AN INTERMEDIATE INVOLVED IN THE FORMATION OF 4-AMINOBENZOIC ACID FROM CHORISMIC ACID IN AEROBACTER AEROGENES

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1. Introduction

The enzymatic conversion of chorismic acid, the branching intermediate of aromatic amino acid biosynthesis, to 4-aminobenzoic acid has been demonstrated by Gibson et al. [1]. Although Huang and Pittard [2] have shown that at least two genes are involved in this pathway, no conclusive evidence concerning the formation of an intermediate was available hitherto. Previous experiments scheduled to isolate such a substance from fermentation cultures of various 4-aminobenzoic acid deficient mutants of *Escherichia coli* [3,4] and *Candida guilliermondii* [5] were not successful. Therefore we felt that an organism producing a high level of chorismic acid might be better suited for this purpose.

2. Materials and methods

Aerobacter aerogenes mutant 62-1 was treated with 1-methyl-3-nitro-1-nitroso-guanidine (MNNG). Subsequently a mutant 62-1 A with an additional block in the common pathway of purine biosynthesis was isolated. Treatment of this mutant with MNNG allowed to isolate a new mutant 62-1 AC exhibiting an additional block between chorismic acid and 4-aminobenzoic acid.

A. aerogenes 62-1 AC was used for accumulation studies. Cultivation and accumulation procedures were essentially as described by Gibson [6]. Regarding the purine requirement of our mutant, guanine (20 mg/l) was added to the media. An intermediate of 4-amino benzoic acid biosynthesis (compound A) could be isolated as follows: The culture medium was centrifuged and placed on a column of acid washed charcoal, which was subsequently eluted with ethanol/2 N ammonia (1:3, v/v). The eluate was evaporated to a small volume in vacuo, followed by lyophilisation. The resulting dry powder was dissolved in 0.1 M Tris-buffer (pH = 8.0) and placed on a column of DEAE-Sephadex A-25. The column was eluted with Tris-buffer. Fractions promoting the growth of *E. coli* mutant K 5151 in 4-aminobenzoic acid-free medium were pooled and lyophilized. The resulting colorless powder was dissolved in water. Following acidification to pH = 3.6 with N acetic acid, the sample was placed on a column of SE-Sephadex C-25 and eluted with 0.1 N sodium acetate buffer (pH = 3.6). The growth-promoting fractions were neutralized by careful addition of 0.1 N NaOH and lyophilized.

3. Results and discussion

We started from a mutant 62-1 of *A. aerogenes* with three genetic blocks in the pathway of aromatic amino acid biosynthesis. Under suitable conditions this mutant accumulates about 0.4 g chorismic acid/l [6]. Following treatment with MNNG we obtained a new mutant 62-1 AC, this one exhibiting two additional blocks in the pathways of purine and 4-aminobenzoic acid biosyntheses (fig. 1).

Formation of an intermediate of 4-aminobenzoic acid biosynthesis (compound A) by *A. aerogenes* 62-1 AC could be demonstrated by growth tests using

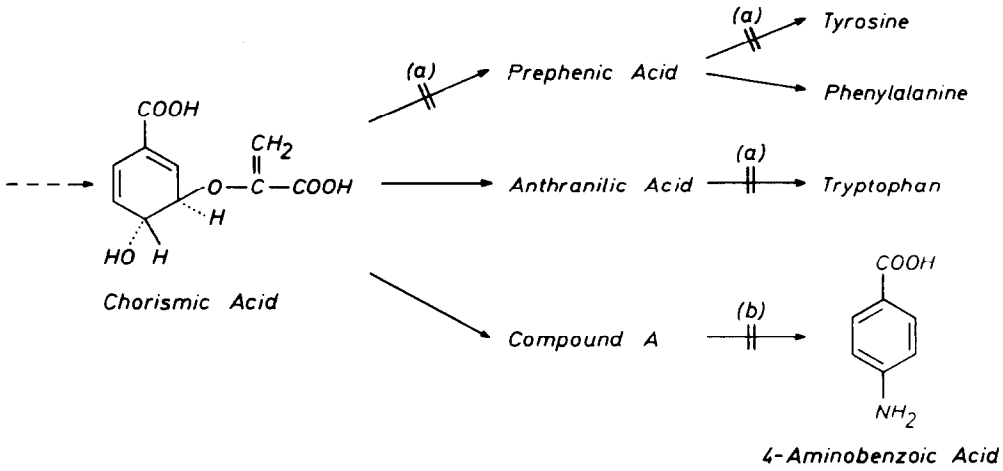

Fig. 1. Pathway of aromatic amino acid biosynthesis. (a) genetic blocks of *A. aerogenes* 62-1 (b) additional block of mutant 62-1 AC.

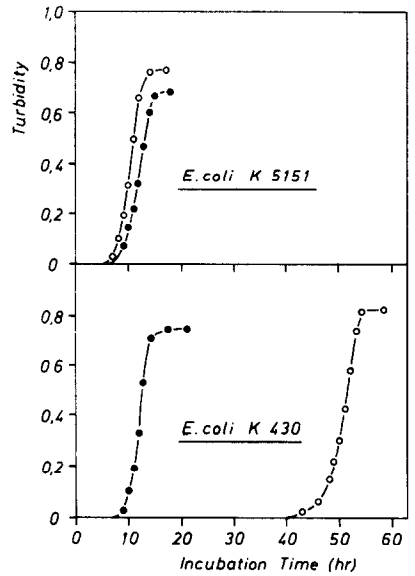

Fig. 2. Growth of 4-aminobenzoic acid deficient mutants of *E. coli* with compound A (○) resp. 4-aminobenzoic acid (●).

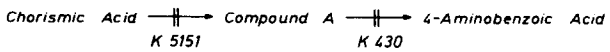

Fig. 3. Genetic blocks of *E. coli* mutants.

4-aminobenzoic acid deficient mutants of *E. coli*, namely K 430 and K 5151. Culture medium of *A. aerogenes* 62-1 AC was sterilized by filtration. Following 100-fold dilution with sterile minimal medium, this solution was inoculated with *E. coli* mutants. Growth curves are shown in fig. 2. *E. coli* K5151 attains full growth within 14 hr. Hence it follows, in agreement with the results of crossfeeding tests [3,4], that this mutant shows a genetic block prior to compound A (fig. 3). *E. coli* K 430 starts to grow not before 42 hr. However, growth begins without delay if sterile accumulate solution is preincubated at 37°C for 2 days, suggesting that decomposition of compound A yields 4-aminobenzoic acid.

Compound A was isolated by chromatographic procedures. The pure compound exhibits a UV-maximum centered at 271 nm in neutral Tris-buffer. On prolonged incubation in citrate-phosphate buffer (pH = 3.5) compound A becomes converted to 4-aminobenzoic acid, which was identified by chromatographic and UV-spectroscopic methods. Therefore it seems reasonable to assume that compound A is a labile intermediate of 4-aminobenzoic acid biosynthesis, which may no longer be converted by *A. aerogenes* 62-1 AC. Studies concerning the structure of compound A are presently undertaken.

Acknowledgements

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