A ROLE FOR BIOTIN IN PURINE BIOSYNTHESIS*

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During studies on the amino acid nutrition of yeast in relation to biotin deficiency (1), our attention was drawn to a report by Chamberlain, Cutts, and Rainbow (2) which described the accumulation of an arylamine by yeast when grown under conditions of biotin deficiency in the presence of an excess of methionine. With use of the strain of yeast with which we had been working in our laboratory, *Saccharomyces cerevisiae*, Fleischmann strain 139, we were able to confirm these basic findings and subsequently to discern the nature of the arylamine, the factors which influence its production, and its relationship to purine biosynthesis by yeast.

**Materials and Methods**

_Cultures—*_S. cerevisiae_, Fleischmann strain 139, was employed as the test organism. The inoculum culture was grown for one transfer (24 hours at 30–32°) in the basal medium at 0.001 γ of biotin per ml. For growth experiments this culture was washed and resuspended to the original volume and then diluted 1:100 in sterile distilled water for use as an inoculum. Tubes containing 10 ml. of medium were inoculated with 0.1 ml. of this dilute suspension. For larger cultures, from which cell suspensions were prepared, a sufficient quantity of the inoculum culture containing 0.001 γ of biotin per ml. was transferred to the fresh medium to give the desired biotin concentration. The medium employed was that of Snell _et al._ (3), modified to include 100 γ of nicotinic acid per liter. _p_-Aminobenzoic acid was omitted since it was not required by our strain of yeast and interfered with the test for aromatic amines.

_Materials—*_4-Amino-5-imidazolecarboxamide* was purchased from the California Foundation for Biochemical Research. A reference sample was obtained from Dr. S. Gurin, Department of Physiological Chemistry, University of Pennsylvania. _4-Amino-5-imidazolecarboxamidine* was purchased from the Earle Laboratories, Peekskill, New York. A reference sample was obtained from Dr. M. E. Balis of the Sloan-Kettering Institute.

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for Cancer Research. 4-Nitroimidazole was purchased from the Earle Laboratories. 4-Aminoimidazole was prepared from 4-nitroimidazole by reduction in the presence of powdered zinc and hydrochloric acid and was used immediately.

Methods—Growth was determined turbidimetrically with an Evelyn colorimeter at 500 to 540 mμ wave length. Spectrophotometric analyses were performed on a model DU Beckman spectrophotometer. Diazotizable amines were determined by the method of Bratton and Marshall (4), and imidazoles by the method of Pauly (5), as modified by Ames and Mitchell (6).

### Table I

Comparison of Absorption Spectra of Unknown Arylamine with Spectra of Known Compounds

<table>
<thead>
<tr>
<th>Compound tested</th>
<th>Wave length of maximal absorption</th>
<th>Visible color of Bratton-Marshall reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ultraviolet region</td>
<td>Visible*</td>
</tr>
<tr>
<td>Unknown arylamine</td>
<td>248 μm</td>
<td>495 μm</td>
</tr>
<tr>
<td>Aminoimidazolecarboxamide</td>
<td>268 μm</td>
<td>545 μm</td>
</tr>
<tr>
<td>Aminoimidazolecarboxamidine</td>
<td>285 μm</td>
<td>545 μm</td>
</tr>
<tr>
<td>Aminoimidazole</td>
<td>248 μm</td>
<td>495 μm</td>
</tr>
<tr>
<td>Nitroimidazole</td>
<td>300 μm</td>
<td>None</td>
</tr>
<tr>
<td>p-Aminobenzoic acid</td>
<td>278 μm</td>
<td>545 μm</td>
</tr>
</tbody>
</table>

* Bratton-Marshall color complex.

Results

The intermediate accumulated by *S. cerevisiae* under the conditions described can be identified as an arylamine by a positive Bratton-Marshall reaction (4) and as an imidazole on the basis of a positive Pauly reaction (5, 6). Its absorption spectrum differs from those of *p*-aminobenzoic acid, 4-amino-5-imidazolecarboxamide, and 4-amino-5-imidazolecarboxamidine. Its extreme lability to heat or oxygen suggested that the compound might be 4-aminoimidazole or a closely related derivative (7–9). Comparison of the absorption spectrum of 4-aminoimidazole with that of the accumulated arylamine strongly indicates that our unknown compound is either identical with, or very closely related to, this substance (Table I). Purification of the arylamine has not progressed to the point where further chemical identification is possible.

Detailed study of the factors which influence accumulation of the arylamine showed that a biotin concentration of $10^{-8}$ to $10^{-4}$ γ per ml., methi-
onine and glutamic acid concentrations in excess of 250 γ per ml., an incubation temperature of 30-32°, and an incubation period of 72 hours were necessary for maximal production. Fig. 1 shows the relationship of arylamine accumulation to time and biotin concentration. Slightly higher

![Graph showing the relationship of arylamine accumulation to time and biotin concentration.](image)

**Fig. 1.** The effect of incubation time and biotin concentration on the production of arylamine by growing cultures of *S. cerevisiae*. Cultures were incubated at 30-32° in the medium described in the text, supplemented with 250 γ per ml. of methionine and glutamic acid. X, 24 hours; Δ, 48 hours; ●, 72 hours; O, 168 hours.

![Graph showing the effect of methionine, glutamic acid, and adenine on the accumulation of arylamine.](image)

**Fig. 2.** The effect of methionine, glutamic acid, and adenine on the accumulation of arylamine by growing cultures of *S. cerevisiae*. Cultures were incubated at 30-32° in the basal medium described for an incubation period of 72 hours. (For the purposes of reference, it should be noted that aspartic acid produced an effect similar in magnitude to that of adenine at concentrations from 250 to 500 γ per ml.)
yields of arylamine were obtained if the culture was incubated at room temperature, but only after inconveniently long incubation periods of 96 to 140 hours. Fig. 2 shows the effects of methionine, glutamic acid, and adenine on the accumulation of aminimidazole. The increased yields with glutamic acid or methionine appear to result independently of each other,
since yields of arylamine are greater in the presence of both amino acids than in the presence of equivalent concentrations of either one alone. Casein hydrolysate also increased the yield of the arylamine, presumably because of its methionine and glutamic acid content.

Certain amino acids, especially aspartic acid,1 inhibited arylamine formation. This effect may correlate with the fact that aspartic acid "spares" biotin in the nutrition of this yeast (1) or with its antagonistic action toward glutamic acid utilization noted in other systems (10). Cysteine and cystine, which also inhibit the growth of the organism, prevent arylamine accumulation. Purines decrease the yield, even in the presence of methionine and glutamic acid.

Surprisingly, glycine did not stimulate production of arylamine. However, a high concentration of glycine is present in the free amino acid pool of this yeast when it is grown under the conditions of biotin deficiency.2 Thus, glycine is not the limiting factor for arylamine production under our conditions.

Arylamine production by washed cell suspensions of biotin-deficient yeast also occurs during short time intervals. Again, arylamine production is optimal in the presence of both methionine and glutamic acid (Fig. 3).

DISCUSSION

The observations described here may be related to purine biosynthesis by this yeast in the following manner: (a) Under the conditions of biotin deficiency, the conversion of aminoimidazole to 4-amino-5-imidazolecarboxamide is blocked (Fig. 4). (b) The effect of methionine and glutamic acid in causing an increase in the amount of aminoimidazole accumulation may be visualized as stimulating the series of reactions leading up to this point. That these two amino acids are causing further accumulation by virtue of posing additional inhibitory conditions upon the cell seems less likely in view of the fact that both methionine and glutamic acid are known to contribute to purine formation (11, 12). (c) Whether biotin is concerned directly in the conversion of aminoimidazole to the carboxamide or is operating through its connection with the formation of aspartic acid is not delineated by the evidence which has been presented here. However, a direct effect is possible since biotin has been implicated in both carbon dioxide fixation and in the assimilation of ammonia (13). The possible role of 4-amino-5-imidazolecarboxylic acid as an intermediate in this series of reactions is suggested by the studies of Rabinowitz (14); a single step conversion of 4-aminoimidazole to the aminocarboxamide by carboxylation is equally as tenable at this point. Regardless of whether the action of

1 The effect of aspartic acid is comparable to that of adenine, as shown in Fig. 2.

biotin is direct or indirect, it is evident that this vitamin exerts a definite and strong influence on the synthesis of purine bases by yeast.

Additional evidence that aminoimidazole is a precursor to the purine bases in yeast is derived from the fact that an adenine-requiring mutant of *S. cerevisiae* (mutant 8553) obtained from Dr. C. C. Lindegren (15) also accumulates an arylamine which exhibits the same physical and chemical properties as aminoimidazole. Aminoimidazole accumulates in cultures of this mutant if either adenine or biotin is present at suboptimal concentrations. Further studies with this adenine-requiring yeast may shed further light on the action of biotin in purine biosynthesis.

![Diagram](attachment:biotin_in_purine_biosynthesis.png)

**Fig. 4.** Potential sites of biotin action in the conversion of 4-aminoimidazole to 4-amino-5-imidazolecarboxamide by *S. cerevisiae*.

**SUMMARY**

1. Under the conditions of biotin deficiency and in the presence of an excess of methionine and glutamic acid, *Saccharomyces cerevisiae* accumulates an aromatic amine.

2. This aromatic amine has been identified as 4-aminoimidazole by its chemical reactions, its absorption spectrum, and its extreme lability to heat and oxygen.

3. Biotin controls further utilization of this arylamine for purine formation by this yeast.

4. Production of the arylamine can be demonstrated in both growing cultures and in washed cell suspensions of biotin-deficient yeast.

**BIBLIOGRAPHY**

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