

# Sensitivity of a Phototrophic Bacterium to the Herbicide Sulfometuron Methyl, an Inhibitor of Branched Chain Amino Acid Biosynthesis

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Z. Naturforsch. **41c**, 1037–1039 (1986); received July 16, 1986

Acetohydroxy Acid Synthase, Branched Chain Amino Acids, *Rhodospirillum*, Sulfonyl-Urea Herbicides, Imidazolinone Herbicides

Sulfonyl urea-herbicides (sulfometuron methyl, chlorsulfuron) strongly inhibit phototrophic growth of the bacterium *Rhodospirillum rubrum* in the absence of L-valine as a feedback inhibitor of the enzyme acetohydroxy acid synthase. The latter enzyme was shown to be highly sensitive to the sulfonyl urea and, to a much lesser extent, to an imidazolinone herbicide (Arsenal®).

The enzyme acetohydroxy acid synthase (AHA-synthase, EC 4.1.3.18) catalyses the following reactions:

$2 \text{ pyruvate} \longrightarrow \alpha\text{-acetolactate} + \text{CO}_2$ , and  $\text{pyruvate} + 2\text{-oxobutyrate} \longrightarrow \alpha\text{-acetohydroxybutyrate} + \text{CO}_2$ , thereby initiating the two parallel biosynthetic sequences leading to L-valine and L-isoleucine. The enzyme has been found in a variety of eu- and prokaryotic organisms including green plants, fungi and various bacteria, and purified from some sources [1–3].

Interestingly, both plant and bacterial AHA-synthases are targets of some newly developed herbicides of the imidazolinone (Arsenal) and the sulfonylurea-type (chlorsulfuron, sulfometuron methyl) [4, 5]. Since, in the enteric bacterium *Salmonella typhimurium* only one of the isoenzymes of AHA-synthase, namely the L-valine-insensitive one is inhibited by chlorsulfuron and sulfometuron methyl, inhibition of growth by these herbicides can only be achieved in the presence of L-valine as a feedback-inhibitor of the other isoenzyme(s) [6]. Thus, analysis of growth retardation by these compounds, to-

gether with enzyme inhibition experiments, can be very helpful in the study of the AHA-synthase isoenzyme pattern of an organism. In this communication we report on studies of AHA-synthase in the phototrophic prokaryote *Rhodospirillum rubrum*. According to our data, *R. rubrum*, unlike enteric bacteria, does not contain AHA-synthase isoenzymes with different sensitivities to herbicides.

As shown in Fig. 1, the sulfonylurea herbicide sulfometuron methyl strongly inhibited growth of *R. rubrum* SI in photosynthetic batch culture. Importantly, the inhibition of growth occurred even in the absence of L-valine, a feedback inhibitor of AHA-synthase. L-valine enhanced only slightly the growth retardation by SM. Reversal of growth inhibition by L-isoleucine plus L-valine (see Fig. 1) suggested that the herbicide interferes with branched chain amino acid biosynthesis. Isoleucine alone did not reverse growth inhibition by SM. Similar results were obtained with chlorsulfuron (data not shown).

Interestingly, SM-inhibited *R. rubrum* SI cultures recovered from growth retardation after about 35 h. Since spontaneous gene mutations which alter sensitive AHA-isoenzymes to resistant forms are readily obtained in enteric bacteria [6], recovery from SM-inhibition in *R. rubrum* SI cultures could be due to selection of resistance mutants. In order to test this possibility, samples of “recovered” cultures were inoculated in fresh media supplemented with the herbicide, and bacterial growth was followed. Significantly, also in such cultures growth was initially inhibited by SM. We conclude, therefore, that recovery from growth inhibition is not due to selection of mutants with altered AHA-synthase properties, but related to secondary metabolic events occurring as a consequence of AHA-synthase inhibition (for example

**Abbreviations:** AHA, acetohydroxy acid; AS, Arsenal® (2-[4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]nicotinic acid); CS, chlorsulfuron (2-chloro-N[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzene-sulfonamide); SM, sulfometuron methyl (N-[(4,6-dimethyl-pyrimidin-2-yl)]aminocarbonylbenzenesulfonamide); *R.*, *Rhodospirillum*.

Arsenal (American Cyanamid Company).  
SM/CS (E. I. du Pont de Nemours & Co.).

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen  
0341–0382/86/1100–1037 \$ 01.30/0



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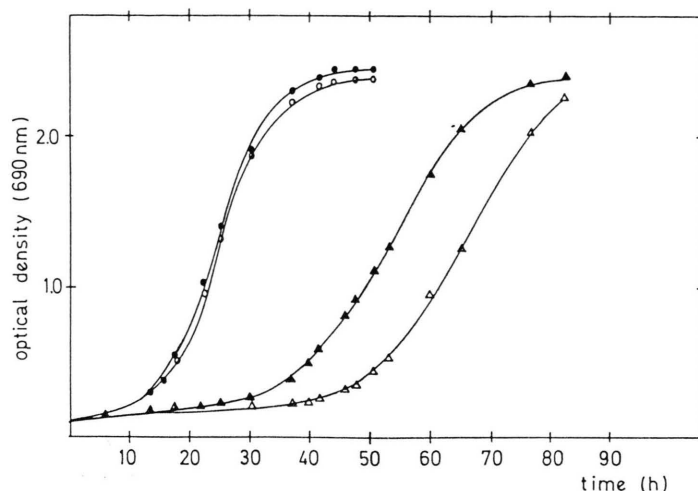


Fig. 1. Effect of sulfometuron methyl on photosynthetic growth of *Rhodospirillum rubrum* strain SI in the presence and absence of L-valine.

● Growth in the absence of herbicide and amino acids;  
○ growth in the presence of SM (40  $\mu\text{g/ml}$ ), L-isoleucine and L-valine (1 mM each);  
▲ growth in the presence of SM (40  $\mu\text{g/ml}$ );  
△ growth in the presence of SM (40  $\mu\text{g/ml}$ ) and L-valine (1 mM).

The bacteria were grown in 13 ml-screw cap tubes containing  $(\text{NH}_4)_2\text{SO}_4$ -malate-medium [8], in a light cabinet ( $30 \pm 2^\circ\text{C}$ ; tungsten lamps). Bacterial growth was monitored by directly measuring the increase in  $\text{OD}_{690}$  in the tubes. Stock solutions of the herbicides were prepared in dimethylsulfoxid. The solvent, up to the concentration used in the experiment (0.2 ml per 13 ml culture) had no effect on growth.

accumulation of 2-oxobutyrate and possible interference with aspartate formation [7]).

*In vitro* inhibition profiles of AHA-synthase from *R. rubrum* SI obtained with the herbicides sulfometuron methyl, chlorsulfuron, Arsenal, and the feedback-inhibitors L-valine and L-isoleucine are depicted in Fig. 2. The two sulfonylurea herbicides (SM

and CS) acted as very potent inhibitors of the enzyme with  $I_{0.5}$ -values of 0.03 (SM) and 0.08 ( $\mu\text{M}$ ). Arsenal, a newly developed imidazolinone herbicide, with  $I_{0.5}$ -value of about 1 mM, was even less inhibitory than L-valine ( $I_{0.5} = 7 \times 10^{-5} \text{ M}$ ).

Note that the extent of AHA-synthase inhibition reached the 100%-level with very low concentrations

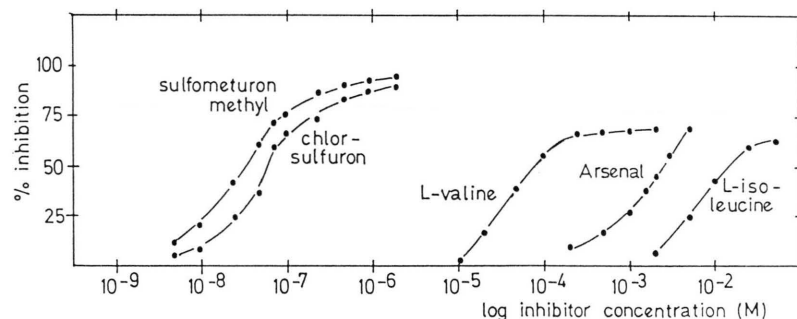


Fig. 2. Effect of L-valine, L-isoleucine, Arsenal, chlorsulfuron and sulfometuron methyl on the activity of acetoxy acid synthase in extracts of *Rhodospirillum rubrum* strain SI. Phototrophically grown cells (in 20 mM K-phosphate, pH 7.2, supplemented with 10 mM Na-pyruvate, 1 mM  $\text{MgCl}_2$ , 0.5 mM dithiothreitol, 0.1 mM thiamine pyrophosphate, 10  $\mu\text{M}$  FAD and 20% (w/v) glycerol) were ruptured by ultrasonic treatment and the resulting homogenates were centrifuged at  $22,000 \times g$  to remove unbroken cells. AHA-synthase-activity was assayed at  $30^\circ\text{C}$  in reaction mixtures containing 20 mM K-phosphate, pH 7.2, 200  $\mu\text{g}$  TPP, 20 mM Na-pyruvate in a total volume of 1 ml. The reaction was stopped by the addition of 0.1 ml of 50% trichloroacetic acid, the mixture was heated at  $60^\circ\text{C}$  for 15 min to convert  $\alpha$ -acetolactate to acetoin, which was determined by the method of Westerfield [9]. Stock solutions of the herbicides were prepared in ethanol or acetone and control activities without inhibitor were corrected for enzyme inhibition by the solvent. Specific activities of uninhibited controls were in the range of 10 munits/mg protein (1 unit is the enzyme activity catalyzing the production of 1  $\mu\text{mol}$   $\alpha$ -acetolactate per min).

(1  $\mu\text{M}$  range) of SM and CS. This finding, together with the fact that SM and, to a lesser extent CS (not shown in Fig. 1), inhibit growth in the absence of L-valine, suggests that *R. rubrum* SI, contrary to enteric bacteria, does not contain AHA-synthase isoenzymes with different sensitivities to sulfonylurea herbicides and/or L-valine.

#### Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft. The authors thank Gisela Streich for skillful technical assistance and Dr. D. Scheel, Max-Planck-Institut für Züchtungsforschung, Köln, for kindly providing the herbicides.

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