The involvement of 5-phosphoribosylamine in the biosynthesis of glycinamide ribotide*

The synthesis of glycinamide ribotide[1,2,3], an early intermediate in the biosynthesis of purine nucleotides, is considered to proceed via the following reactions:

1. R5P** + ATP → PRPP + AMP[4]
2. PRPP + glutamine → PRA** + Glutamic acid + P-P
3. PRA + Glycine + ATP → Glycinamide ribotide + ? ADP + P

The synthesis of the glycinamide ribotide is measured by the fixation of glycine-1-^{14}C into a form which does not liberate $^{14}CO_2$ on reaction with ninhydrin[5]. Extracts of pigeon liver acetone powder have been shown to require R5P, ATP, glutamine and glycine for the synthesis of glycinamide ribotide[2]. The enzyme system has been fractionated by acidification to pH 5.5; the precipitate carries out reaction (1) while the supernatant fraction catalyzes reactions (2) and (3). PRPP*** can be substituted for R5P and the acid precipitate (Table I) thus providing evidence for reaction (1). However, with PRPP, ATP is still required for the synthesis of the glycinamide ribotide[3].

TABLE I

REQUIREMENT FOR PRPP AND ATP IN THE SYNTHESIS OF GLYCINAMIDE RIBOTIDE

Additions	*Acid Supernatant*	*Acid Precipitate*	*μmoles glicinamide ribotide formed*	
			Expt. 1	*Expt. 2*
R5P + ATP	—	+	0.50	—
R5P + ATP	+	—	0.09	0.04
PRPP	—	—	0.03	0.19
PRPP + ATP	+	—	0.30	0.90
PRPP (apyrase treated)§	+	—	—	0.03

R5P 5 μmoles; PRPP Expt. 1, 1.0 μmoles, Expt. 2, 1.1 μmoles; Glutamine 10 μmoles; ATP 1.2 μmoles; PGA 14 μmoles, 0.6 mg of an $(NH_4)_2SO_4$ fraction of a rabbit muscle extract. (S. RATNER AND A. PAPPAS, *J. Biol. Chem.*, 179 (1949) 1183); $MgCl_2$, 5 μmoles; tris buffer pH 8.0, 20 μmoles; glycine-1-^{14}C 5 μmoles; lyophilized acid supernatant fraction, 20 mg; acid precipitate, 1.2 mg; final volume, 1.1 ml; incubation 30′ at 37°. § PRPP was preincubated 10′ at 37° with 0.1 ml apyrase (P. S. KRISHNAN, *Arch. Biochem.*, 16 (1948) 474) to destroy ATP contamination. This did not hydrolize PRPP. Additions were then made for the 30′ incubation.

The reaction of PRPP with glutamine (2), catalyzed by a 0–20% alcohol fraction of the acid supernatant fraction, can be followed either by the disappearance of PRPP, as measured spectrophotometrically by its condensation with orotic acid to form uridylic acid[6], or by the liberation of orthophosphate[7]. Because of the presence of inorganic pyrophosphatase in the enzyme preparation, it cannot be stated definitely whether the initial product is pyrophosphate or orthophosphate. PRPP does not require ATP in reaction (2) and it does not react with glycine.

5-phosphoribosyl amine (PRA) was suggested as a product of reaction (2) by the results of a balance study (Table II). The appearance of 1.02 μmoles of orthophosphate (equivalent to 0.50 μmoles PRPP) was associated with the utilization of 0.64 μmoles of glutamine and the production of 0.60 μmoles of glutamic acid[8]. The product of reaction (2) has not yet been isolated from the reaction mixture. However, 5-phosphoribosylamine, a rather unstable compound, was synthesized by treating dry potassium ribose-5-phosphate with liquid ammonia[9]. This compound was found to substitute for PRPP and glutamine in the synthesis of glycinamide ribotide (Table III). This substitution resulted in a 3–4 fold increase in this synthesis. The product of the reaction of PRA with glycine and ATP (3) has been shown to migrate on paper chromatograms identically with glycinamide ribotide in 3 solvents. Since reaction (2) may involve a displacement of the pyrophosphate by the amide nitrogen of glutamine and subsequent hydrolysis of the glutamyl ribotide derivative, several

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** Abbreviations: R5P, ribose-5-phosphate; ATP, adenosine triphosphate; AMP, adenosine monophosphate; PRPP, 5-phosphoribosyl pyrophosphate; PRA, 5-phosphoribosyl amine; P-P, inorganic pyrophosphate; P, orthophosphate; GAR, glycinamide ribotide.

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enzymic steps may be involved. Likewise, in reaction (3), the requirement for ATP suggests that an activation mechanism occurs similar perhaps to that required for peptide bond synthesis[10].

TABLE II

REACTION OF PRPP WITH GLUTAMINE

Additions	*Pi formed µmoles*	*Glutamic acid formed µmoles*	*Glutamic utilized µmoles*
PRPP	0.34	—	—
Glutamine	—	0.05	0.13
PRPP + glutamine	1.36	0.65	0.77
Δ with both substrates	+ 1.02§	+ 0.60	+ 0.64

Additions: PRPP, 2.4 µmoles; glutamine, 2.0 µmoles; glycine, 5 µmoles; $MgCl_2$, 2.5 µmoles; tris buffer, pH 8.0, 25 µmoles; ethanol fraction of acid supernatant, 7.0 mg; final volume 0.6 ml; 20′ 37°. § 1.02 µmoles orthophosphate equals 0.50 µmoles PRPP.

TABLE III

FORMATION OF GLYCINAMIDE RIBOTIDE FROM PRA

Additions	*Glycinamide ribotide formed, µmoles*	
	Expt. 1	*Expt. 2*
PRPP + glutamine + ATP	0.32	0.13
R5P + glutamine + ATP	0.12	0.01
R5P + NH_3 + ATP	0.07	—
PRA + ATP	1.10	0.48
PRA + glutamine + ATP	0.78	—
PRA	—	0.01

Additions: PRPP 1.3 µmoles; R5P 5 µmoles; PRA 2 µmoles; glutamine 10 µmoles; ATP 1.2 µmoles; PGA 14 µmoles; 0.6 mg of $(NH_4)_2SO_4$ fraction of rabbit muscle extract; $MgCl_2$ 5 µmoles; tris buffer pH 8.0, 50 µmoles; NH_4Cl_2 2.5 µmoles; glycine-1-^{14}C 5 µmoles; ethanol fraction of acid supernatant, 0.2 ml. Final vol. 0.75 ml. Incubation 37°, Expt. 1, 30 min; Expt. 2, 20 min.

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Sur deux constituants hormonaux nouveaux du corps thyroïde: la 3:3′-diiodothyronine et la 3:3′:5′-triiodothyronine

L'existence dans le corps thyroïde et dans le produit de sa sécrétion de la L-thyroxine (3:5:3′:5′-tetraiodothyronine) et de la L-3:5:3′-triiodothyronine suggère l'hypothèse que ces hormones y sont accompagnées d'autres corps de la même série. Si, comme il est probable, la biosynthèse des iodothyronines s'opère par condensation des L-3-mono- et L-3:5-diiodotyrosines, avec élimination d'un reste d'alanine, on peut penser que la 3:3′-diiodothyronine et la 3:3′:5′-triiodothyronine doivent être présentes dans la glande avec leurs deux homologues déja connus.

Nous avons tout d'abord synthétisé la 3:3′-diiodothyronine et la 3:3′:5′-triiodothyronine[1], qui nous ont servi de corps de référence, et étudié par ailleurs leur identification chromatographique au moyen de solvants adaptés à leur séparation[2]. Les plus efficaces de ceux-ci se sont révélés être le mélange de *n*-butanol et de dioxane (4:1) à + 16° C et celui de méthanol et d'acétate d'ammonium 0.2 *M* (1:2.5) à pH: 6.1 et à + 3° C. Seule la 3:3′-diiodothyronine migre dans ce dernier solvant (R_F = 0.23), alors que les autres corps de la même série demeurent sur la ligne de départ, dans les