

RELAXATION OF CATABOLITE REPRESSION AND LOSS OF VALINE SENSITIVITY IN *ESCHERICHIA COLI* K-12

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1. Introduction

The inhibition of growth of *Escherichia coli* K-12 in a minimal medium containing valine [1] has been attributed to isoleucine limitation resulting from the extreme feedback sensitivity of acetohydroxy acid synthetase (AHAS) to valine [2]. Strains of *E. coli* capable of growth in a minimal medium supplemented with valine contain AHAS activity which is less sensitive to inhibition by valine. Recently, it has been demonstrated that the AHAS of *E. coli* K-12 is a single component (AHAS-I) whereas a valine-resistant W strain of *E. coli* contains an additional component (AHAS-II) which is not sensitive to inhibition by valine [3,4].

As reported previously [5] the AHAS of *E. coli* B is subject to catabolite repression. Since the growth of *E. coli* B in minimal medium is not inhibited in the presence of valine this strain presumably contains AHAS I and II, either or both of which may be sensitive to catabolite repression [5]. In the present work, we have established that the (single) AHAS activity of *E. coli* K-12 is sensitive to catabolite repression and that relaxation of this control permits cell growth in the presence of valine.

2. Materials and methods

Escherichia coli K-12 ('wild-type') was used for these studies. Cells were grown on a shaking water bath at 37°C in a minimal salts medium with supplements as indicated. The sodium salt of adenosine 3',5'-monophosphate (cAMP) (Calbiochem) was used.

The cells were harvested in log phase by centrifugation and washed with 0.1 M phosphate buffer, pH 7.0. The washed cells were suspended in 0.1 M Tris-HCl buffer, pH 8.0, and disrupted by sonic treatment for 4 min in a Bronwill 20 kHz sonic oscillator. The sonic extract was assayed immediately for AHAS activity as described previously [6]. For the determination of end-product inhibition by valine the assay procedure was modified by decreasing the concentration of the sodium pyruvate substrate to 0.025 M. Protein was determined by the method of Lowry et al. [7] using crystalline bovine serum albumin as standard.

For the determination of amino acids in culture media, the centrifuged cell-free medium was concentrated to dryness. The residue was dissolved in a small vol. of distilled water, adjusted to pH 2 and desalted using AG 50W-X2 (H⁺) (100–200 mesh). A Technicon amino acid analyser was used for the quantitative analysis of the resulting desalted solution.

3. Results and discussion

3.1. Inhibition of cell growth by valine; its dependence on catabolite repression

The degree of catabolite repression was modified by the use of cAMP and by variation of the carbon substrate. For maximum repression, glucose was employed as the carbon source; glycerol provided an intermediate degree of catabolite repression and acetate, or cAMP supplementation, the lowest degree of repression.

Inhibition of cell growth by valine was most marked

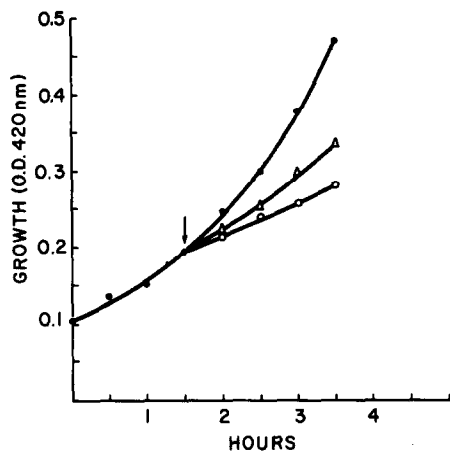


Fig. 1. Reversal of valine inhibition of growth of *E. coli* K-12 by cAMP. The bacteria were grown at 37°C with shaking on glucose minimal salts medium supplemented with cyclic AMP as follows: 5×10^{-3} M cAMP (●—●); 2.5×10^{-3} M cAMP (△—△); no cAMP (○—○). L-Valine (final concentration, 10^{-3} M) was added at the time indicated by the arrow. Growth curve of the control (no cAMP; no valine) was identical with that obtained with 5×10^{-3} M cAMP.

when *E. coli* K-12 cells were grown in a glucose-salts medium, that is, under conditions of maximum catabolite repression (fig. 1). In contrast, the growth of cells in an acetate-salts medium was insensitive to valine (fig. 2) whereas growth in a glycerol-salts

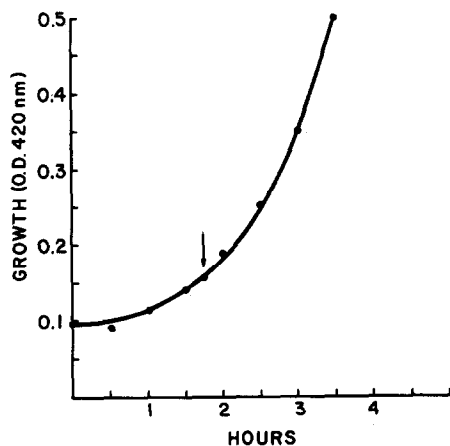


Fig. 2. Insensitivity to valine of *E. coli* K-12 growing in acetate medium. The bacteria were grown with shaking on acetate minimal salts medium at 37°C. L-Valine (final concentration, 10^{-3} M) was added at the time indicated by the arrow.

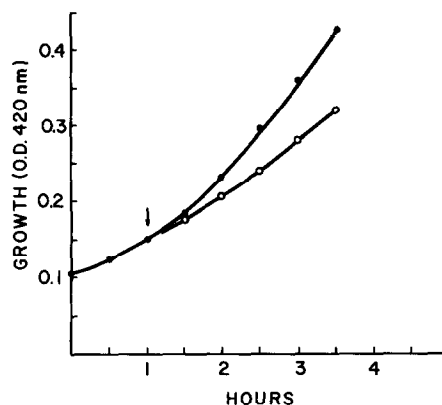


Fig. 3. Effect of addition of valine to *E. coli* K-12 growing in glycerol medium. The bacteria were grown with shaking on a glycerol-minimal salts medium at 37°C (●—●). L-Valine (final concentration, 10^{-3} M) was added at the time indicated by the arrow (○—○).

medium (fig. 3) was inhibited to a lesser degree than that observed with glucose as carbon source. The presence of exogenous cAMP prevented the inhibition of growth by valine which was observed when cells were grown under conditions of catabolite repression (fig. 1).

3.2. Regulation of acetohydroxy acid synthetase by catabolite repression

As shown in table 1, the AHAS of *E. coli* K-12 was sensitive to catabolite repression; the activity of the enzyme in crude extracts was inversely related to the degree of catabolite repression during growth of the cells. In addition, the increased activity of AHAS observed in cells grown on glucose-salts supplemented

Table 1
Effect of carbon source on AHAS in crude extracts of *E. coli* K-12

Carbon source	Specific activity (μ moles acetoin/hr /mg protein)
Glucose (0.4%)	0.97
Glucose (0.4%) + cAMP (5×10^{-3} M)	1.8
Sodium acetate (0.8%)	3.2
Sodium acetate (0.8%) + L-valine (1×10^{-3} M)	3.3
Glycerol (0.4%)	1.3

Table 2
Effect of cAMP on excretion of branched chain amino acids by *E. coli* K-12

Amino acids	Medium		Ratio B/A
	A Glucose salts $\mu\text{moles}/100\text{ ml}$ of medium	B Glucose salts + cAMP ($5 \times 10^{-3}\text{ M}$) $\mu\text{moles}/100\text{ ml}$ of medium	
L-valine	0.074	0.31	4.2
L-isoleucine	0.015	0.042	2.8
L-leucine	0.034	0.053	1.6

with cAMP was accompanied by an increased excretion of branched chain amino acids — especially valine — into the culture medium (table 2). This observation provides support for the suggestion [8] that the formation of valine may provide an alternate end-product of carbohydrate metabolism in *E. coli* under some conditions.

The sensitivity to end-product inhibition of AHAS in extracts was not altered when the cells were grown in a glucose-salts medium containing cAMP nor when acetate was supplied as the carbon source. Thus, a

reduction in the degree of catabolite repression does not induce the preferential synthesis of a second valine-insensitive AHAS. Since the inhibition of AHAS by valine did not exceed 80% (see fig. 4), relaxation of catabolite repression was sufficient to allow cell growth even at relatively high concentrations of valine.

Acknowledgements

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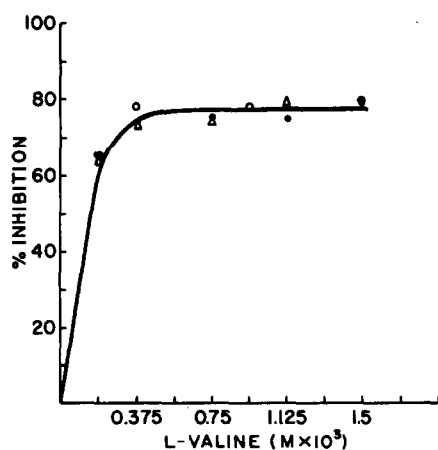


Fig. 4. Constancy of feed-back sensitivity of acetohydroxy acid synthetase in sonic extracts of *E. coli* K-12 grown on minimal salts medium with different supplements: 0.4% glucose (○—○); 0.4% glucose plus $5.0 \times 10^{-3}\text{ M}$ cAMP (△—△); 0.8% acetate (●—●).

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