The Effect of Azetidine-2-Carboxylic Acid on the Synthesis of Proline in *Escherichia coli*

Azetidine-2-carboxylic acid (A2C) is found in plants[1] and has a structure similar to that of proline, except that it lacks 1 methylene group[1,2]. It seemed reasonable that this analogue would show some of the same biological properties as proline, including the ability to exert end-product control over the synthesis of the natural amino acid. The effect of A2C on the conversion of glutamic acid to pyrroline-5-carboxylic acid (PC) was measured, because control of the synthesis of proline occurs at this step[3,4].

Cells derived from the wild type (55-1) and a proline excreting strain (WP1-30), lacking PC reductase (L-proline: NADP 5-oxidoreductase, EC 1.5.1.2) were used. It was found that PC formation in 55-1 was sensitive to A2C inhibition, although the amount of inhibition was less than that by proline. Under the conditions used in these experiments[3], $8 \times 10^{-4} M$ A2C inhibited the production of PC by 80%. TRISTRAM and THURSTON reported[4] 20–28% inhibition of PC production, using different conditions. Cells of a strain whose production of PC was insensitive to inhibition by proline were equally insensitive to the effects of A2C (Figure 1).

In order to examine quantitatively the effect of A2C on the control system, the concentration of glutamic acid was varied, and the rate of PC production measured. As is shown in Figure 2, A2C is a non-competetive inhibitor of the reaction, as is also proline. It may be calculated that the Km[5] for glutamic acid in the presence of $1 \times 10^{-4} M$ A2C is $9.6 \times 10^{-5} M$, and $4.6 \times 10^{-5} M$ in the absence of the analogue. In the presence of $1.7 \times 10^{-7} M$ proline, the observed Km is $5.6 \times 10^{-5} M$. Inhibition constants were calculated to be $5.2 \times 10^{-7} M$ for proline and $4 \times 10^{-5} M$ for A2C. The internal concentrations of proline and A2C were estimated from the uptake of radioactive substrates to be $5 \times 10^{-4} M$ and $6 \times 10^{-5} M$, respectively.

A2C acts as a false end-product inhibitor of the synthesis of proline. The loss of a methylene group in this analogue apparently changes the inhibition constant for the sensitive reaction by a factor of 100, and limits uptake into the cell by a factor of 10.

The rate growth of *Escherichia coli* W is slowed in the presence of low concentrations of A2C; in the absence of exogenous proline, $10^{-4} M$ A2C inhibits the growth rate by 70% (Table). No effect of A2C on growth could be seen in strain WP1, a proline excreting strain. Growth inhibition by A2C can be explained as an environmentally induced inability to synthesize proline, which is overcome in strains insensitive to control of the synthetic pathway[6].

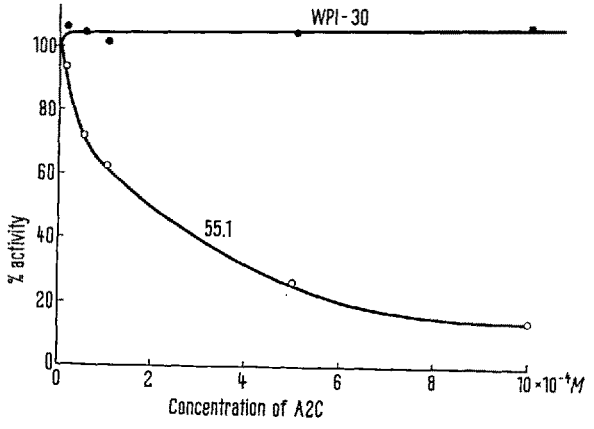

Fig. 1. The effect of A2C on the production of GSA in cells of *E. coli* which are sensitive (o) and resistant to (●) control by proline.

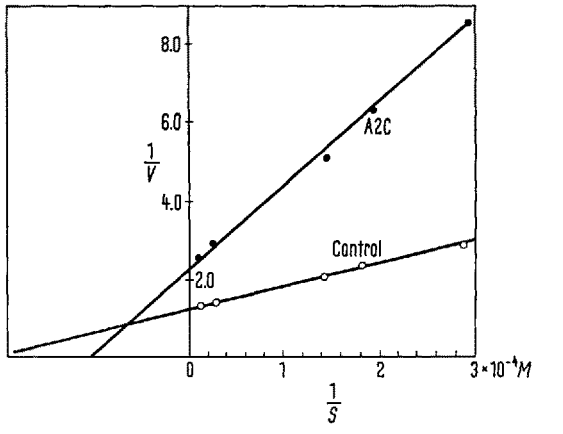

Fig. 2. A LINEWEAVER-BURK analysis of the effect of varying glutamic acid concentration on the rate of GSA production. The open circles (o) indicate the system in the absence of inhibitor, the closed circles (●) show the effect of $1 \times 10^{-4} M$ A2C.

Effect of azetidine-2-carbonylate on the growth of strain W

Proline (M/l)	A2C (M/l)	Generation time (min)	Inhibition (%)
0	0	72	0
0	1.0×10^{-4}	246	70.4
8.7×10^{-5}	0	72	0
8.7×10^{-5}	1.0×10^{-4}	73	0

Zusammenfassung. Die Beeinflussung der Prolinsynthese in *Escherichia coli* durch Azetidin-2-carbonsäure wird untersucht.

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