ROLE OF 4-AMINOIMIDAZOLE-5-CARBOXYAMIDE IN PURINE SYNTHESIS BY ESCHERICHIA COLI

BY ERNST D. BERGMANN, RUTH BEN-ISHAI,* AND BENJAMIN E. VOLCANI

(From The Weizmann Institute of Science, Rehovoth, Israel)

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In a previous communication (1) it has been shown that the accumulation of 4-aminoimidazole-5-carboxamide ("amine") in sulfadiazine-inhibited Escherichia coli is suppressed by the addition of methionine and catalytic quantities of p-aminobenzoic acid (PABA), and it was assumed that the "labile methyl" group of the sulfur-containing amino acid is used, with the help of PABA as carrier, for the completion of the "amine" structure to the purine skeleton. The implication that the "amine" is a precursor of the purines still required experimental proof.

In the present investigation, an attempt was made to solve the question by the use of purine-requiring mutants of E. coli. If it could be shown that these mutants respond to the "amine" in a similar manner as to purines, it could be concluded that the "amine" is converted into purines. This is, indeed, the case.

The utility of 4-formamidoimidazole-5-carboxamide, 4-acetamidoimidazole-5-carboxamide, formamidomalonamidamidine hydrochloride, and aminomalonamidamidine dihydrochloride as precursors of the purine bases also was tested, and the effect of PABA, folic acid, and vitamin B₁₂ on the biosynthesis of the purines was studied.

Some of the results have been briefly reported elsewhere (2).

EXPERIMENTAL

Cultures and Media—The E. coli purine-requiring mutants M55B-46 (fast adenine, slow xanthine), M55B-75 (fast adenine or xanthine), M45B-4 (fast adenine, negative xanthine), and M43-25 (adenine slow), kindly supplied by Dr. B. D. Davis, were carried as slant cultures on the mineral medium described by Davis and Mingioli (3), supplemented with 0.2 per cent yeast extract. For the preparation of inocula, a small amount of surface growth from an 8 hour culture was suspended in sterile 0.9 per cent sodium chloride solution to a turbidity reading 60 on the Klett-Summerson photoelectric colorimeter (red filter No. 64) and was then further diluted

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1:33 in saline. Each experimental culture of 5 ml. was inoculated with 1 drop of this suspension. The basal medium used was that of Davis and Mingioli (3) but without agar.

Tested materials were added in the desired concentrations to 2.5 ml. of the double strength medium in 18 × 150 mm. Pyrex culture tubes. The cultures were then diluted with distilled water, capped, and autoclaved at 15 pounds pressure for 15 minutes. 4-Aminoimidazole-5-carboxamide hydrochloride (“amine” (4)), 4-formamidoimidazole-5-carboxamide (5), 4-acetamidoimidazole-5-carboxamide, formamidomalonamidamidine hydrochloride (4), aminomalondiamidine dihydrochloride (4), and vitamin B₁₂ were added aseptically from glass-filtered sterile solutions freshly prepared in distilled water. The cultures were incubated at 37° for 18, 20, 24, or 40 hours and their turbidity was determined as described above. All experiments were carried out in duplicate.

4-Acetamidoimidazole-5-carboxamide—A mixture of 700 mg. of 4-aminoimidazole-5-carboxamide hydrochloride, 340 mg. of sodium acetate, 0.5 ml. of acetic anhydride, and 1.5 ml. of glacial acetic acid was heated for half an hour at 70° and for a further half hour at 100°. The solution was concentrated to dryness in vacuo; 5 ml. of water were added and the solid product filtered and recrystallized from water; m.p. 220°.

Analysis—C₅H₇O₃N₄. Calculated. C 42.6, H 4.7, CH₂CO 25.5

Found. C 42.3, H 5.0, CH₂CO 24.5

The compound does not react with diazonium salts.

Results

Growth Response of Mutants to “Amine” in Presence and Absence of Purine Bases—Mutants M55B-46 and M55B-75 utilized the “amine” instead of adenine; the utilization was somewhat enhanced by 4 γ per ml. of adenine (Table I). Other purine bases showed effects similar to adenine. Mutants M45B-4 and M43-25 did not utilize the “amine,” even after 64 hours of incubation.

Rate of Utilization of Adenine and “Amine” by Mutant M55B-46—Of the various mutants tested, M55B-46 responded most actively to the “amine;” it was, therefore, used to compare the rates of utilization of the “amine” and of adenine. When the organism was grown in the presence of adenine, full growth was obtained after 18 hours of incubation. Fig. 1 shows that the utilization of the “amine” takes much longer than that of adenine.

Fig. 2 shows that the utilization of the “amine” is enhanced by vitamin B₁₂, especially at levels above 15 per cent of full growth. The vitamin had no effect on the utilization of any of the purine bases.
Neither PABA (0.02 to 2 γ per ml.) nor folic acid (0.005 to 0.5 γ per ml.) showed any influence on the utilization of the "amine" similar to that of vitamin B₁₂, nor did either enhance the stimulatory effect of the latter.

**TABLE I**

*Growth Response of Purineless Mutants of E. Coli to "Amine" in Presence and Absence of Adenine*

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Time of incubation</th>
<th>Turbidity readings*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amine, 240 γ per ml.</td>
<td>Amine, 500 γ per ml.</td>
<td>Amine, 30 γ per ml.; adenine, 4 γ per ml.</td>
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</tr>
<tr>
<td>M55B-46</td>
<td>20</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>M55B-75</td>
<td>20</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>M45B-4</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M43-25</td>
<td>48</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Full growth = a turbidity reading of 90.

**FIG. 1.** Growth of an E. coli purineless mutant in the presence of "amine" at various periods of incubation. Curve 1, after 18 hours; Curve 2, after 20 hours; Curve 3, after 24 hours.

In view of the recently emphasized analogy between the action of methionine and vitamin B₁₂ (3) and the ability of the former to counteract the accumulation of the "amine," the effect of DL-methionine on the utili-
zation of "amine" by the mutant M55B-46 was studied. Fig. 3 shows that methionine decreases the utilization of the "amine" significantly, that of adenine slightly. The effect is not significantly altered by PABA, folic acid, or vitamin B₁₂.

Growth Response of Mutant M55B-46 to 4-Formamido- and 4-Acetamidoimidazole-5-carboxamide—The utilization of the formyl derivative, a possible intermediate in the synthesis of the purines, is greater than that of the "amine," leading to 60 per cent of full growth with 60 γ per ml. of "formylamine" in 20 hours; larger quantities of the formyl derivative failed to raise the growth above this level (Fig. 4). Suboptimum doses of the purine bases enhance the utilization of the "formylamine," while vitamin B₁₂, PABA, and folic acid have no effect.

The mutant did not respond to the acetyl derivative of the "amine" in concentrations of 20 to 500 γ per ml., either alone or in combination with purine bases, vitamin B₁₂, folic acid, or PABA.

Aminomalonalaminamidine dihydrochloride and formamidomalonalaminamidine hydrochloride, which differ from the "amine" by 1 carbon atom in the case of the former and by the absence of the ring in the case of the latter, might be precursors of the "amine" and thus of the purine bases. In some experiments, concentrations of 400 to 600 γ per ml. of the former yielded full growth after 40 hours of incubation. These results, however, were not constant, and may be due to back-mutations induced by this compound. The formamidomalonalaminamidine yields growth only to the extent to which it is actually converted into the "amine" during incubation.
**Fig. 3.** Effect of D,L-methionine on the growth of an *E. coli* purineless mutant in the presence of "amine" or adenine. Curves 1 and 2, with 120 and 150 γ per ml. of "amine," respectively; Curve 3, with 8 γ per ml. of adenine.

**Fig. 4.** Growth response of an *E. coli* purineless mutant to 4-formamidoimidazole-5-carboxamide in the presence and absence of adenine. Curve 1, 14 γ per ml. of adenine (full growth); Curve 2, formyl compound; Curve 3, 4 γ per ml. of adenine plus the formyl compound.
Of the four purineless mutants of *E. coli* investigated, two utilized the "amine;" *viz.*, one-fifteenth and one-thirtieth as efficiently as they did the purine bases. In the presence of suboptimum amounts of the purines, a sparing effect exists.

These findings appeared to contradict those of Gots (6); however, more recently, when the same strain as that employed in the present investigation was used, he also found the "amine" to be utilizable (7). The conclusion that the "amine" can serve as a precursor of the purines in *E. coli* appears valid, as it has also proved correct for yeast (8), *Lactobacillus arabinosus* (9), pigeon liver homogenates (10), and in a purineless mutant of *Ophiostoma* (11).

The completion of the purine skeleton by incorporation of C₂, which resembles a known chemical synthesis of purines (12–14), is most probably an enzymatic effect, similar to that produced by the formylase of Knox and Mehler (15); it appears that for this reaction a coenzyme is required, which might be vitamin B₁₂. This vitamin increases the rate of utilization of the "amine," but has no stimulating effect on the growth of the purineless mutant in a medium containing purine bases.

It seemed of interest to pursue the question one step further and to study the utilization of 4-formamidoimidazole-5-carboxamide by the mutant. The substance was found to be twice as active as the "amine," but, in contradistinction to the "amine," it failed to cause more than 60 per cent of full growth. The, however limited, utilizability of the 4-formamidoimidazole-5-carboxamide recalls the results of Schulman et al. (10) with pigeon liver homogenates; these authors have demonstrated the participation of formic acid in the synthesis of hypoxanthine from the "amine." Also Eakin (16) has stated that for some bacteria the effectiveness of the "amine" as a purine substitute is enhanced by formic acid.

In the conversion of 4-formamidoimidazole-5-carboxamide into purine bases, not only are folic acid and PABA inactive, but, in contradistinction to the case of the "amine," vitamin B₁₂ is also inactive. From these observations, it can be concluded that there are two possible pathways in the synthesis of the purine nucleus: either the "amine" is converted into the purines with the help of vitamin B₁₂, by-passing the formyl compound, or the "amine" is converted into the purine bases through its N-formyl derivative and vitamin B₁₂ is necessary for the formylation, but not the cyclization, step. Indeed, vitamin B₁₂ has already been recognized as a mobilizer of methyl groups and seems particularly apt to facilitate the participation of C₁ compounds in biosyntheses.

In view of the easy cyclization of the formyl compound to hypoxanthine under laboratory conditions, it was necessary to prove that the observa-
tions recorded here are not due to the presence of hypoxanthine in the preparation of 4-formamidomidazole-5-carboxamide used in the experiments. However, neither bioassay nor chromatography of the formyl compound, according to Vischer and Chargaff (17) and Shaw (5), showed any trace of inhomogeneity. In control experiments, the presence of 10 per cent of hypoxanthine in the formyl compound could be detected by the chromatographic method.

The authors are indebted to Dr. B. D. Davis for the cultures of the purine mutants used in this investigation.

SUMMARY

1. Purineless mutants of *Escherichia coli* have been found which utilize 4-aminoimidazole-5-carboxamide ("amine") instead of purines. Vitamin B$_{12}$ increases the rate of this utilization.

2. 4-Formamidimidazole-5-carboxamide is twice as active as the "amine" in replacing the purine bases. Vitamin B$_{12}$ has no effect on the utilization of the formyl compound.

3. 4-Acetamidomidazole-5-carboxamide, aminomalonicamidamide, and formamidomalonicamidamide are not utilized by the purineless mutants of *E. coli*.

4. It is concluded that the "amine" is the precursor of the purine bases in *E. coli*.

BIBLIOGRAPHY