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# NOTES

## β-Chloro-L-Alanine Inhibition of the *Escherichia coli* Alanine-Valine Transaminase

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**β-Chloro-L-alanine, an amino acid analog which inhibits a number of enzymes, reversibly inhibited the *Escherichia coli* K-12 alanine-valine transaminase, transaminase C. This inhibition, along with the inhibition of transaminase B, accounted for the isoleucine-plus-valine requirement of *E. coli* in the presence of β-chloro-L-alanine.**

β-Chloro-L-alanine (BCA) is a bacteriostatic amino acid analog which inhibits a number of enzymes, including threonine deaminase (1), the branched-chain amino acid transaminase (transaminase B) (1), L-aspartate-β-decarboxylase (11), alanine racemase (2, 8), and probably *O*-acetylserine sulfhydrylase (5). Arfin and Koziell (1) showed that *Salmonella typhimurium* LT2 will grow in the presence of BCA if isoleucine and valine are added to the medium and presumed that the growth requirement was due to the inhibition of transaminase B, an enzyme which catalyzes the final step in isoleucine, valine, and leucine biosynthesis. A second transaminase, transaminase D, which also catalyzes this step in leucine synthesis, is probably not BCA sensitive since BCA-inhibited strains do not require leucine. A third transaminase, transaminase C, also catalyzes this step in valine synthesis (10) but has been thought to play a minor role in most *Escherichia coli* strains (9). Therefore, the inhibition of transaminase B has been thought to account for the isoleucine-plus-valine requirement found in the presence of BCA. More recently, however, it has been shown that transaminase C is active in *S. typhimurium* and in most strains of *E. coli* K-12 (4, 6, 12). Therefore, the isoleucine-plus-valine requirement observed when BCA is added to the medium suggested to us that BCA inhibits transaminase C, as well as transaminase B. We show here that this dual inhibition accounts for the valine-plus-isoleucine requirement of *E. coli* in the presence of BCA. We also show that the BCA inhibition is reversible at a slow rate.

Eight *E. coli* K-12 strains and one plasmid were used in this study (Table 1). Mini-Mu::*avtA*$^+$ plasmid strains have more than 20 times as much transaminase C activity in crude extracts as do haploid strains (M.-D. Wang and C. M. Berg, manuscript in preparation). Cultures containing Mu were grown at 30°C to prevent phage induction. Other cultures were grown at 37°C.

Lennox (L) complex medium and Vogel and Bonner glucose-medium E salts were employed. They were supplemented as described previously (3, 12). Chloramphenicol (20 μg/ml) and BCA (1 mM in plates, 10 mM in enzyme assays) were added where indicated. Specialized chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

To characterize growth requirements, about 0.05 ml of stock solutions of L-isoleucine (76 mM), L-valine (85 mM), α-ketoisovalerate (72 mM), and α-keto-β-methylvalerate (66 mM) were spotted approximately 4 cm apart on medium E plates which had been spread with approximately $10^7$ cells. The presence of growth radiating from or between the spots was scored after 24 to 48 h of incubation.

Cells were grown and crude extracts prepared as described previously (12), except that chloramphenicol was added to the growth medium of CBK741(pIL001) to prevent plasmid loss. Crude extracts were incubated for 15 min at 37°C in the presence or absence of 10 mM BCA. BCA was

TABLE 1. *E. coli* K-12 strains

| Bacterial strain | Genotype | Derivation (reference) |
|---|---|---|
| CBK699 | *thyA* Δ(*proB-lac*) | (12) |
| CBK700 | *thyA* Δ(*proB-lac*) *ilvE720*::Tn*5* | (12) |
| CBK708 | *thyA* Δ(*proB-lac*) Δ*ilvGEDA*::Tn*5*-131 (Tet) | (12) |
| CBK740 | *thyA* Δ(*proB-lac*) *ilvE12* | Transduction of CBK708 to Val$^+$, using P1 · CU2 (9) |
| CBK741 | *thyA* Δ(*proB-lac*) *ilvE12* *avtA23*::Tn*5* | Tn*5* mutagenesis of CBK740 Val$^-$ Kan$^r$ |
| CBK748 | *thyA* Δ(*proB-lac*) *ilvE12* *avtA21*::Mu d1 | Transduction of CBK740 to Amp$^r$, using P1 · CBK703$^{tr}$ (12). |
| CBK793 | *thyA* Δ(*proB-lac*) *avtA21*::Mu d1 | Transduction of CBK748 to Ilv$^+$, using P1 · CBK699 |
| CBK741(pIL001) | CBK741(Mu dII4042::*avtA*$^+$) | Mini-Mu cloning (7; Wang and Berg, in preparation) |

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TABLE 2. Effect of BCA on growth requirements of strains lacking one or both valine transaminases

| *E. coli* K-12 strain | Relevant genotype | | Transaminase missing[b] | BCA added | Growth with following addition to medium[a]: | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *ilvE* | *avtA* | | | None | Ile | KMV + Val | Ile + KIV | Ile + Val |
| CBK699 | + | + | Neither | − | + | + | + | + | + |
| | | | | + | − | − | − | − | + |
| CBK793 | + | − | TrC | − | + | + | + | + | + |
| | | | | + | − | − | − | − | + |
| CBK740 | − | + | TrB | − | − | + | − | + | + |
| | | | | + | − | − | − | − | + |
| CBK741 | − | − | TrB, TrC | − | − | − | − | − | + |
| | | | | + | − | − | − | − | + |

[a] Basic medium contains thymine and proline. Abbreviations: Ile, isoleucine; KMV, α-keto-β-methylvalerate (isoleucine precursor); Val, valine; KIV, α-ketoisovalerate (valine precursor).

[b] TrC, Transaminase C (the alanine-valine transaminase); TrB, transaminase B (the branched-chain amino acid transaminase).

removed by filtration, and transaminase C was assayed as described in the legend to Fig. 1.

We found that *E. coli*, like *S. typhimurium* (1), requires both isoleucine and valine in the presence of 1 mM BCA and that the α-ketoacid precursors of isoleucine and valine do not overcome BCA inhibition (Table 2). These data suggested that transaminase C, as well as transaminase B, is inhibited by BCA in vivo. To test this, transaminase C activity was assayed after incubation of a crude extract from CBK741(pIL001) in the presence of BCA. CBK741(pIL001) is devoid of transaminase B activity and carries the gene encoding transaminase C on a multicopy plasmid. Incubation of the crude extract in the presence of 10 mM BCA resulted in 80% inhibition of transaminase C activity, which recovered slowly over a period of 4 h (Fig. 1). Since there may have been some recovery from inhibition during the time it took to remove the BCA (more than 1.5 h), the initial inhibition was probably greater. The addition of 1 mM pyridoxal phosphate to the washed extracts did not increase the rate of recovery from BCA inhibition (data not shown).

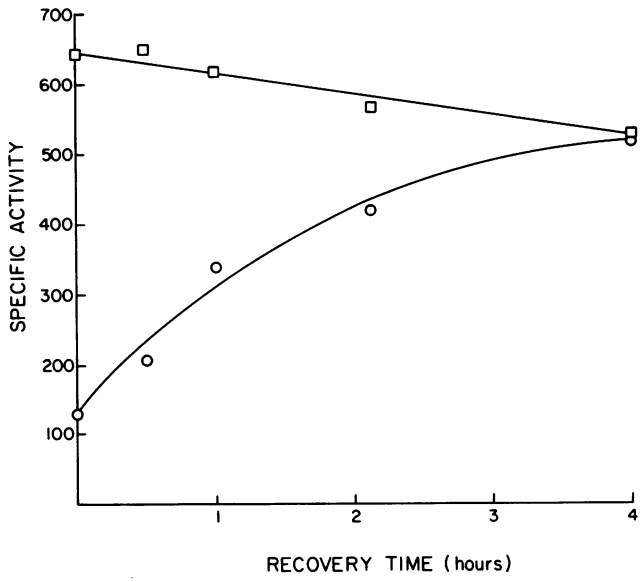


FIG. 1. Inhibition of transaminase C by BCA. Two milliliters of a crude extract from CBK741 Mu cts(pIL001) was incubated for 15 min in the presence (○) or absence (□) of 10 mM BCA. The extracts were then centrifuged in a Centricon-30 microconcentrator (Amicon Corp., Lexington, Mass.) at 5,000 × *g* at 10°C for 45 min. The retentate was resuspended in 2 ml of sonication buffer (0.05 M potassium phosphate, pH 7.2, containing $2 \times 10^{-4}$ M dithiothreitol) and recentrifuged for 45 min before collection and resuspension in 4 ml of sonication buffer (to yield a final BCA concentration of less than 0.01 mM). The washed retentate was incubated at 37°C and assayed for transaminase C activity at intervals to measure recovery from BCA inhibition. Transaminase C was assayed by measuring pyruvate production in an α-ketoisovalerate-dependent conversion of alanine to pyruvate, and activity was expressed as nanomoles of pyruvate produced per minute per milligram of protein (9, 12).

Both the physiological (Table 2) and the enzymological (Fig. 1) data show that BCA inhibited transaminase C. This inhibition of transaminase C, along with the inhibition of transaminase B, accounted for the dual requirement for isoleucine and valine when *E. coli* was grown in the presence of BCA. The slow reversibility suggests that BCA binds tightly, but reversibly, to the active site.

We used the BCA sensitivity of *E. coli* to clone genes whose products, when overproduced, confer BCA resistance and the ability to grow without alanine or valine to a BCA-hypersensitive leaky alanine- or valine-requiring auxotroph. We used a Mini-Mu plasmid vector to clone short random segments of the *E. coli* genome in a multicopy plasmid (7). Plasmids carrying *ilvE* (transaminase B), *avtA* (transaminase C), and another gene, probably *tyrB* (transaminase D ), were obtained. Suppression of the BCA sensitivity and alanine or valine requirement in the plasmid-containing strains is a result of overproduction of an enzyme involved in alanine or valine synthesis. We define multicopy suppression as the ability of a gene cloned on a multicopy plasmid to complement a mutant phenotype caused by mutation in a different gene (M.-D. Wang and C. Berg, manuscript in preparation).

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