THE INHIBITION BY SULPHANILAMIDE OF THE METABOLISM OF HISTIDINE IN GERMINATING SEEDS

V. M. SIVARAMAKRISHNAN AND P. S. SARMA

University Biochemical Laboratory, Madras-25

THE catabolism of histidine is mediated by the specific enzyme histidase,[1] which occurs in animal livers (especially cat liver), and also in the bacterium *Pseudomonas fluorescens*.[2] The presence of histidase in plants has not been reported. By the action of liver histidase, histidine is hydrolytically decomposed into ammonia, and a product, which on treatment with strong alkali or acid, yields a molecule of glutamic acid, one molecule of formic acid, and one more molecule of ammonia.[1] Thus glutamic acid or a derivative of it, is a product of the decomposition of histidine. Subsequent investigations (reviewed by Tabor[3]) have established urocanic acid, Lα-formamidinoglutaric acid, and N-formyl glutamic acid as intermediates in the conversion of histidine to glutamic acid, formic acid and ammonia.

Since the decomposition of histidine gives rise to formic acid, a single carbon unit, the vitamins, *p*-aminobenzoic acid and folic acid may be expected to take part in the degradation of histidine. Recently, Tabor and collaborators[4] have reported that feeding of histidine to folic acid-deficient rats increases the urinary excretion of a compound, which, on decomposition, gives a molecule each of glutamic acid, formic acid, and ammonia. This compound has now been identified to be α-formamidinoglutaric acid.[5] Our previous investigations[6] have shown that, during germination of greengram seeds (*Phaseolus radiatus*), the addition to the growth medium, of sulphanilamide, an antivitamin for p-aminobenzoic acid, produces an accumulation of histidine, due presumably to an inhibition of its catabolism. With the availability of histidine labelled with C^{14} in the α-carbon atom from the Tracerlab Inc., Boston, the degradation of histidine in germinating seeds, its possible conversion to glutamic acid, the inhibition by sulphanilamide of this conversion, and also the quantitative significance of this conversion in the overall metabolism of histidine have been investigated.

EXPERIMENTAL

The experiment consists essentially in germinating greengram seeds with radioactive histidine in the medium, isolating the glutamic acid as the hydrochloride, and histidine as nitranilate from the seedlings, and measuring the radioactivity in them.

25 g. of greengram seeds divided into five 5 g. lots were used for the germination. Each lot, after surface sterilization with 0·1% $HgCl_2$ solution and washing, was allowed to germinate inside a sterile 11 cm. petri dish containing a filter circle, and the sterilized medium. The medium consisted of 1·232 mg. of radioactive histidine, with an activity equal to $4{\cdot}128 \times 10^6$ c.p.m., and sterile water to 24 ml., which is just enough for 72 hr. germination. Germinations were carried out in a sterile chamber in diffuse light for 72 hr. at room temperature, which varied between 28 to 31° C.

Similar germinations with medium containing sulphanilamide also (640 μg./24 ml.) were conducted simultaneously to study the effect of sulphanilamide.

At the end of the germination, the seedlings as well as the petri dishes were washed, and the activity in the collected washings was determined. The seedlings were then hydrolysed with 6 N HCl for 22 hr., the excess HCl was removed by distillation *in vacuo*, the solution was diluted to precipitate out the humin, and then made up to a known volume. A sample of this solution was removed for the estimations of the amino acids, histidine according to Macpherson[7] and glutamic acid according to Meister, Sober and Tice,[8] as well as the total radioactivity after suitable dilution. The rest of the solution was used for the isolations. Carrier histidine (400 mg.) was added to facilitate the isolation of radioactive histidine.

From this solution, glutamic and aspartic acids were precipitated as their barium salts by Foremann-precipitation,[9] and the glutamic acid then separated as the hydrochloride according to conventional procedures given by Block and Bolling.[10] The glutamic acid hydrochloride was purified by repeated reprecipitations to constant activity. From the solution remaining after Foremann-precipitation, the barium was removed as sulphate, the solution acidified strongly with nitric acid, and then excess of silver nitrate solution added. The precipitated silver chloride was removed and the pH of the solution then adjusted to 7·4 with barium hydroxide solution, to precipitate the histidine as the silver salt. The histidine-silver salt was

then converted into pure histidine nitranilate, following closely the details given by Block and Bolling.[10] Radioactivity measurements were carried out with 0·1 mg. samples spread out as uniform thin layers in stainless steel planchets, 2·4 cm. in diam., with a windowless gas-flow counter, connected to an autoscaler and an Eagle Present Counter. All counts were taken with a probable statistical error not exceeding 2%. No corrections were made for self-absorption.

Results and Discussion

Table I

Distribution of radioactivity in greengram seeds germinated with α-C^{14}-labelled histidine in the medium, for 72 hr.

	Control	Sulphanilamide treated
Activity supplied as histidine	$2·064 \times 10^7$ c.p.m.	$2·064 \times 10^7$ c.p.m.
Activity in washings ..	Negligible	Negligible
Activity in the hydrolysate	$1·168 \times 10^7$ c p m.	$1·506 \times 10^7$ c.p.m.
Activity of histidine in the seedlings	$1·164 \times 10^7$ c.p.m.	$1·424 \times 10^7$ c.p.m
Degradation of histidine as % of activity supplied	43·6	31·0
Activity in glutamic acid	$3·373 \times 10^4$ c p.m.	$2·148 \times 10^4$ c p.m.
Activity in glutamic acid as % of total activity	0·1634	0·1041

It will be seen from Table I that, under normal conditions, there is a degradation of histidine to the extent of 43·6% in 72 hr. On the other hand, chemical determinations of histidine in the ungerminated seeds and 72. hr.-germinated seedlings actually point to a net synthesis of histidine to the extent of about 19%. This would mean that, during 72 hours' germination, there is a degradation of histidine by about 43·6%, and a concomitant synthesis of about 62·6%, resulting in the observed net synthesis of 19%. Histidine, then, is in a metabolically active state in germinating seeds. Similar results have been obtained with glutamic acid also.[11] While estimations of glutamic acid in the ungerminated seeds and 72 hr.-germinated seedlings showed a net fall in glutamic acid of only 35%, isotopic investigations revealed a degradation amounting to no less than 95%, pointing to a concomitant synthesis of about 60%, and hence to the high metabolic activity of glutamic acid in germinating seeds. The investigations with these amino acids suggest, that other amino acids too, are probably in a metabolically active state, undergoing both degradation and synthesis at the same time.

The glutamic acid isolated has been found to be radioactive. Thus, there is a conversion of histidine to glutamic acid in plants also, even as in animals[1] and bacteria.[2] When sulphanilamide is added to the medium, there is a greater retention of radioactivity, and a lesser degradation of histidine. Sulphanilamide, thus, inhibits the catabolism of histidine. At the same time, there is a decrease in the radioactivity of glutamic acid. This suggests that sulphanilamide inhibits the conversion of histidine to glutamic acid.

While this conversion seems to be an established one, a comparison of the activities in histidine and glutamic acid throws doubt as to the importance of this conversion as a major pathway of histidine metabolism in this species. A loss of 43·6% of the total activity in histidine is accompanied by a gain in glutamic acid of only 0·1634%. An accumulation of 12·6% of total activity in histidine by sulphanilamide inhibition is accompanied by a loss of only 0·0593% of total activity in glutamic acid. Thus, the changes in the radioactivity of glutamic acid are only minor fractions of the changes in the activity of histidine. As such, this conversion does not seem to be a major pathway in the metabolism of histidine, even when due allowance is made for the rapid catabolism of glutamic acid during germination.[11]

1. Edlbacher, S., *Z. Physiol. Chem.*, 1926, **157**, 106; *Ergeb. Enzymforsch.*, 1943, **9**, 131.
2. Tabor, H. and Hayaishi, O., *J. Biol. Chem.*, 1952, **194**, 171.
3. Tabor, H., in "*A Symposium on Amino Acid Metabolism*" (Ed. McElroy & Glass), The Johns Hopkins Press, 1955, p. 373.
4. —, Silverman M., Mehler, A. H., Daft, F. S. and Bauer, H., *J. Amer. Chem. Soc.*, 1953, **75**, 756.
5. Seegmiller, J. E., Silverman, M., Tabor, H. and Mehler, A. H., *Ibid.*, 1954, **76**, 6205.
6. Sivaramakrishnan, V. M. and Sarma, P. S., *J. Sci. Industr. Res.*, 1953, **12B**, 157.
7. Macpherson, H. T., *Biochem. J.*, 1946, **40**, 472.
8. Meister, A., Sober, H. A. and Tice, S. V., *J. Biol. Chem.* 1951, **189**, 591.
9. Foremann, F. W., *Biochem. J.*, 1914, **8**, 463.
10. Block, R. J. and Bolling, D., *The Amino Acid Composition of Proteins and Foods*, C. C. Thomas, Springfield, Ill., 1951.
11. Sivaramakrishnan, V. M. and Sarma, P. S., *Biochem. J.* (In Press).