Biosynthesis of Chloramphenicol. IVa*. Isolation and Some Properties of 3-Deoxy-D-*arabino*-heptulosonate--7-phosphate Synthetase (E. C. 4. 1. 2. 15) of *Streptomyces sp. 3022a*

[a]B. ŠKÁRKA,** [b]D. W. S. WESTLAKE and [b]M. A. PICKARD**

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

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

Received July 7, 1969

In revised form September 2, 1969

Some properties of 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthetase from crude cell-free extracts from *Streptomyces sp. 3022a* were studied. The modification of the method for determination of the enzyme activity is described. It was found that the dilution does not lead to the loss of the enzyme activity. The activity of this enzyme changes during the growth, the minimum was found the second day of cultivation. By its response to the inhibitors and some other compounds the 3-deoxy-D-*arabino*-heptulosonate--7-phosphate synthetase (DAHP-synthetase) from *Streptomyces sp. 3022a* is similar to the DAHP-synthetase from *Bacillus subtilis*.

3-Deoxy-D-*arabino*-heptulosonate-7-phosphate synthetase (DAHP-synthetase) is the first enzyme in the aromatic biosynthesis and the biosynthesis of chloramphenicol. In the last years the properties of this enzyme in *Escherichia coli* [1, 2, 11—13], *Bacillus subtilis* [7, 8, 10], *Saccharomyces cerevisiae* [2, 5], *Neurospora crassa* [1], *Staphylococcus epidermis*, some *Algeae* and other species were studied. Recently *Doy* [3] summarized the published results. DAHP-synthetase in different species of microorganisms or plants shows different composition, properties and structure.

In this work some properties of DAHP-synthetase from chloramphenicol producing *Streptomyces sp. 3022a* are described.

Materials and methods

The origin of *Streptomyces sp. 3022a* and methods used for its maintenance and culture have been reported [15].

Protein was determined by *Lowry* [9].

---

* *I.* Vining L. C., Westlake D. W. S., *Can. J. Microbiol.* ***10***, 705 (1964).

II. Siddiquellah M., McGrath R., Vining L. C., Sala F., Westlake D. W. S., *Can. J. Biochem.* ***45***, 1881 (1967).

III. McGrath R., Vining L. C., Sala F., Westlake D. W. S., *Can. J. Biochem.* ***46***, 587 (1968).

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

Preparation of crude cell-free extracts

Cells were obtained by centrifugation (10,000 × *g*, 10 minutes, 4°C). Pellets were washed with 0.1 M sodium-potassium buffer pH 7.0 and centrifuged under the same conditions. The cells were disrupted by sonication (Biosonic, 6 minutes, ice bath). Cell debris were removed by centrifugation (10,000 × *g*, 10 minutes, 4°C).

Determination of the DAHP-synthetase activity

The activity of the DAHP-synthetase was determined by the chemical determination of DAHP produced by the enzymatic condensation of erythroso-4-phosphate and phosphoenol pyruvate [13, 14] with refinements by *Doy* [4] and ours.

As very important in the DAHP-synthetase activity determination have been found the followings: pH, temperature, preincubation of substrates, time of boiling and time of reading of optical density after boiling.

The influence of pH on the DAHP-synthetase activity is shown in Fig. 1. pH Optimum is 7.2.

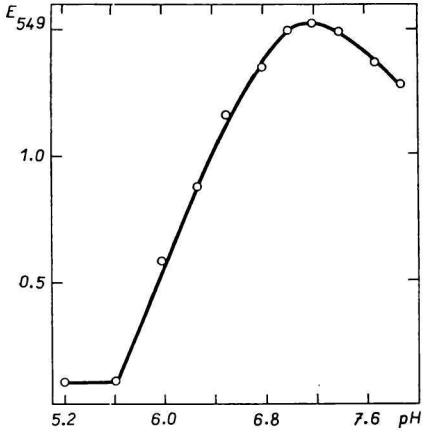

Fig. 1. The influence of pH on DAHP-synthetase activity.

The influence of temperature on the DAHP-synthetase activity is shown in Fig. 2. The optimum temperature is 41°C.

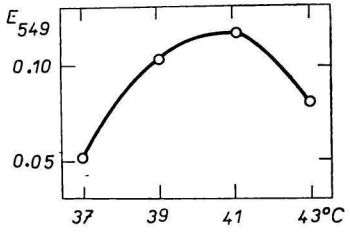

Fig. 2. The influence of temperature on DAHP-synthetase activity.

The influence of boiling on the results of the DAHP-synthetase activity determination is shown in Fig. 3. Apparently it is necessary to choose and to use the same time of boiling.

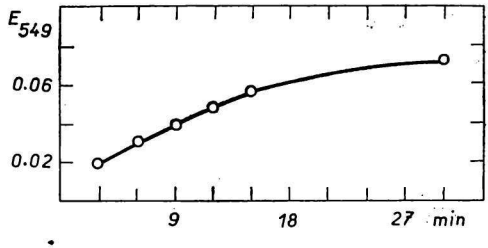

Fig. 3. The influence of time of boiling on the colour during the DAHP-synthetase activity determination.

The evolution of colour before reading of optical density is shown in Fig. 4. It looks useful to measure optical density 15—25 minutes after boiling.

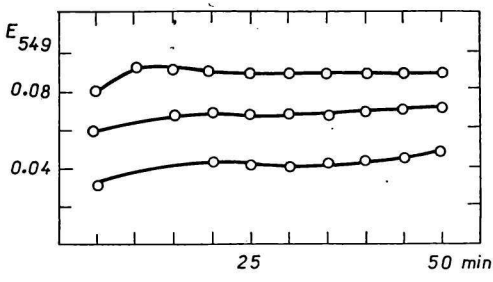

Fig. 4. Evolution of the colour after boiling during the DAHP-synthetase activity determination.

It was also found possible to change to a certain extent the volume ratio of the solutions without danger of inaccuracy.

The procedure used for DAHP-synthetase activity determination was: 0.25 ml of 2 mM erythroso-4-phosphate in 0.1 M sodium-potassium phosphate buffer pH 7.2, 0.25 ml of 2 mM phosphoenol pyruvate in 0.1 M sodium-potassium phosphate buffer pH 7.2 and 0.25 ml of 0.1 M sodium-potassium phosphate buffer pH 7.2 was left for 5 minutes in water bath at 41°C. After preincubation 0.25 ml of the enzyme sample was added and left to incubate 15 minutes, 30 and 60 minutes, respectively.

The reaction was terminated with 0.1 ml of 10% trichloroacetic acid. Protein was sedimented by centrifugation (10,000 × *g*, 10 minutes) and 0.25 ml of the supernatant was preincubated 5 minutes in water bath at 37°C. After preincubation 0.25 ml of 0.025 M sodium periodate in 0.15 N-H_2SO_4 was added to the sample and left for 30 minutes. The subsequent addition of 0.5 ml of 2% sodium arsenite in 0.5 N-HCl was followed by the addition of 2 ml of 0.3% 2-thiobarbituric acid. The colour was developed by heating over 95°C for 8 minutes (timer started when the temperature in the tubes increased to 95°C). The tubes were cooled to room temperature and optical density was read up at 549 nm 15—25 minutes after boiling. We used E_m 3.34 × 10^4 [4]. A mixture of substrates and buffer, having passed through all procedures, was used as blank. For control a mixture of substrates, trichloroacetic acid and enzyme sample was used. The results were expressed in mμM DAHP/mg of protein × 15 minutes (specific activity).

As a possible cause of occasional troubles with cloudiness of the cooled samples before reading of optical density the concentration of buffer was found and therefore only 0.01 M sodium-potassium buffer pH 7.2 was preferred.

Because of very low optical density in the samples in many cases 0.65 ml of supernatant after incubation, only 0.15 ml of periodate, 0.30 ml of arsenite and 0.90 ml of 2-thiobarbituric acid were used.

Results and Discussion

Relationship between the relative enzyme concentration and its activity

Jensen [6] found a linear relationship between enzyme concentration and enzyme activity up to the use of about 30% of the substrates. He worked with extracts from *Bacillus subtilis* and used 41—410 μg of protein in the samples. We repeated his experiment with different extracts from *Streptomyces sp. 3022a* and we found the similar relations (Fig. 5). It seems that dilution had no effect on the loss of enzyme activity.

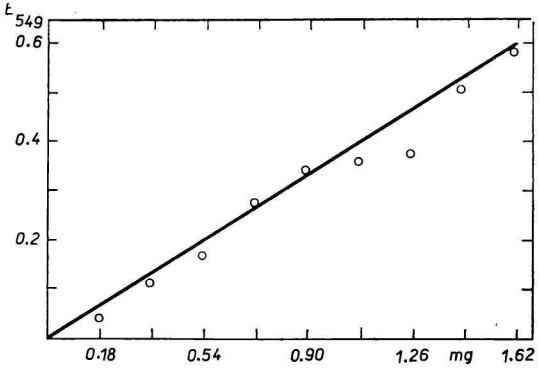

Fig. 5. The relation between the amount of protein and E_{549}.

Change of enzyme activity during the cultivation

The change of enzyme activity was followed during four days of the cultivation. The minimum of activity was found the second day. The results are shown in Fig. 6.

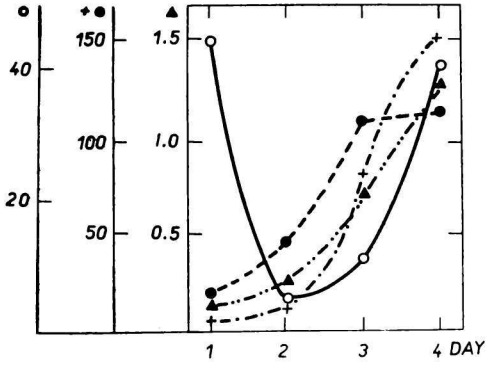

Fig. 6. The change of DAHP-synthetase activity during the growth. ▲ protein in mg/ml; + chloramphenicol in μg/ml; ● chloramphenicol in μg/mg of protein; ○ specific activity.

The sensibility of DAHP-synthetase to the presence of some compounds

The sensibility of DAHP-synthetase to the presence of some intermediates of the aromatic biosynthesis and of some common inhibitors was studied. The results are shown in Table 1.

Table 1

The sensibility of DAHP-synthetase from *Streptomyces sp. 3022a*

Compound added (1 mM)	Inhibition [%]
p-chloromercuribenzoate	100
iodoacetate	100
2-mercaptoethanol	100
thiothreitol	100
glutathione	100
EDTA	100
shikimic acid	70
chorismic acid	75
prephenic acid (0.3 mM)	33
p-aminophenylalanine	61
L-*threo*-2-amino-1-(*p*-nitrophenyl)-1,3-propanediol	83
threo-*p*-aminophenylserinol	0
phenylalanine	56
tyrosine	100
tryptophan	58
p-aminobenzoic acid	56
chloramphenicol	6

The comparison of these results with data in papers [1, 2, 6—8] shows some relationship with the sensibility of DAHP-synthetase from *Bacillus subtilis*. Therefore a similar regulation of the aromatic biosynthesis for *Streptomyces sp. 3022a* and for *Bacillus subtilis* is suggested.

References

1. Doy C. H., *Biochim. Biophys. Acta* ***151***, 293 (1968).
2. Doy C. H., *Biochim. Biophys. Acta* ***159***, 352 (1968).
3. Doy C. H., *Rev. Pure Appl. Chem.* ***18***, 41 (1968).
4. Doy C. H., Brown K. D., *Biochim. Biophys. Acta* ***104***, 377 (1965).
5. Doy C. H., Cooper J. M., *Biochim. Biophys. Acta* ***127***, 302 (1966).
6. Jensen R. A., Nester E. W., *J. Mol. Biol.* ***12***, 468 (1965).
7. Jensen R. A., Nester E. W., *J. Biol. Chem.* ***241***, 3365 (1966).
8. Jensen R. A., Nester E. W., *J. Biol. Chem.* ***241***, 3373 (1966).
9. Lowry O. H., Rosebrough N. J., Farr L. L., Randall R. J., *J. Biol. Chem.* ***193***, 265 (1951).
10. Nester E. W., Lorence J. H., Nasser D. S., *Biochemistry* ***6***, 1553 (1967).
11. Smith L. C., Ravel J. M., Lax S. R., Shive W., *J. Biol. Chem.* ***237***, 3566 (1962).
12. Smith L. C., Ravel J. M., Lax S. R., Shive W., *Arch. Biochem. Biophys.* ***105***, 424 (1964).
13. Srinivasan P. R., Katagiri M., Sprinson D. B., *J. Biol. Chem.* ***234***, 713 (1959).
14. Srinivasan P. R., Sprinson D. B., *J. Biol. Chem.* ***234***, 716 (1959).
15. Vining L. C., Westlake D. W. S., *Can. J. Microbiol.* ***10***, 705 (1964).

Translated by B. Škárka