Enzymic Synthesis of Xanthosine- and Guanosine-5-phosphate from Inosine-5-phosphate

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The studies of Greenberg and Buchanan *et al.*[1,2] have established IMP-5 * as a key intermediate in the biosynthesis of hypoxanthine in pigeon liver and considerable information has been obtained regarding the synthesis of this substance from smaller molecules *via* the formation of AIC-ribotide. Until recently very little was known, however, about the possible role of IMP-5 in the biosynthesis of other purine nucleotides. Carter and Cohen [3,4] have recently obtained evidence for a transformation of AIC-ribotide (or IMP-5) to AMP-5 by way of succinyl-AMP-5. With the discovery of this interesting reaction a connection has been established between IMP-5 and the biosynthesis of adenine nucleotides.

In view of the possibility that IMP-5 might be an intermediate in the biosynthesis of other purine nucleotides a study of the metabolism of this substance in pigeon liver extracts was carried out. When IMP-5-^{14}C was incubated with a dialyzed extract of acetone-dried pigeon liver in the presence of DPN, ATP, PGA or HDP, L-glutamine and L-glutamate, Mg^{++}-ions and phosphate buffer pH 7.4, two new radioactive peaks were obtained after chromatography on Dowex-2 with formic acid according to the method of Hurlbert *et al*[5]. These two peaks were tentatively identified as guanosine-5-phosphate and xanthosine-5-phosphate, respectively. Their identification rests on the following points: 1) A positive analysis for ribose and phosphate; 2) Acid hydrolysis yields guanine and xanthine, respectively, that can be crystallized to constant radioactivity after addition of authentic guanine or xanthine; 3) Complete dephosphorylation with *Crotalus* venom 5-phosphatase.

Further studies showed that the synthesis of GMP-5 occurred in two steps. In the first one, IMP-5 was oxidized to XMP-5 in the presence of DPN and phosphate buffer. ATP did not stimulate the reaction. If DPN was omitted there was no formation of XMP-5 (Table 1).

Table 1.

Expt. No.	Modifications of the medium		μmoles of XMP-5 formed
	DPN Nicotinamide	ATP HDP	
14	+	+	0.19
15	—	+	0.00
16	+	—	0.33
20			0.44

In expts. 14—16 a dialyzed extract of 500 mg acetone-dried pigeon liver was incubated at 37° C for one hour with 1 μmole IMP-5-^{14}C, 4 μmoles ATP, 100 μmoles HDP, 20 μmoles DPN, 120 μmoles nicotinamide, 150 μg pyridoxal phosphate, 120 μmoles L-aspartate, 240 μmoles L-glutamate, 60 μmoles L-glutamine, 120 μmoles NH_4Cl, 120 μmoles $MgSO_4$ and 1 000 μmoles K-phosphate buffer pH 7.4 with the modifications indicated in the table. Final volume 20 ml.

In expt. 20 a dialyzed extract was incubated under the same conditions with 1 μmole IMP-5-^{14}C, 20 μmoles DPN, 120 μmoles nicotinamide and 1 000 μmoles K-phosphate buffer pH 7.4. Final volume 15 ml.

When XMP-5-^{14}C obtained from this reaction was incubated with the extract in the presence of ATP, PGA, L-glutamine and L-glutamate, Mg^{++}-ions and phosphate buffer GMP-5 was obtained. Under these conditions no formation of IMP-5 could be detected. Further experiments indicated that with L-glutamine as amino group donor optimal synthesis of GMP-5 was obtained at lower concentrations of the amino acid than in corresponding experiments with L-glutamate. (Fig. 1) It was therefore tentatively concluded that

* The following abbreviations are employed: AIC-ribotide = 4-amino-5-imidazole-carboxamide ribotide, AMP-5 = adenosine-5-phosphate, ATP = adenosine triphosphate, DPN = diphosphopyridine nucleotide, GMP-5 = guanosine-5-phosphate, HDP = hexose diphosphate, IMP-5 = inosine-5-phosphate, PGA = 3-phosphoglyceric acid, XMP-5 = xanthosine-5-phosphate.

L-glutamine rather than L-glutamate was the specific amino group donor. Substituting L-aspartate or NH_4Cl for L-glutamine gave a very poor yield of GMP-5. When ATP was omitted no synthesis of GMP-5 was obtained (Table 2).

Table 2.

Expt. No.	Modifications of the medium					μmoles of GMP-5 formed
	ATP PGA	L-aspartate	L-glutamate	L-glutamine	NH_4Cl	
23	+	+	+	+	+	0.17
24	—	+	+	+	+	0.00
29	+	+	—	—	—	0.01
30	+	—	+	—	—	0.09
31	+	—	—	+	—	0.14
32	+	—	—	—	+	0.02

In expts. 23—24 a dialyzed extract of 500 mg acetone-dried pigeon liver was incubated at 37° C for one hour with 0.36 μmoles XMP-5-^{14}C, 2 μmoles ATP, 100 μmoles PGA, 200 μg pyridoxal phosphate, 120 μmoles L-aspartate, 240 μmoles L-glutamate, 60 μmoles L-glutamine, 120 μmoles NH_4Cl, 120 μmoles $MgSO_4$ and 1 000 μmoles K-phosphate buffer pH 7.4 with the modifications indicated in the table. Final volume 20 ml.

In expts. 29—32 a dialyzed extract was incubated under the same conditions with 0.20 μmoles XMP-5-^{14}C, 2 μmoles ATP, 100 μmoles PGA, 200 μg pyridoxal phosphate, 120 μmoles $MgSO_4$, 1 000 μmoles K-phosphate buffer pH 7.4 and 100 μmoles of either L-aspartate, L-glutamate, L-glutamine or NH_4Cl. Final volume 15 ml.

In view of these findings it seems justified to assume that the biosynthesis of GMP-5 from IMP-5 in pigeon liver goes by way of an oxidation to XMP-5 and a subsequent amination of this substance (eqn. 1).

$$\text{IMP-5} \xrightarrow{\text{DPN}} \text{XMP-5} \xrightarrow[\text{ATP}]{\text{L-glutamine}} \text{GMP-5} \quad (1)$$

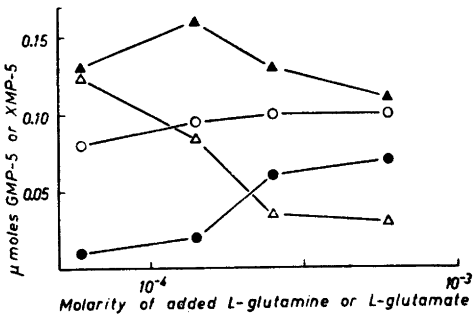

Fig. 1. Dependence of GMP-5 synthesis on L-glutamine or L-glutamate.

—○—○— *GMP-5 synthesized in experiments with L-glutamine.*
—△—△— *XMP-5 reisolated in experiments with L-glutamine.*
—●—●— *GMP-5 synthesized in experiments with L-glutamate.*
—▲—▲— *XMP-5 reisolated in experiments with L-glutamate.*

A dialyzed extract of 300 mg acetone-dried pigeon liver was incubated at 37° C for one hour with 0.30 μmoles XMP-5-^{14}C, 2 μmoles ATP, 50 μmoles PGA, 200 μg pyridoxal phosphate, 120 μmoles $MgSO_4$, 1 000 μmoles K-phosphate buffer pH 7.4 and L-glutamine or L-glutamate as indicated in the figure. Final volume 13 ml.

A full report of these findings will be given later.

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