Influence of *p*-Aminobenzoic Acid, *p*-Hydroxybenzoic Acid and L-Tryptophan on Biosynthesis of Chloramphenicol

[a]B. ŠKÁRKA*, [b]D. W. S. WESTLAKE and [c]L. C. VINING

[a]*Department of Microbiology and Biochemistry, Slovak Technical University, Bratislava 1*

[b]*Department of Microbiology, University of Alberta, Edmonton, Alberta, Canada*

[c]*National Research Council, Atlantic Regional Laboratory, Halifax, Nova Scotia, Canada*

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Westlake et al. found or verified the part of the biosynthetic pathway of chloramphenicol to shikimic acid and from L-*p*-aminophenylalanine to chloramphenicol. The part between shikimic acid and L-*p*-aminophenylalanine is still unknown. The influence of some aromatic compounds on the production of chloramphenicol was studied using the derivatives of chorismic acid (tryptophan, *p*-aminobenzoic acid and *p*-hydroxybenzoic acid) and prephenic acid (tyrosine and phenylalanine). The derivatives of chorismic acid stimulated the production of chloramphenicol while the derivatives of prephenic acid did not. The biosynthetic pathway of chloramphenicol from shikimic acid appears to be very close to the pathways for tryptophan, *p*-aminobenzoic and *p*-hydroxybenzoic acids or common to an unknown branch point.

Although chloramphenicol is a compound of relatively small molecule and is produced in great quantities by a number of synthetic methods, still there is not known all about its biosynthesis.

Gottlieb [2], resp. *Carter* [1] found aromatic amino acids not to be direct precursors of chloramphenicol. Later *Gottlieb* [3, 5] found that addition of various amino acids increased the antibiotic synthesis. Only some years ago *Robbins* [9] summarized experiments with labelled compounds to discover the origin of the atoms of the chloramphenicol molecule. Very important contribution to this problem were the works by *Gottlieb* [4] and *Vining* and *Westlake* [12], showing that chloramphenicol, phenylalanine and tyrosine have a common biosynthetic pathway till the shikimic acid. *Siddiquellah* [10] and *McGrath* [7, 8] found *p*-aminophenylalanine and *threo*-*p*-aminophenylserine to be the specific precursors of the aromatic portion of chloramphenicol. *Westlake* [13] finally solved also the origin of the nitrogen atom in this molecule.

*Postdoctoral Fellow U. of A. 1968—1969.

Today it is possible to imagine the first part of the biosynthetic pathway of chloramphenicol to the shikimic acid and the final part from *p*-aminophenylalanine. There is no satisfactory knowledge about the part between.

To get some information about the unknown steps of chloramphenicol biosynthesis from the shikimic acid, the influence of the derivatives of chorismic acid and prephenic acid on the production of chloramphenicol was made the subject of the present studies.

Materials and methods

The origin of *Streptomyces sp. 3022a* and methods used for its maintenance and culture have been reported [12].

Chloramphenicol and *p*-aminobenzoic acid have been determined by [11].

Results and Discussion

The metabolism of *p*-aminobenzoic acid for chloramphenicol producing *Streptomyces sp. 3022a* was studied [11]. The first experiment compared the effect of *p*-aminobenzoic and *p*-hydroxybenzoic acids on the production of chloramphenicol. 500 ml of the medium in 2000 ml flasks were used. The concentrations of the additives were

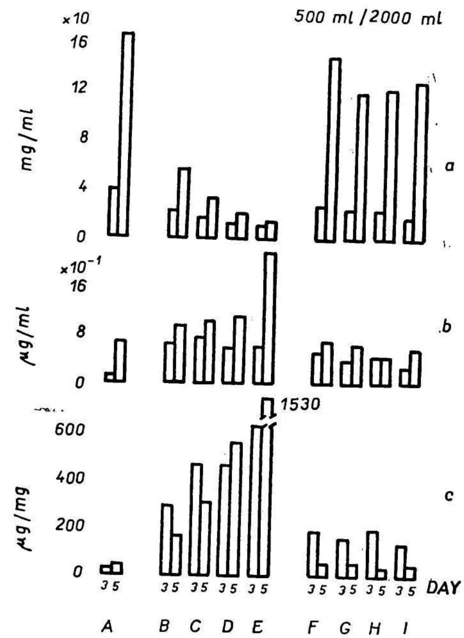

Fig. 1. Influence of the addition of *p*-aminobenzoic (*p*-ABA) and *p*-hydroxybenzoic (*p*-HBA) acids on the production of chloramphenicol.
a) protein mg/ml; *b*) chloramphenicol μg/ml; *c*) chloramphenicol μg/mg prot.
A. control; *B*–*E*. *p*-ABA; *F*–*I*. *p*-HBA.
B, *F* = 3.75 mM; *C*, *G* = 7.5 mM; *D*, *H* = 15 mM; *E*, *I* = 30 mM.

from 3.75 to 30 mM. Fig. 1 shows the results from 3rd and 5th days of the cultivation. The results show the inhibitory effect of *p*-aminobenzoic acid on the growth of mycelium but the simultaneous stimulation of the chloramphenicol production.

In the next experiment also tryptophan, tyrosine, phenylalanine and chloramphenicol were used; again 500 ml of the medium in 2000 ml flasks. The concentrations of the additives were 0.1 or 0.2 mM. These concentrations were chosen as they did not show any effect on the growth of mycelium. The values were recorded for the days 3—7 of cultivation (Fig. 2). The results confirmed that the derivatives of prephenic acid (tyrosine and phenylalanine) do not stimulate the production of chloramphenicol, but the derivatives of chorismic acid (tryptophan, *p*-aminobenzoic and *p*-hydroxybenzoic acids) have a stimulating effect. The amount of the added chloramphenicol was not subtracted in Fig. 2. The results are in good agreement with the paper [6].

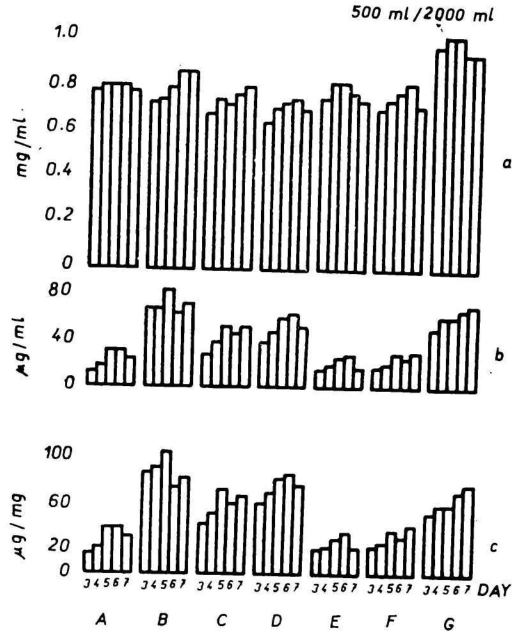

Fig. 2. Influence of the addition of *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, tryptophan (TR), tyrosine (T), phenylalanine (P) and chloramphenicol (Chap) on the production of chloramphenicol.

a) protein mg/ml; *b*) chloramphenicol µg/ml; *c*) chloramphenicol µg/mg prot.
A. control; *B*. *p*-ABA 0.2 mM; *C*. *p*-HBA 0.2 mM; *D*. TR 0.1 mM; *E*. T 0.1 mM; *F*. P 0.1 mM; *G*. Chap 0.2 mM.

With regard to Table 1 on the influence of aeration on the production of chloramphenicol in the next experiment the ratio of the volume of medium to the volume of flask was changed to 200 ml and 2000 ml. The results (Fig. 3) show similar relations as before. In the fourth experiment 100 ml of the medium in 500 ml flasks were used and the addition of tyrosine and phenylalanine was excluded. The relations between the groups remained similar as before (Fig. 4).

Table 1

Influence of aeration on the production of chloramphenicol
(*Westlake*, unpublished results)

ml of the medium in 1 l flask	pH	Chloramphenicol [μg/ml]	Glycerol (residual) [mg/ml]
60	8.6	89	0.03
100	8.5	100	0.04
125	7.8	85	0.03
175	7.5	64	0.04
225	6.4	52	0.03
325	5.6	38	1.00
450	5.5	29	2.60

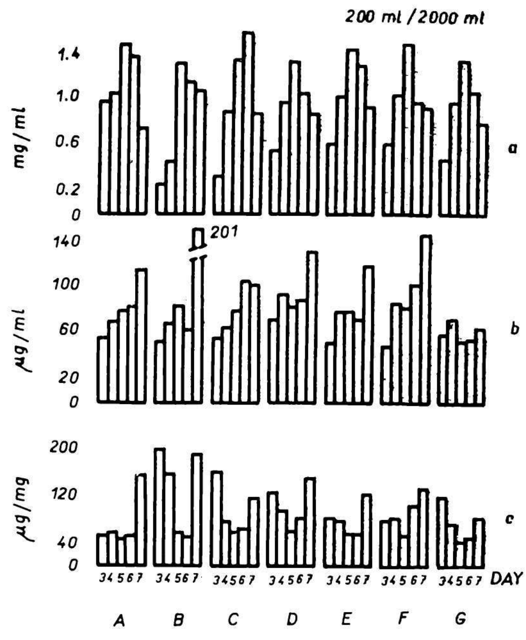

Fig. 3. Influence of the addition of *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, tryptophan, tyrosine, phenylalanine and chloramphenicol on the production of chloramphenicol by using 200 ml of the medium in 2000 ml flasks.
a) protein mg/ml; *b*) chloramphenicol μg/ml; *c*) chloramphenicol μg/mg prot.
A—*G* as in Fig. 2.

At last the combination of all additives was applied. To the first group *p*-aminobenzoic acid was added, to the second *p*-aminobenzoic acid, *p*-hydroxybenzoic acid and tryptophan, to the third group all 5 additives. The results in Figs. 5 and 6 show again that the derivatives of prephenic acid do not influence the production of chloramphenicol, but the derivatives of chorismic acid have a clear stimulating effect.

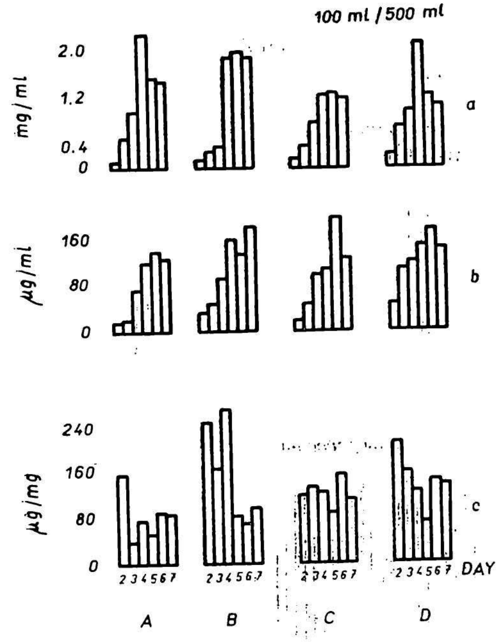

Fig. 4. Influence of the addition of *p*-aminobenzoic acid, *p*-hydroxybenzoic acid and tryptophan on the production of chloramphenicol by using 100 ml of the medium in 500 ml flasks.
a) protein mg/ml; *b*) chloramphenicol µg/ml; *c*) chloramphenicol µg/mg prot.
A. control; *B*. *p*-ABA 0.2 mM; *C*. *p*-HBA 0.2 mM; *D*. TR 0.1 mM.

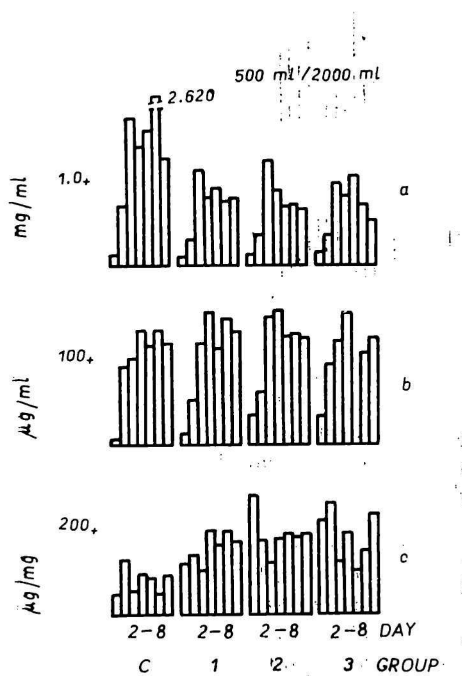

Fig. 5. Influence of the addition of combinations of the tested compounds on the production of chloramphenicol by using 500 ml of the medium in 2000 ml flasks.
1. *p*-aminobenzoic acid; *2*. *p*-aminobenzoic acid, *p*-hydroxybenzoic acid and tryptophan; *3*. as before plus tyrosine and phenylalanine.
a) protein mg/ml; *b*) chloramphenicol µg/ml; *c*) chloramphenicol µg/mg prot.

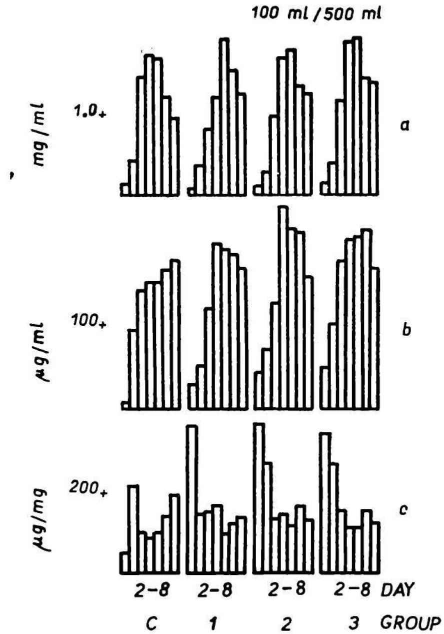

Fig. 6. Influence of the addition of combinations of the tested compounds on the production of chloramphenicol by using 100 ml of the medium in 500 ml flasks.
a) protein mg/ml; *b*) chloramphenicol μg/ml; *c*) chloramphenicol μg/mg prot.

From these results it may be suggested that the biosynthesis of chloramphenicol from shikimic acid leads *via* chorismic acid by the same way or very close to it as for some other aromatic compounds to a till now unknown derivative and from it to L-*p*-aminophenylalanine as it is shown on Scheme 1:

Erythroso-4-phosphate
+
Phosphoenol pyruvate
↓
Shikimic acid ⟶ { Tryptophan; *p*-Aminobenzoic acid; *p*-Hydroxybenzoic acid; Chloramphenicol }
↓
Chorismic acid
↓
Prephenic acid
↙ ↘
Tyrosine Phenylalanine

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