Submitted to Biochimica et Biophysica Acta

UCRL-19888
Preprint

c.2

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

RECEIVED
LAWRENCE
RADIATION LABORATORY
SEP 2 1970
LIBRARY AND
DOCUMENTS SECTION

Joyce Yueh Hsu

July 1970

AEC Contract No. W-7405-eng-48

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY

UCRL-19888 c.2

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

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.

Acknowledgements: The work described in this paper was sponsored by the U.S. Atomic Energy Commission. The author is grateful for stimulating discussions with Professor Melvin

Calvin and Dr. Edward L. Bennett during the course of this work.

Laboratory of Chemical Biodynamics
Lawrence Radiation Laboratory
University of California
Berkeley, California 94720
U.S.A.

Joyce Yueh Hsu

References

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

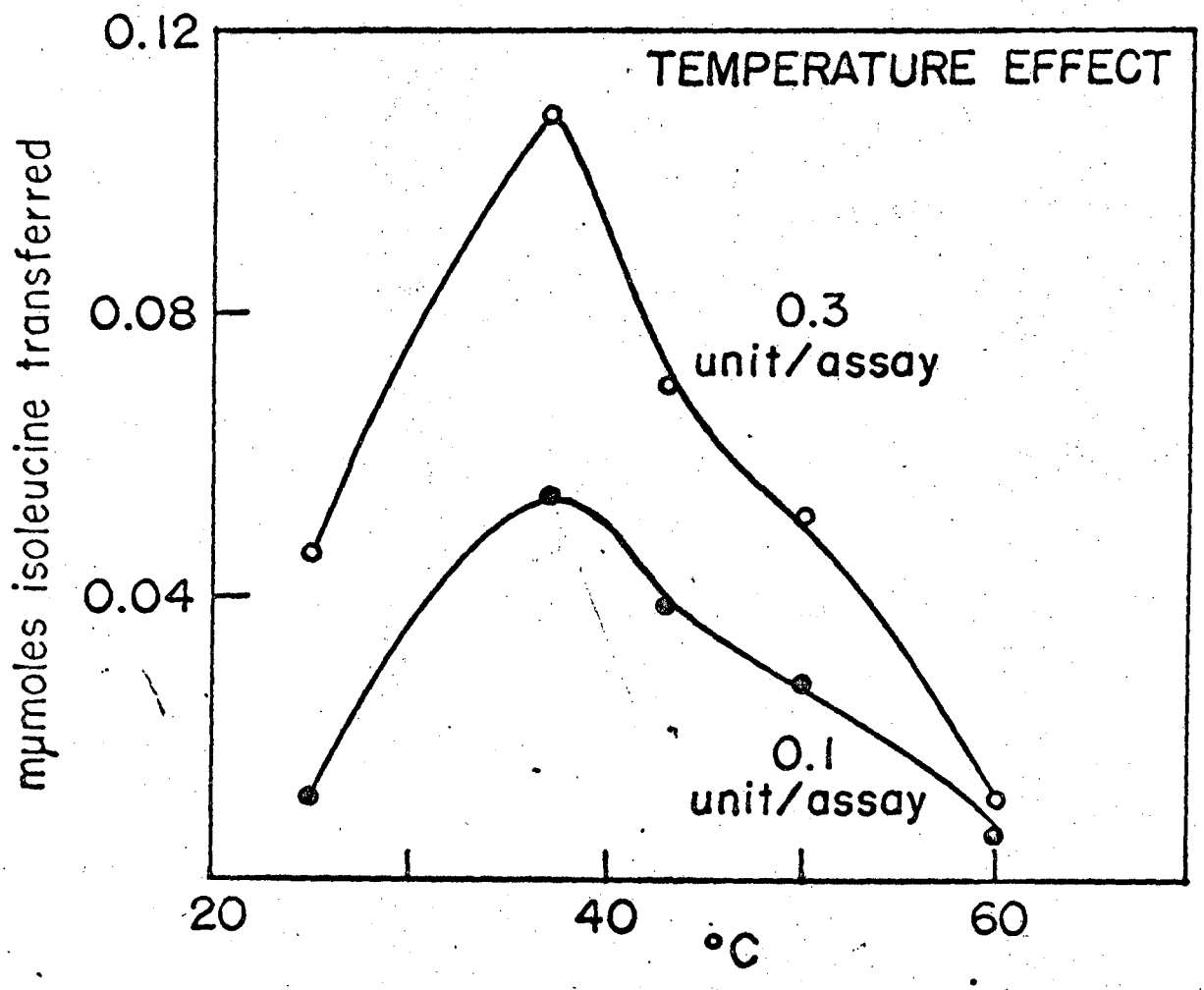
2. M. Arca, L. Frontali and G. Tecce, Biochim. Biophys. Acta, 108 (1965) 326.

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.



XBL6811-6671

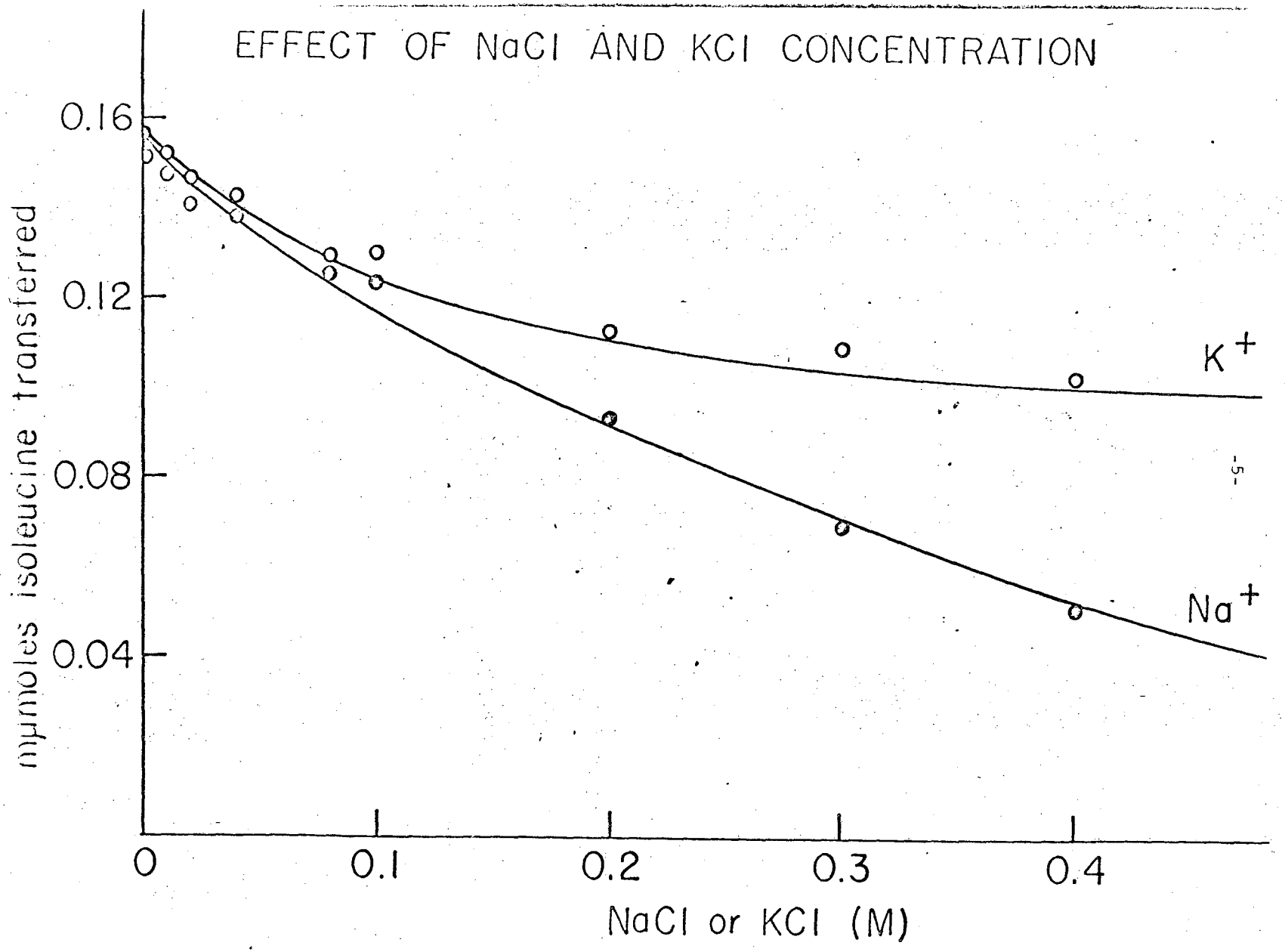

EFFECT OF NaCl AND KCl CONCENTRATION
mμmoles isoleucine transferred
0.16
0.12
0.08
0.04
0
0.1
0.2
0.3
0.4
NaCl or KCl (M)
K+
Na+

Fig. 2

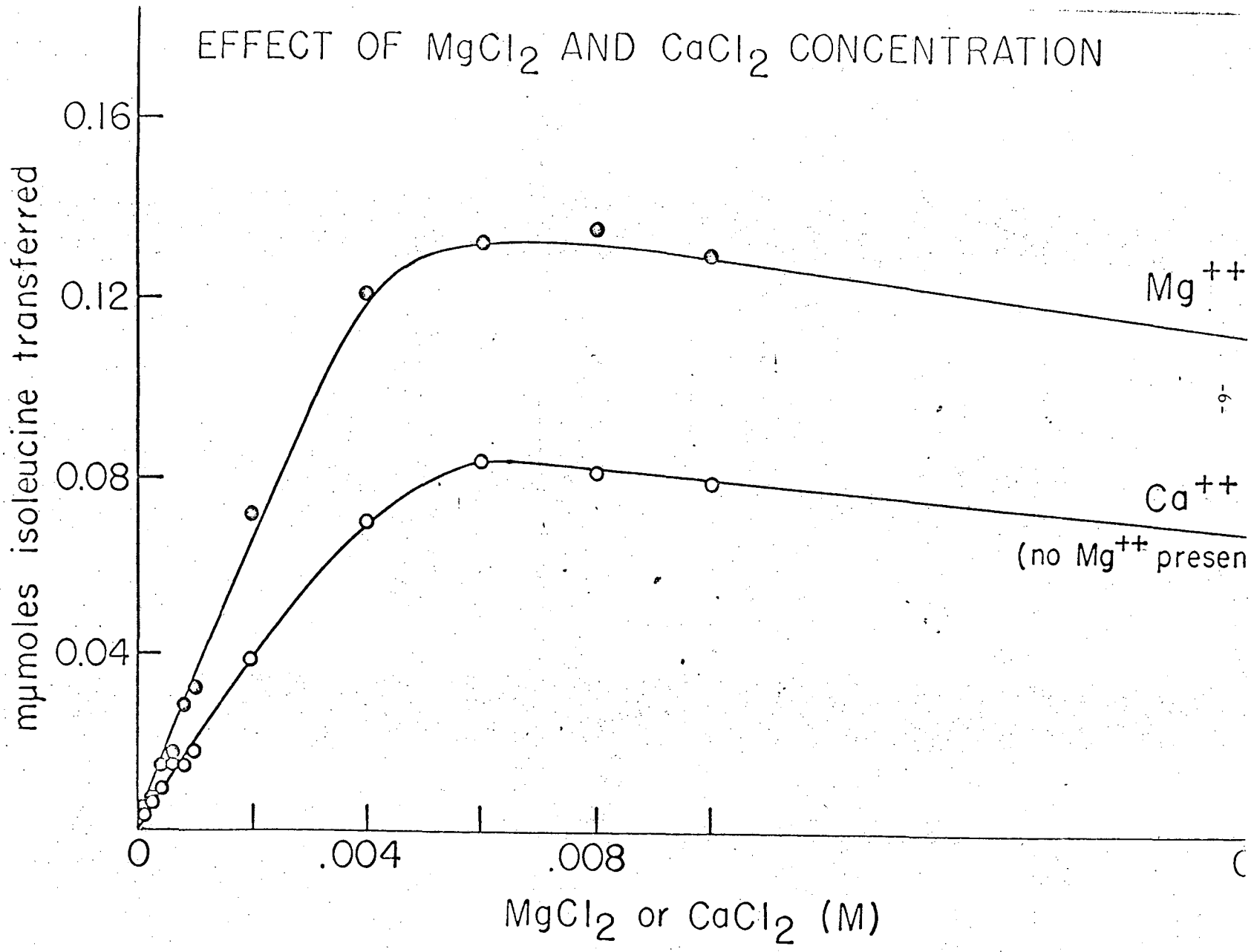


Fig. 3

XBL 6811

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

TECHNICAL INFORMATION DIVISION
LAWRENCE RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720