## Title

ENVIRONMENTAL EFFECTS ON THE SPECIFICITY OF ISO-LEUCYL-tRNA SYNTHETASE ISOLATED FROM ESCHERICHIA COLI



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## DISCLAIMER

# ENVIRONMENTAL EFFECTS ON THE SPECIFICITY OF ISOLEUCYL-tRNA SYNTHETASE ISOLATED FROM ESCHERICHIA COLI

Berg et al.[1] demonstrated that the isoleucyl-tRNA synthetase could activate valine but did not successfully transfer it to tRNA.

Similar results were also found by Arca et al.[2] when they worked with preparations from a thermophilic organism, Bacillus stearothermophilus, at 50°. However, when they increased the temperature to 70° they found that the isoleucyl-tRNA formation underwent a rapid decline, and that there was almost no formation at 80°. On the other hand, while there was little or no formation of valyl-tRNA from 50° to 60°, its formation increased sharply between 65° and 80°.[2] They suggested that above 70° some conformational change takes place in the tRNA, or in the enzyme, resulting in loss of ability to transfer isoleucine and gain of ability to transfer valine.

We were interested in finding out whether the isoleucyl-tRNA synthetase from E. coli could undergo a similar directional change in specificity under various environmental conditions.

The enzyme was purified 270-fold, using the procedure described by Berg et al.[1] It appeared homogeneous by acrylamide gel electrophoresis. It showed a single symmetric boundary by velocity sedimentation, with an $s_{20,w}$ of 5.6S.

It could activate valine to 50% but all other naturally-occurring amino acids less than 5%. Also, as Berg had found, it did not transfer valine to tRNA as assayed by the method based on the rate of formation of aminoacyl-$^{14}$C-tRNA,

$$Enz + ATP + AA_1 + tRNA^1 = AA_1\text{-}tRNA^1 + Enz + PP_i + AMP;$$

one unit of enzyme is equivalent to the formation of 1 μmole of aminoacyl-tRNA in 10 min at 37°.

(I) Temperature Effect on the Enzyme

A temperature test was carried out on the E. coli enzyme. The enzyme activity curve in transferring isoleucine appeared to be a normal bell shape with its maximum at 37°. Valine did not get transferred through the temperature range from 25° to 70° (Fig. 1).

II. Ion Effect on the Enzyme

$K^+$ and $Na^+$ effect. The formation of isoleucyl-tRNA declined gradually with the increase of either $K^+$ ion concentration or $Na^+$ ion concentration in the assay mixture. Valyl-tRNA was not formed through the ion concentration from $10^{-5}$ M to 1 M; results are shown in Fig. 2.

$Mg^{++}$ and $Ca^{++}$ effect. Both $Mg^{++}$ and $Ca^{++}$ served as activators to the enzyme, $Mg^{++}$ being more effective (Fig. 3). Again, no valyl-tRNA was formed while the ion concentration changed.

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References

1. F. H. Berman, P. Berg and M. Dieckmann, J. Biol. Chem. 236 (1961) 1735.

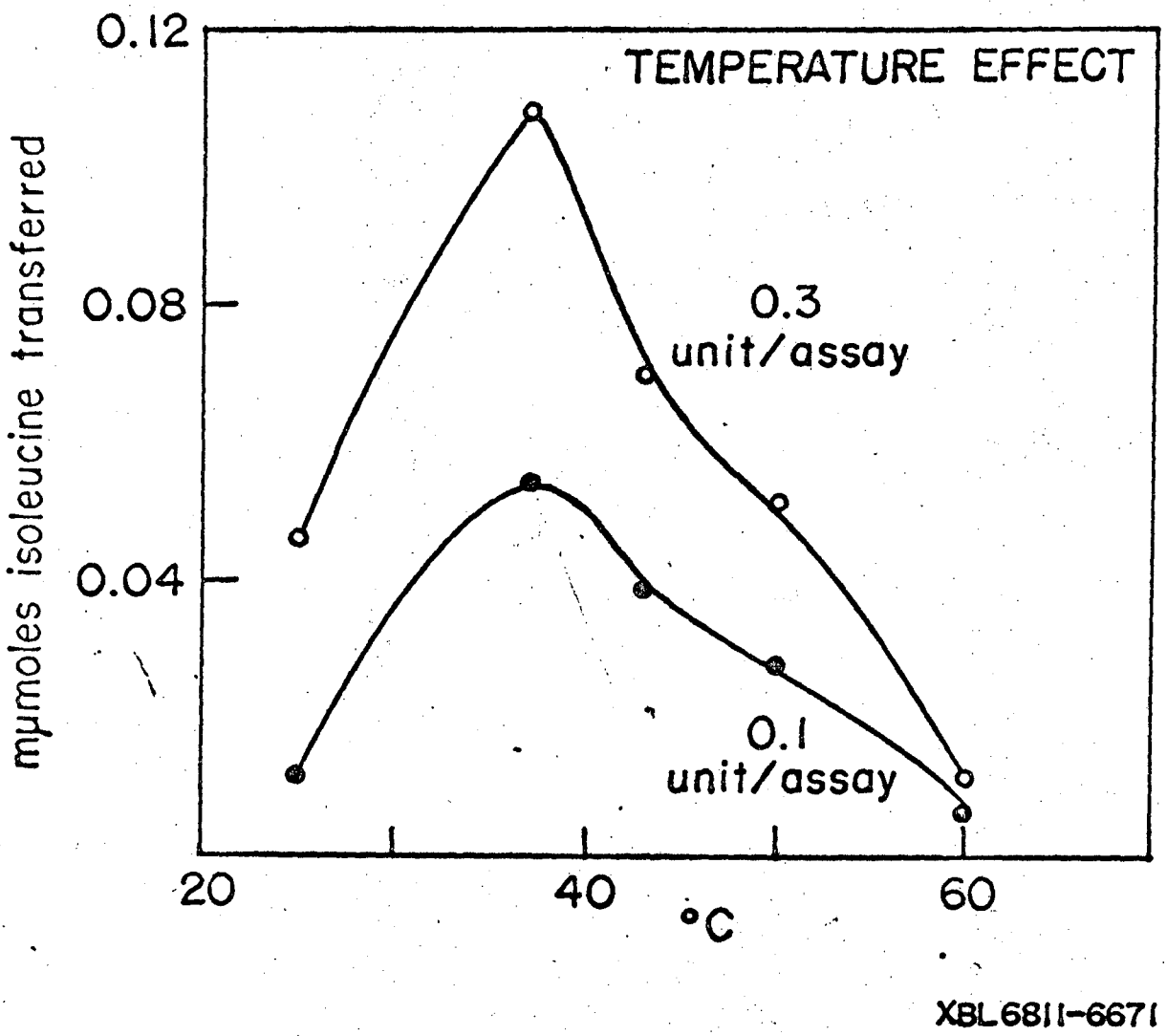
2. M. Arca, L. Frontali and G. Tecce, Biochim. Biophys. Acta, 108 (1965) 326.

Figure Captions

Fig. 1. Effect of temperature on the formation of $^{14}$C-valyl-tRNA (O-O) and $^{14}$C-isoleucyl-tRNA (X-X) by purified isoleucyl-tRNA synthetase from E. coli B.

Fig. 2. Effect of $K^+$ (O-O) and $Na^+$ (●-●) on the formation of $^{14}$C-isoleucyl-tRNA. Valyl-tRNA was not formed.

Fig. 3. Effect of $Mg^{++}$ (O-O) and $Ca^{++}$ (●-●) on the formation of $^{14}$C-isoleucyl-tRNA. Valyl-tRNA was not formed.

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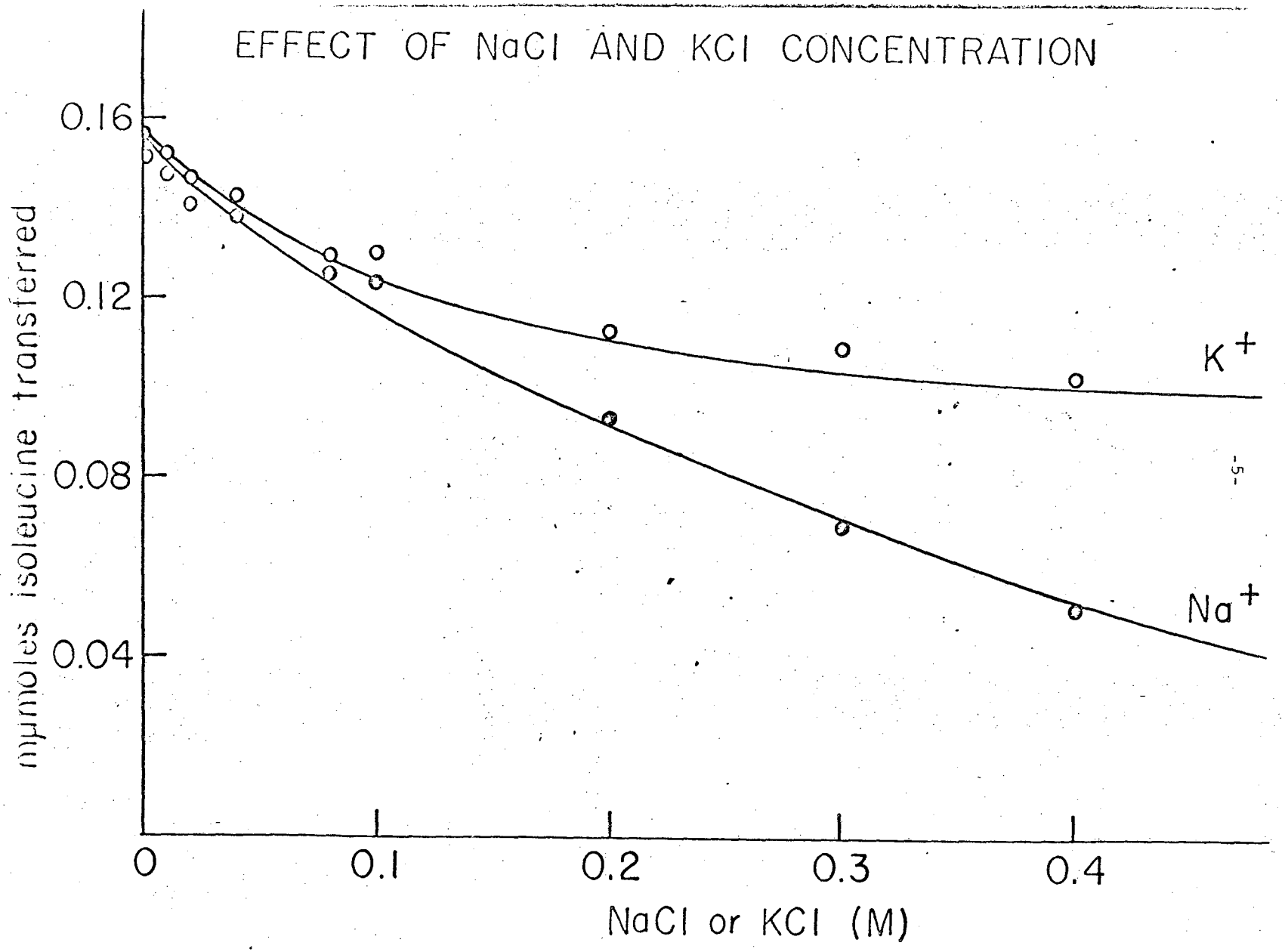
EFFECT OF NaCl AND KCl CONCENTRATION
mμmoles isoleucine transferred
0.16
0.12
0.08
0.04
0
0.1
0.2
0.3
0.4
NaCl or KCl (M)
K+
Na+

Fig. 2

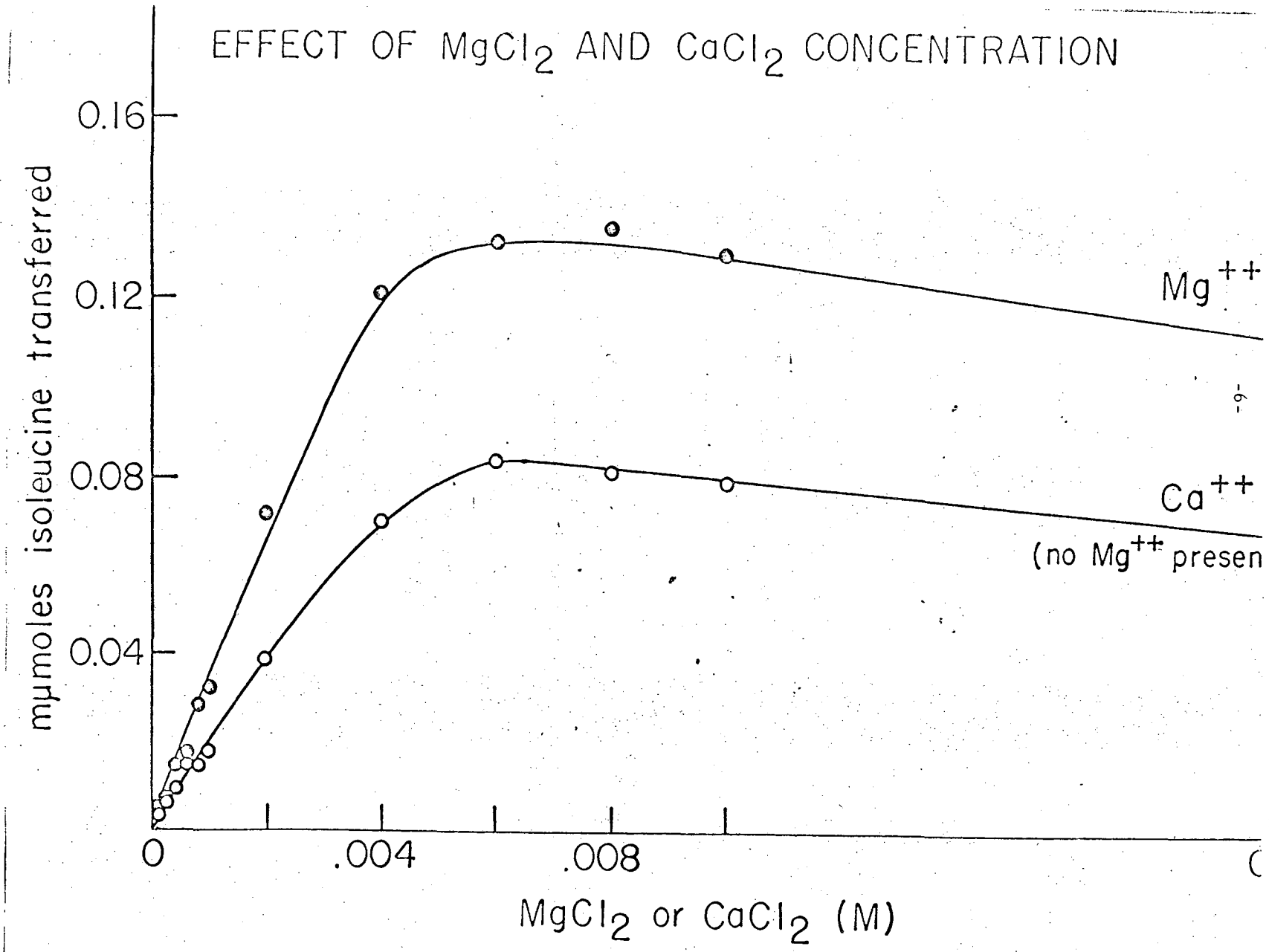


Fig. 3

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