymes [1–4]. The amino acid sequences are substantially diverged but retain sequence similarity around the active site residues [4]. The high affinity enzymes have attracted a great deal of interest due to their utility as anti-tumour agents in the treatment of childhood acute lymphocytic leukemia [5]. This is due to the efficient depletion of exogenous L-asparagine, which leukemic cells depend on for growth. More recently it has been reported in *S. enterica* Typhimurium that L-asparaginase II (AnsB) has a role in infection and immunity by inhibiting T-cell responses [6] and it also contributes to virulence in the human pathogens *Campylobacter jejuni*, *Helicobacter pylori* and *S. enterica* Typhimurium [6–8] via a role in colonisation.

As well as two L-asparaginase isozymes, *E. coli* also possess two distinct systems for L-asparagine uptake distinguishable on the basis of specificity and regulation [9,10]: a low-affinity system (K_m = 80 μM), and a high affinity system (K_m = 3.5 μM) which is repressed by the presence of L-asparagine in the growth medium in concentrations greater than 1 mM. These systems have not been studied at the molecular level; However, an asparagine permease, AnsP, has also been reported in *S. enterica* and *E. coli* [11,12].

There is evidence that L-asparaginase I (AnsA) plays a role in *E. coli* in the utilisation of L-asparagine as a nitrogen source [13] and it presumably functions to degrade L-asparagine when it has accumulated to an appropriate intracellular concentration. However, the role of the high affinity L-asparaginase II has never been determined. While L-asparaginase I is expressed constitutively, L-asparaginase II expression requires co-dependent activation by both anaerobiosis, via the Fnr transcriptional activator, and by the cyclic-AMP receptor protein (Crp) [3,14,15]. Several possible roles are consistent with the activity, localisation, and regulation of the L-asparaginase II. Firstly, L-asparaginase II may serve to utilise L-asparagine as a carbon source under unfavorable conditions, as in the case of, for example, exported phosphatases, proteases and lipases. Such a role is consistent with regulation by Crp. Secondly, this enzyme may be required for growth on low concentrations of L-asparagine as a nitrogen source. Thirdly, it has been suggested that during anaerobic growth on a non-fermentable carbon source, hydrolysis of L-asparagine, could lead to the provision of fumarate as a terminal electron acceptor for anaerobic respiration since the resulting L-aspartate could be taken up by an L-aspartate transporter and catabolised to fumarate by L-aspartase (see Fig. 2) [3]; this function is consistent with the anaerobic regulation of L-asparaginase II in both *E. coli*, via Fnr, and *S. enterica* by an unknown mechanism, [15] and with the anaerobic regulation of aspartase and dicarboxylate transporters [16–18].

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Table 1
Strains and plasmids used in this study. CGSC: Coli Genetic Stock Centre.

Name	Relevant genotype	Source/Reference
E. coli strains		
HB94	Wild type	Del Casale et al. (1983)
HB94K	*ansB*::*kan*	This work
RC614	*ansA*	Del Casale et al. (1983)
RC614K	*ansA, ansB*::*kan*	This work
CGSC6355(P1)	P1 lysogen	CGSC
Plasmids		
pMJ13	*ansB*	Jennings et al. (1990)
pMJ13kan	*ansB*::*kan*	This work
pPJ1	*ansA*	Jerlstrom et al. (1989)

2. Materials and methods

2.1. Media and growth conditions

Strains and plasmids used in this study are detailed in Table 1. HB94 (wild type) and its three mutant derivatives (RC614 (*ansA*$^-$); HB94K (*ansB*$^-$); RC614K (*ansA*$^-$, *ansB*$^-$) were grown as follows. For Fig. 1A, HB94 was grown in 1× M9 medium and supplemented with 10 mM Ca, 100 mM Mg, 20% glycerol, 100 mM tryptophan and 18 mM NH_4Cl. Cultures were grown anaerobically at 37 °C. Cells were harvested by centrifugation (45,000 × *g*, 15 min, RT) after overnight growth, washed twice with 1× M9 medium and resuspended in 2 ml of the same medium. This was used as a starter

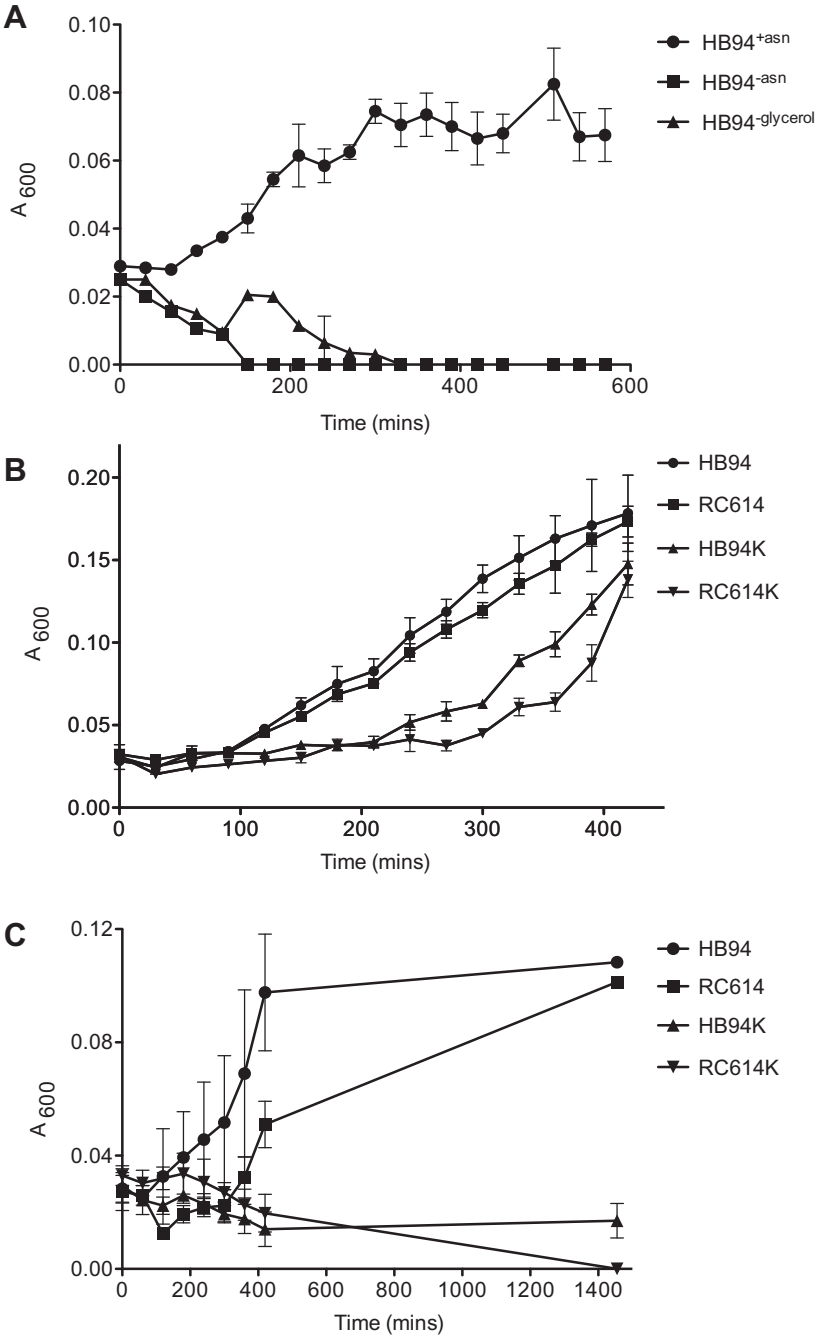

Fig. 1. The role of L-asparaginase II in anaerobic respiration. (A) Growth of HB94 in the presence or absence of asparagine and glycerol. (B) Growth of HB94 and the isogenic mutants RC614, HB94K and RC614K in the presence of asparagine and absence of NH_4Cl. (C) Growth of HB94 and the isogenic mutants RC614, HB94K and RC614K in the presence of asparagine and NH_4Cl. Growth conditions are detailed in Section 2.