UTILIZATION OF PURINES AND THEIR DERIVATIVES BY *GAFFKYA HOMARI*[1]

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Gaffkya homari, a micrococcus isolated from septicemic lobsters, has an obligate purine requirement (Aaronson, 1956). Further study shows this requirement to be satisfied by several natural purines as well as by several purine analogues. This broad response suggested that *G. homari* might be useful for studying purine biosynthesis. We wished especially to know whether *G. homari* utilized 4-amino-5-imidazolecarboxamide ("carboxamide") and 4-amino-5-imidazolecarboxamidine ("amidine") in a manner similar to that reported for several other purine requiring microorganisms (Aaronson and Nathan, 1954; Aaronson, 1955).

MATERIALS AND METHODS

G. homari strain ATCC 10400 was used. The culture methods were essentially those used by Baker *et al.* (1953) and outlined in a previous communication (Aaronson, 1956).

The chemically defined medium used for these studies is the same as that described by Aaronson (1956), supplemented with a mixture of stimulatory amino acids.

Cells for incubation experiments were prepared by harvesting a 72 hr culture of *G. homari* grown on a shaker in a chemically defined medium containing hypoxanthine, 2.0 mg/100 ml and a mixture of amino acids. Cells were washed once in trishydroxymethylaminomethane ("Tris") buffer and then added to 5.0 ml of incubation mixture in a 15 by 120 mm tube to give a cell suspension with an optical density between 2.6 and 2.8. Tubes were incubated at 34 C on a shaker for 3 to 22 hr. The incubation mixtures were centrifuged at the end of each experiment and 0.02 ml of each supernatant was spotted on Whatman no. 1 chromatographic paper. Chromatograms were developed in an *iso*amyl-Na_2HPO_4 system described below. Ultraviolet absorbing spots were resolved with a "Mineralight."

All chemicals were obtained from commercial sources except where indicated. The purines, their derivatives and possible intermediates, appeared homogeneous by single dimension ascending paper chromatography with the following solvent systems: $Na_2HPO_4 \cdot 12H_2O$, 5 per cent (w/v) saturated with *iso*amyl alcohol and butanol:glacial acetic acid:water (4:1:5, v/v).

We thank Dr. M. E. Balis for imidazole-4, 5-dicarboxylic acid, ethyl-4-amino-5-imidazolecarboxylate, 4-hydroxy-5-imidazolecarboxamide, 4-amino-5-imidazolecarboxamide, and 4-amino-5-imidazolecarboxamidine; Dr. M. Eidinoff for ureidosuccinic acid; Dr. G. B. Elion for the thioguanine, 2,6-diaminopurine, and 8-azaguanine; Dr. G. H. Hitchings for 1-methyl guanine; Dr. K. Pfister for the imidazole-4-carboxamide, phenylazomalonamidine, malonamidine, and formamidomalonamidine; and Dr. E. Shaw for aminomalonamidine.

RESULTS

The ability of various purines, their ribosides and their ribotides to satisfy the purine requirement is shown in table 1. Not only is there less growth with purine ribotides (table 1), but it is appreciably slower. To determine if the ribosides and ribotides were utilized as such, *G. homari* cells were incubated for varying lengths of time with adenine, its riboside, and its several ribotides (table 2). In 3 to 5 hr, adenosine is completely broken down to adenine; the ribotides are only slightly converted to adenine in 22 hr. This slower conversion of the ribotides to their purine bases may account for the slower and poorer growth response made by *G. homari* when

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TABLE 1

The ability of compounds to satisfy the purine requirement of Gaffkya homari

Compound	Conc Range	Effect on Growth*
	mg/2ml	
Adenine	0.002–0.2	++
Guanine	0.002–0.2	++
Hypoxanthine	0.002–0.2	++
Xanthine	0.002–0.2	++
Uric acid	0.002–0.2	−
1-Methyl guanine	0.002–0.2	+
Thioguanine	0.002–0.2	+
Isoguanine	0.002–0.2	+
2,6-Diaminopurine	0.002–0.2	+
Theobromine	0.002–0.2	−
Theophylline	0.002–0.2	−
Caffeine	0.002–0.2	−
8-Azaguanine	0.002–0.2	−†
Adenosine	0.004–0.4	++
Guanosine	0.004–0.4	++
Inosine	0.004–0.4	++
Xanthosine	0.004–0.4	++
Adenosine-2′-phosphate	0.005–0.5	+
Adenosine-3′-phosphate	0.005–0.5	+
Adenosine-5′-phosphate	0.005–0.5	+
Guanylic acid	0.005–0.5	+
Inosine-5′-phosphate	0.005–0.5	+

* ++ = maximum growth; + = partial (50–75% of maximum growth); − = not utilized and does not spare growth in presence of utilized purines.

† Inhibitory.

TABLE 2

*Products formed from purines and their derivatives by incubation with Gaffkya homari cells**

Incubation Mixture	Rf of Ultraviolet Absorbing Spots	
	No cells	Cells
0.05 g/5 ml		
Tris buffer	none	none
Tris +		
Adenine	0.42	0.46
Adenosine	0.54	0.47
Adenylic acid (2′)	0.76	0.45†, 0.80
Adenylic acid (2′ + 3′)	0.72, 0.78	0.47†, 0.74, 0.80
Adenylic acid (5′)	0.78	0.79

* These are the results of a 5 hr incubation. Similar results obtained with 3 to 22 hr incubations.

† Faint spot.

TABLE 3

Some possible purine precursors and their ability to satisfy the purine requirement of Gaffkya homari

Compound*	Effect on Growth†
4-Amino-5-imidazolecarboxamide·HCl	+
(carboxamide)	+
4-Amino-5-imidazolecarboxamidine·2HCl (amidine)	+
4-Imidazolecarboxamide	−
4-Hydroxy-5-imidazolecarboxamide	−
4,5-Imidazoledicarboxylic acid	−
Ethyl-4-amino-5-imidazolecarboxylate·HCl	−
Phenylazomalonamidine·2HCl	−
Malonamidine·2HCl	−
Aminomalonamidine	−
Formamidomalonamidine·2HCl	−
Ureidosuccinic acid	−
2,4,6-Triaminopyrimidine	−
2,4,5,6-Tetraminopyrimidine	−

* Concentration range tested 0.002–0.2 mg/2 ml.

† + = partial (50–75% of maximum growth); − = not utilized and does not spare growth in presence of utilized purines.

TABLE 4

The ability of imidazoles and their respective purines to satisfy the purine requirement of Gaffkya homari

Compound	Conc	Optical Density
	μmoles/ml	
No purine		0
Adenine	2.2	0.12
	7.4	0.35
	22.2	0.60
	74.0	0.98
	222.0	1.06
Hypoxanthine	2.2	0.32
	22.2	0.53
	74.0	1.02
	222.0	1.08
Carboxamide	48.0	0.12
	144.0	0.33
	480.0	0.40
	1,440.0	0.54
Amidine	32.0	0.40
	96.0	0.28
	320.0	0.65
	960.0	0.63

the ribotides are the sole source of required purine.

Several imidazoles as well as other compounds perhaps involved in purine synthesis were tested for their ability to support growth (table 3); only carboxamide and amidine were active. In table 4 these two compounds are compared with their purine counterparts, hypoxanthine and adenine respectively, for their ability to satisfy the purine requirment. While they did not permit as rapid and abundant growth as purines, they nevertheless gave a definite growth response (one half growth for carboxamide and amidine). Samples were taken from experimental flasks after autoclaving and after incubation to assess the likelihood that the conditions of sterilization and incubation resulted in ring closure of the imidazoles to their respective purines. These samples were assayed by chromatography followed by ultraviolet absorption to identify the presence of purines. There is no ring closure of imidazoles to purines after either sterilization or incubation.

DISCUSSION

G. homari is unusual in the variety of purines, purine ribosides, and purine ribotides which satisfy its purine requirement. The growth with ribosides and ribotides may be attributed to its ability to split these compounds to their respective purine bases. The wide variety of purines utilized by this microorganism suggests its use in the study of the conversion of various purines and their derivatives to nucleic acid adenine and guanine, and possibly nucleic acid with purine analogue markers.

The purine ribotides exist as several isomers. Balis and co-workers (1953, 1955) have found that only the 3′ isomer of various ribotides were effectively incorporated into the nucleic acid of *Lactobacillus casei* and mutant of *Escherichia coli*, the 2′ and 5′ isomers being poorly or not at all incorporated. Weinfeld *et al.* (1955), however, found that both the 2′ and 3′ isomers of adenylic acid were incorporated into rat nucleic acid while the 5′ isomer was incorporated to a smaller extent. The nutritional experiments described here (table 1) indicate no difference in the utilization of 2′, 3′, or 5′ isomers of ribotides either as the ribotide itself or by their purine base hydrolysis products. *Staphylococcus flavocyaneus* was also able to utilize all three isomers (Aaronson, 1955). Although it is likely that in both *G. homari* and *S. flavocyaneus* the several ribotide isomers are utilized via preliminary hydrolysis to free purine base, it is also possible that they may be incorporated as ribotides. This must await testing by means of tracer experiments.

Some attempts have been made to determine the utilization of compounds which are involved in *in vitro* purine synthesis such as malonic acid derivatives, imidazole derivatives, etc. Bergmann *et al.* (1952) found that purine requiring mutants of *E. coli* were unable to utilize 4-acetoamidoimidazole-5-carboxamide, aminomalonamidine, and formamidomalonamidine while they did use carboxamide. Buchanan *et al.* (1955) found that labeled aminomalonic acid, aminomalonamide, aminomalonamidine, and 5-amino-4-imidazolecarboxylic acid, ethylester were not precursors of nucleic acid adenine and guanine in the rat. The malonic acid derivatives mentioned above were also very inefficient precursors of inosinic acid in a pigeon liver system.

Imidazoles have been implicated in both *in vitro* and *in vivo* purine synthesis: in yeast (Williams and Buchanan, 1953); in the rat (Miller *et al.*, 1950); in pigeon liver homogenates (Schulman and Buchanan, 1952).

Among the several compounds involved in the chemical synthesis of purines only 4-amino-5-imidazolecarboxamide and 4-amino-5-imidazolecarboxamidine are utilized by *G. homari*. The role of carboxamide in purine biosynthesis has been discussed elsewhere (Greenberg, 1953); we would like merely to note that amidine may function similarly particularly in adenine biosynthesis. This is suggested by the more efficient use of amidine rather than carboxamide by *G. homari*; amidine is twice as active as carboxamide for *S. flavocyaneus* (Aaronson, 1955); amidine but not carboxamide satisfies the purine requirement of *Crithidia fasciculata* (Aaronson and Nathan, 1954); amidine satisfies the purine requirement of 12 different microorganisms representing 8 genera while carboxamide was used by only 5 of these microorganisms (Nimmo-Smith, 1954); amidine but not carboxamide is the only imidazole which replaced adenine for *Strigomonas oncapelti* (Nathan, 1957, *personal communication*).

SUMMARY

The purine requirement of *Gaffkya homari* is satisfied by a wide array of purines, their derivatives, or their analogues. The purine bases and

ribosides were more active than the ribotides. The 2′, 3′, and 5′ ribotides were utilized equally well.

Of several potential intermediates in the biosynthesis of purines only 4-amino-5-imidazolecarboxamidine (amidine) and 4-amino-5-imidazolecarboxamide (carboxamide) were utilized. *G. homari* utilized amidine more efficiently than carboxamide indicating a role for a compound of this configuration in purine biosynthesis.

REFERENCES

AARONSON, S. 1955 The purine requirement of *Staphylococcus flavocyaneus*. J. Gen. Microbiol., **12**, 147–155.

AARONSON, S. 1956 A biochemical-taxonomic study of a marine micrococcus, *Gaffkya homari*, and a terrestrial counterpart. J. Gen. Microbiol., **15**, 478–484.

AARONSON, S. AND NATHAN, H. A. 1954 Utilization of imidazole counterparts of purines in microbial systems. Biochim. et Biophys. Acta, **15**, 306–307.

BAKER, H., SOBOTKA, H., AND HUTNER, S. H. 1953 Growth requirements of some thermophilic and mesophilic bacilli. J. Gen. Microbiol., **9**, 485–493.

BALIS, M. E., LEVIN, D. H., AND BROWN, G. B. 1953 The utilization of purine ribotides by *Lactobacillus casei*. J. Biol. Chem., **200**, 1–6.

BALIS, M. E., LARK, C. T., AND LUZZATI, D. 1955 Nucleotide utilization by *Escherichia coli*. J. Biol. Chem., **212**, 641–645.

BERGMANN, E. D., BEN-ISHAI, R., AND VOLCANI, B. E. 1952 Role of 4-amino-imidazole-5-carboxamide in purine synthesis by *Escherichia coli*. J. Biol. Chem., **194**, 531–537.

BUCHANAN, J. M., LEVENBERG, B., FLAKS, J. G., AND GLADNER, J. A. 1955 Interrelationships of amino acid metabolism with purine biosynthesis. In *A symposium on amino acid metabolism*, pp. 743–764. Edited by W. D. McElroy and H. Bentley Glass. The Johns Hopkins Press, Baltimore.

GREENBERG, G. R. 1953 Mechanisms involved in the biosynthesis of purines. Federation Proc., **12**, 651–659.

MILLER, C. S., GURIN, S., AND WILSON, D. W. 1950 C^{14} Labeled 4(5)-amino-5(4)-imidazolecarboxamide in the biosynthesis of purines. Science, **112**, 654–655.

NIMMO-SMITH, R. H. 1954 The function of folic acid in the biosynthesis of purine and pyrimidine derivatives. In *Ciba Foundation symposium on chemistry and biology of pteridines*, pp. 237–249. Edited by G. E. W. Wolstenholme and M. P. Cameron. J. A. Churchill, Ltd., London.

SCHULMAN, M. AND BUCHANAN, J. M. 1952 Biosynthesis of the purines. II. Metabolism of 4-amino-5-imidazolecarboxamide in pigeon liver. J. Biol. Chem., **196**, 513–26.

WEINFELD, H., ROLL, P. M., AND BROWN, G. B. 1955 The utilization of nucleotides by the mammal. III. Isomeric purine nucleotides labeled with C^{14}. J. Biol. Chem., **213**, 523–531.

WILLIAMS, W. J. AND BUCHANAN, J. M. 1953 Biosynthesis of the purines. IV. The metabolism of 4-amino-5-imidazolecarboxamide in yeast. J. Biol. Chem., **202**, 253–262.