RESPONSE OF AEROBACTER AEROGENES METHIONINE AUXOTROPHS TO
ADENINE THIOMETHYL COMPOUNDS

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Received for publication August 9, 1952

It has been shown that many microorganisms produce thiomethyl adenosine from methionine (Smith and Schlenk, 1952). This prompted biochemical studies on the relationship between methionine and thiomethyl adenosine, which resulted in the discovery of methionyl adenosine as the key intermediate (Catoni, 1952; Schlenk, 1952). Replacement of methionine by thiomethyl adenosine has not been demonstrated with methionine auxotrophs of Escherichia coli (Davis and Mingioli, 1950). However, thiomethyl adenosine has been shown to replace the adenine requirement of Lactobacillus arabinosus (Schlenk and Gingrich, 1944) and the adenine requirement of adenine auxotrophs of E. coli (Davis and Mingioli, 1950). The successful isolation of methionine auxotrophs of Aerobacter aerogenes which utilize thiomethyl adenosine or methionyl adenosine in place of methionine will be reported here.

MATERIALS AND METHODS

Aerobacter aerogenes, strain NRRL 199, was used as the parent strain. Auxotrophs were produced by exposure of cells suspended in 0.85 per cent NaCl to ultraviolet light emitted by a Hanovia lamp for 15 seconds at a distance of 15 cm from the light source. This exposure killed 99.99 per cent of the cells. The auxotrophs among the survivors were concentrated selectively by use of the penicillin method (Lederberg and Zinder, 1948; Davis, 1948), and methionine auxotrophs were detected by use of replica plating (Lederberg and Lederberg, 1952).

The minimal medium used had the following composition: glucose, 0.1 per cent; K2HPO4, 0.7 per cent; KH2PO4, 0.2 per cent; sodium citrate, 0.05 per cent; MgSO4, 0.01 per cent; (NH4)2SO4, 0.1 per cent (and 1.5 per cent agar when used). Homocysteine and methionyl adenosine were sterilized by filtration through sintered glass. All other supplements were added to the minimal medium and sterilized by autoclaving.

One drop of a distilled water suspension of cells from a 18 to 24 hour culture was used to inoculate 10 ml of minimal broth containing various supplements. The tubes were incubated for 24 hours at 30 C, and growth was measured in an Evelyn photoelectric colorimeter with a 620 mμ filter.

All chemicals used were commercial products except thiomethyl pentose, thiomethyl adenosine, and methionyl adenosine which were generously supplied by Dr. F. Schlenk.

RESULTS

The majority of the methionine auxotrophs obtained did not utilize adenosine thiomethyl ribose in place of methionine. Of the auxotrophs that could utilize thiomethyl adenosine, there were two distinct groups differing in the types of compounds that would permit growth. Table 1 illustrates typical results that were obtained with five representative cultures. Culture 10 is an adenine auxotroph which is included as a control culture. It will be observed that it grows equally well on all compounds used in equimolar concentrations that supply adenine, but does not grow on any other compounds. Culture 72 grows only on methionine, and as yet no other simple supplement has been found that replaces methionine. This latter culture is included for comparative purposes and will be reported on in greater detail in future reports. Culture 26 is interesting in that it grows equally well in methionine or any adenine containing compound including thiomethyl adenosine and methionyl adenosine.

Cultures 56 and 68 are similar in that they respond only to methionine and not at all to adenine. These cultures show about 60 per cent of maximal growth in equimolar concentrations of homocysteine, thiomethyl adenosine, and methionyl adenosine. The response to the latter two compounds does not seem due to any free methionine contamination of the compounds since culture 72 does not respond to these compounds.
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at all. It is interesting to note that cultures 56 and 68 respond very poorly if at all to thiomethyl pentose alone or adenine alone, yet show a significant growth response to adenine thiomethyl ribose. Both cultures also respond fairly well to homocysteine and methionyl adenosine supports slightly better growth of culture 56 than either compound alone. However, culture 68 shows significantly better growth in the mixture than in either homocysteine or methionyl adenosine alone. The other three cultures respond to the mixture to the same degree that they respond to methionyl adenosine.

None of the cultures responded to the addition

| TABLE 1 |
| Response of Aerobacter aerogenes auxotrophs to various compounds |
| Per cent light transmission in minimal medium plus supplements after 24 hours' incubation. |
| **SUPPLEMENT, 0.05 MICROMOLES/ML** | **AUXOTROPH** |
| | 10 | 25 | 56 | 68 | 72 |
| None | 100 | 100 | 100 | 100 | 100 |
| DL-Methionine | 99 | 73 | 66 | 71 | 79 |
| Adenine | 74 | 75 | 99 | 100 | 99 |
| Thiomethyl adenosine | 73 | 74 | 88 | 86 | 99 |
| Thiomethyl ribose | 99 | 100 | 95 | 96 | 99 |
| Methionyl adenosine | 71 | 75 | 83 | 86 | 99 |
| DL-Homocysteine | 97 | 90 | 85 | 86 | 99 |
| Homocysteine + methionyl adenosine | 72 | 74 | 81 | 74 | 98 |
| Vitamin B₁₂* | 100 | 100 | 100 | 100 | 100 |
| p-Aminobenzoic acid* | 100 | 100 | 100 | 100 | 100 |

* Vitamin B₁₂ and p-aminobenzoic acid were used at concentrations of 25 μg/ml and 50 μg/ml, respectively.

of vitamin B₁₂ or p-aminobenzoic acid to the medium. Furthermore, B₁₂ did not enhance growth when added to homocysteine.

DISCUSSION

The ability of adenine thiomethyl ribose and methionyl adenosine to replace partially the methionine requirement of some auxotrophs implicates a dual role for methionine in the metabolism of these organisms. One may speculate that methionine is required for protein synthesis as well as for the production of some transmethylating agent. If the synthesis of such a compound from methionine proceeded through an irreversible step, then this could explain the fact that the two nucleosides do not support complete growth since the protein requirement for methionine is not fulfilled. Davis and Mingioli (1950) reported that some auxotrophs of E. coli grew equally well on B₁₂ or methionine but did not respond to homocysteine or thiomethyl adenosine. This led to the suggestion that B₁₂ is necessary for the methylation of homocysteine. Dubnoff (1952) showed that one of these auxotrophs responded to homocysteine under anaerobic conditions. This response to homocysteine was enhanced by the addition of B₁₂. Thus, he postulated that the genetic block in this mutant involved the synthesis of a methyl donor, which step involved B₁₂. In attempts to discover the methyl donor in the system, Dubnoff showed that dimethyl-β-propiothetin would increase the effect of homocysteine in promoting growth to a greater extent than B₁₂. Without homocysteine dimethyl-β-propiothetin was ineffective. However, an addendum to this paper indicated that the methyl donor could be replaced by p-aminobenzoic acid, indicating that in the absence of B₁₂, p-aminobenzoic acid synthesis is impaired and thus B₁₂ may not be directly involved in the synthesis of the methyl donor.

Gibson and Woods (1952) using E. coli auxotrophs that require p-aminobenzoic acid showed that methionine synthesis from homocysteine would occur only in the presence of p-aminobenzoic acid. No methyl donor was needed in these systems. Davis (1951) demonstrated a sparing effect of vitamin B₁₂ on the p-aminobenzoic acid requirement of E. coli mutants. He suggested that p-aminobenzoic acid is involved in B₁₂ synthesis, which in turn is required for the conversion of homocysteine to methionine. It is interesting to note that some of the methionine auxotrophs (cultures 56 and 68) reported here do respond to homocysteine, thiomethyl adenosine, as well as methionyl adenosine, but none of the cultures reported here responded to vitamin B₁₂ or p-aminobenzoic acid. This suggests that this type of mutant is partially blocked in its ability to transmethylate homocysteine to methionine. The addition of either homocysteine alone or thiomethyl adenosine or methionyl adenosine alone does not permit full growth. The addition of homocysteine to one of the methylated nucleosides does permit better growth than either compound alone (culture 68).
The fact that culture 72 does not respond to homocysteine at all with or without the addition of B12 or methionyl adenosine poses an interesting problem. The site of the genetic block in the biosynthesis of methionine in this organism is being investigated.

The ability of culture 26 to utilize adenine or methionine equally well points to an interesting relationship between the pathways of biosynthesis of these two compounds. A relationship among the purines, methionine, and p-aminobenzoic acid has been reported by several groups of investigators (Snell and Mitchell, 1943; Shive and Roberts, 1946; Cutts and Rainbow, 1951). This problem is being investigated further.

In an attempt to determine whether the different methionine auxotrophs are blocked at different genetic sites, experiments were designed to mix combinations of two strains on solid media and in liquid media to test for syntrophy. As yet, results with these tests under various conditions have not yielded any definite results that merit reporting here.

SUMMARY

Three groups of methionine auxotrophs of Aerobacter aerogenes are reported. One group can utilize adenine or methionine for complete growth. A second group will respond to homocysteine, thiomethyl adenosine, or methionyl adenosine. One culture has been isolated for which no simple single replacement compound has yet been found.

The significance of these results in relation to the problems of transmethylation systems in bacteria is discussed.

REFERENCES


