Short Communications and Preliminary Notes

ON ORNITHINE AND PROLINE SYNTHESIS IN *ESCHERICHIA COLI**

by

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Evidence has recently been obtained for the following path of ornithine synthesis in *Escherichia coli*: glutamic acid —> N-acetyl-glutamic acid —> N-acetylglutamic γ-semialdehyde —> N$^{\alpha}$-acetyl-ornithine —> ornithine[1,2]. The role of N-acetylglutamic acid was inferred in part from the finding[3] that extracts of *E. coli* contain an enzyme catalyzing the acetylation of glutamic acid, but the utilization of N-acetylglutamic acid in ornithine formation has thus far not been demonstrated. By the use of tracer methods as previously described[4], results have now been obtained indicating an *in vivo* utilization of N-acetylglutamic acid for ornithine synthesis.

For the utilization experiments, N-acetyl-L-glutamic acid was prepared from random-^{14}C-labelled L-glutamic acid and unlabelled acetic anhydride in aqueous alkali[5]. Labelled N-acetyl-L-glutamic acid (0.5 mg) and unlabeled L-glutamic acid (2.0 mg) were added to an aerated culture (20 ml) of the B strain of *E. coli* growing exponentially at 37° C in unlabelled glucose-salt medium. The bacteria were permitted to grow from an optical density of about 0.1 to 0.2, measured at 620 mμ. The organisms were then harvested and extracted successively with cold trichloracetic acid, alcohol, alcohol-ether, and hot trichloracetic acid, essentially as previously described[4]. The resulting protein residue was hydrolyzed and the amino acids obtained were separated by two-dimensional paper chromatography. A radioautogram revealed predominant labelling of arginine with some labelling of glutamic acid and proline, the molar specific activities of these three amino acids being in the ratio 1.00:0.01:0.03 respectively. The radioactivity of the arginine accounted for approximately 90% of that of the protein hydrolysate. The arginine was degraded with arginase, and all the radioactivity was found in the ornithine formed. During growth of the bacteria in the presence of the labelled N-acetyl-L-glutamic acid, 49 μg arginine were incorporated into the protein residue. Out of this amount of arginine, 13 μg were calculated to be derived from the labelled substrate, on the assumption that the five carbon atoms of the glutamic acid moiety of N-acetyl-L-glutamic acid gave rise to the five carbon atoms of the ornithine moiety of arginine. This assumption is consistent with the earlier finding that in similar experiments with labelled L-glutamic acid as supplement, the glutamic acid, ornithine (from arginine), and also proline of the protein residue became labelled at equal specific activity[6]. Since arginine is known to be produced from ornithine, these results provide evidence that N-acetyl-L-glutamic acid can indeed give rise to ornithine without intermediate formation of glutamic acid.

The observation that the labelled N-acetyl-L-glutamic acid led to a higher specific activity of proline than of glutamic acid points to a link other than glutamic acid between the routes of ornithine and proline formation. Thus, the existence of a minor path of proline synthesis, operating in addition to the major one which includes reduction of glutamic acid to glutamic γ-semialdehyde[7], is indicated.

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New evidence for the role of glutamic γ-semialdehyde as a major precursor of proline, but not of ornithine, and for the role of N^{α}-acetylornithine as a major precursor of ornithine has now been obtained from "isotopic competition"[8] experiments.

REFERENCES

1 H. J. VOGEL, *Proceedings of the 6th International Congress of Microbiology*, Rome, in press.
2 H. J. VOGEL, *Proc. Natl. Acad. Sci.*, in press.
3 W. K. MAAS, G. D. NOVELLI AND F. LIPMANN, *Proc. Natl. Acad. Sci.*, to be published.
4 P. H. ABELSON, E. T. BOLTON AND E. ALDOUS, *J. Biol. Chem.*, 198 (1952) 165.
5 V. DU VIGNEAUD AND C. E. MEYER, *J. Biol. Chem.*, 98 (1932) 295.
6 E. T. BOLTON AND P. H. ABELSON, 1952, unpublished.
7 H. J. VOGEL AND B. D. DAVIS, *J. Am. Chem. Soc.*, 74 (1952) 109.
8 R. B. ROBERTS AND I. Z. ROBERTS, *J. Cell. Comp. Physiol.*, 36 (1950) 15;
D. B. COWIE, E. T. BOLTON AND M. K. SANDS, *J. Bact.*, 62 (1951) 63;
P. H. ABELSON, E. T. BOLTON AND E. ALDOUS, *J. Biol. Chem.*, 198 (1952) 173.

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DISTRIBUTION OF XANTHINE OXIDASE IN ORGANS OF THE FROG (*RANA HEXADACTYLA*)

by

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The most common organ containing xanthine oxidase is the liver[1,2]. Pigeon, dog and hedgehog are the only exceptions recorded to date[1]. THUNBERG experiments[3] on the complete breakdown of ATP by liver preparations of various animals showed[4] that added ATP caused no decrease in methylene blue decolorisation time in the case of aqueous frog liver extract. This was traced to the absence of xanthine oxidase in frog liver extracts and homogenates. Results were consistently negative with one day old liver preparations [1:10] from ten well-nourished frogs, using xanthine as substrate and borate as buffer (pH 7.6). The enzyme was also absent from frog spleen, pancreas, lung and ovary. It was present in kidney. The ability of the frog to oxidise xanthine has been known only from *in vivo* studies[5]. Fresh frog liver homogenate contained no xanthine oxidase inhibitor when tested on purified milk xanthine oxidase. Addition of *kochsaft* from fresh frog liver homogenate to the milk enzyme caused slight methylene blue decolorisation. Frog liver may prove a convenient alternative to pigeon liver for studying hepatic purine synthesis. The reducing power of frog liver extract on methylene blue is much less than that of pigeon liver extract. No specimens of *R. temporaria* and *R. esculenta* were available for comparative experiments.

REFERENCES

1 E. J. MORGAN, *Biochem. J.*, 20 (1926) 1282.
2 M. FLORKIN AND G. DUCHATEAU-BOSSON, *Enzymologia*, 11 (1943) 24.
3 H. VON BULER AND B. SKARŻYNSKI, *Z. physiol. Chem.*, 263 (1940) 259.
4 H. C. FRIEDMANN, Thesis, University of Madras, 1950; *J. Madras Univ.*, Sec. B, 23 (2) (1953) in press.
5 ST. J. PRZYLECKI, *Arch. internat. de physiol.*, 24 (1925) 238.

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