INITIAL STEPS OF PURINE BIOSYNTHESIS IN WHEAT GERM

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Purine biosynthesis in bacteria and animal tissue is initiated by an enzymic reaction involving the formation of 5-phosphoribosylamine by amidation of 5-phosphoribosyl-1-pyrophosphate (PP-ribose-P). This highly unstable compound reacts with glycine and ATP to give rise to glycinamide ribonucleotide (GAR) in the presence of glycinamide ribonucleotide kinosynthase (Goldthwait et al 1956).

Hartman et al (1956) have emphasized the role of glutamine as a donor of the amide group of GAR, in pigeon liver. Recently, Nierlich and Magasanik (1961) have reported the formation of phosphoribosylamine directly from ammonia, ribose-5-P and ATP catalyzed by enzyme preparations obtained from Aerobacter aerogenes, E. coli and a chicken liver extract. There is no evidence as to which of these donors participates in a similar reaction in plants.

In the present study viable wheat embryos isolated according to the procedure of Johnston and Stern (1957) and commercial wheat germ (Maple Leaf Milling Company, Winnipeg) were used as sources of enzymes. In addition to glutamine and ammonia which have been used in the previous studies, asparagine

and carbamyl phosphate were also tested. Asparagine is known to accumulate in plant tissues to a considerable extent.

The reaction was conducted in three stages employing four enzyme preparations. The fraction of protein precipitating between 0-20 per cent ethanol obtained from acetone powder of wheat germ (I) and that from wheat embryos precipitating between 20-45 per cent saturation of ammonium sulphate (II) were both required for the formation of phosphoribosylamine in the first step. Fraction II was precipitated twice with ammonium sulphate. All the preparations were dissolved in Tris buffer (0.2M), pH 8.0. The second step was catalyzed by a fraction from wheat germ precipitating between 15-30 per cent ethanol (III). The formation of amino-imidazole carboxamide ribotide from IMP was brought about by a fraction precipitating between 33-65 per cent saturation of ammonium sulphate (IV) from wheat embryos.

The following reaction mixture was incubated for 50 minutes at 37°C: Tris-HCl buffer, pH 8.5, 60 μmoles; $MgCl_2$, 4 μmoles; glutathione, 1 μmole; enzymes I and II, 0.2 ml each (protein 17 and 21 mg/ml respectively); and one of the tested amide donors i.e. glutamine, asparagine, carbamyl phosphate or ammonium chloride, 4 μmoles. The reaction was started by adding 2.68 μmoles of PP-ribose-P to the systems at zero time or alternatively 4 μmoles of ribose-5-P and 2 μmoles of ATP.

After the first stage the pH of the reaction mixture was lowered to 7 by the addition of 100 μmoles of KH_2PO_4. The second stage incubation was then continued with 4.0 μmoles of glycine, 2.0 μmoles of ATP, and 0.2 ml of enzyme III (protein

40 mg/ml) for another 45 minutes. At the end of the second stage the following were added to the reaction mixture: EDTA, 30 μmoles; IMP, 2.0 μmoles and 0.2 ml of enzyme IV (protein 35 mg/ml) and the system incubated for a further 45 minutes. The reaction was then stopped with 0.1 ml of 30 per cent trichloroacetic acid and the diazotizable amine was measured by coupling with N-1-naphthylenediamine dihydrochloride and the amount of GAR determined by a slight modification of the standard procedure (Hartman et al 1958).

In a parallel experiment a two-stage incubation was carried out with the 'complete system' including glycine and enzyme III for 90 minutes, following which IMP was added and GAR assayed as before. Results of two typical experiments using ribose-5-P and PP-ribose-P as acceptors and various amide donors in the three-stage (A) and the two-stage (B) incubation systems are given in Table I. It is evident from the data presented that preincubation enhances the formation of GAR. The presence of glycinamide ribonucleotide was also confirmed by paper chromatography and electrophoresis.

We have reasonable evidence suggesting the possibility that each of these donors is associated with a separate enzyme. PP-ribose-P has been shown to accept the amide group readily from asparagine and less so from carbamyl phosphate and glutamine but it does not react at all with NH_4Cl. On the other hand ribose-5-P reacts most readily with ammonium chloride. Asparagine and carbamyl phosphate participate directly in the reaction and not through conversion to glutamine or ammonia.

Table I

Formation of GAR in the presence of various amide donors

Amide donor	mμmoles of GAR formed with ribose-5-P (A)	ribose-5-P (B)	PP-ribose-P (A)	PP-ribose-P (B)
Glutamine	2.00	0.00	7.20	4.40
Asparagine	10.10	1.40	21.00	17.00
NH_4Cl	12.30	9.90	2.45	0.98
Carbamyl phosphate	0.00	0.00	13.20	7.00

Although glutamine is utilized up to a certain extent, wheat embryos seem to utilize asparagine in preference to any other donor in the first step or purine biosynthesis, differing in this respect from bacteria and animal tissues.

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