On the Specificity of the Herbicide Chlorsulfuron in Intact Spinach Chloroplasts

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In vitro incubation of intact spinach chloroplasts with 1 mM Pyruvate was used to study the specificity of action of the herbicide Chlorsulfuron on the synthesis of valine, alanine and fatty acids. As a result, increasing concentrations of the herbicide strongly inhibited valine synthesis while fatty acid synthesis *via* pyruvate dehydrogenase complex (PDC) and alanine formation by transamination reaction was promoted.

Introduction

In plants and microorganisms the branched chain amino acid valine is formed from pyruvate *via* hydroxyethylthiamindiphosphate → acetolactate. The enzyme acetolactate synthase which is solely localized in the chloroplasts of higher plants [1, 2] is the target of the sulfonylurea herbicide Chlorsulfuron [3, 4]. As one of the imporant criteria of newly developed herbicides their specificity of action has to be proven.

In this context, Chlorsulfuron was checked for its effect on the pyruvate derived plastidic metabolism by comparing the synthesis of valine as a main branched chain amino acid and of fatty acids and alanine from added pyruvate.

Material and Methods

Chlorsulfuron (water soluble form) was a generous gift from Du Pont De Nemours International, Genf. [2-^{14}C]Pyruvate, sodium salt (specific activity 462,5 MBq × mmol^{-1}) was from NEN, Dreieich, FRG. Intact spinach chloroplasts were isolated according to Jensen and Bassham [7]. Such preparations obtained higher metabolic rates as those by the time consuming centrifugation through Percoll [8]. Because the systems studied are exclusively localized in the chloroplast a minimal contamination by peroxisomes and mitochondria was tolerated. The reaction medium (1 ml) contained (in mM):

Sorbitol 330, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES, adjusted with NaOH to pH 7.6) 50, $MgCl_2$ 1, $MnCl_2$ 1, KH_2PO_4 0.5, $NaNO_3$ 2, ascorbate 2, EDTA 2, $NaHCO_3$ 5, glutamate 0.05, pyruvate 1 plus labelled compound. Chloroplast suspensions were illuminated (0.1 J × cm^{-2} × s^{-1}; Osram "Bellaphot") at 20+/−2 °C. Assays of fatty acids and amino acids were done as described in [1]. Thin layer chromatography of the amino acids was carried out on silicagel with *n*-propanol/water 64:36 (v:v) [9]. Incorporated radioactivity was assayed by scintillation counting in methanol/Hydroluma (Baker) 1:4.

Results and Discussion

As demonstrated in Fig. 1 the biosynthesis of valine (and also of alanine; data not shown) passes with a constant rate for about 60 minutes. Furthermore, uptake and herbicidal effect on valine synthesis in chloroplasts occurs without any lag-phase.

Increasing concentrations of the added herbicide exerts a decrease in the rate of valine synthesis (Fig. 2). Correspondingly, alanine synthesis by transamination reaction of pyruvate (Fig. 2) and the synthesis of 16:0/18:0 and 18:1 fatty acids increases

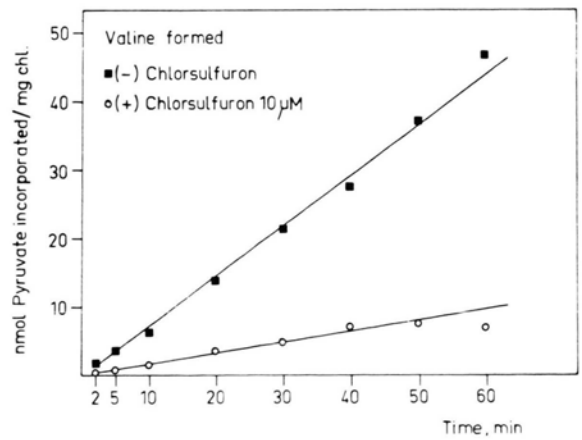

Fig. 1. Time course of the incorporation of exogenously added [2-^{14}C]-pyruvate into valine without (■) and with (○) addition of 10 µM Chlorsulfuron.

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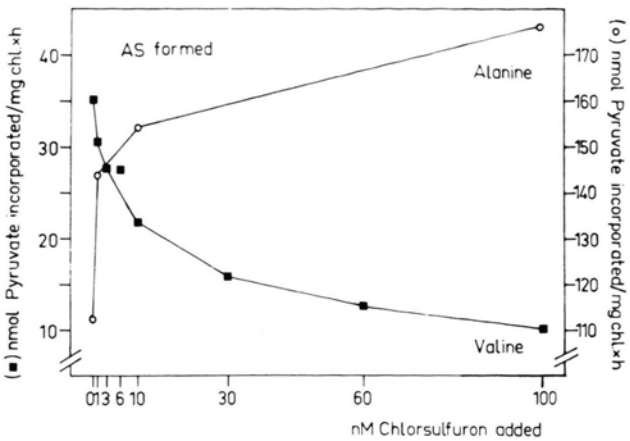


Fig. 2. Effect of increasing concentrations of Chlorsulfuron on valine and alanine synthesis in intact spinach chloroplasts from exogenously added pyruvate (1 mM).

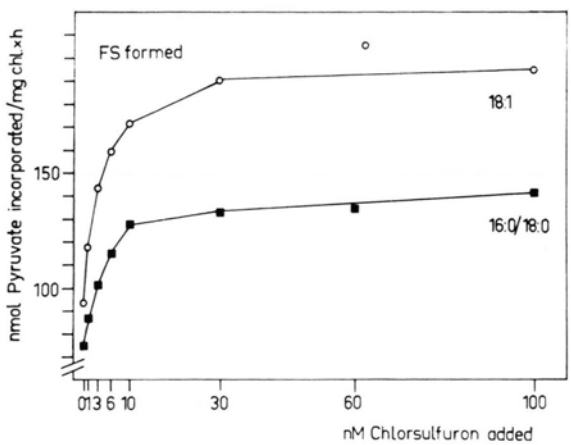
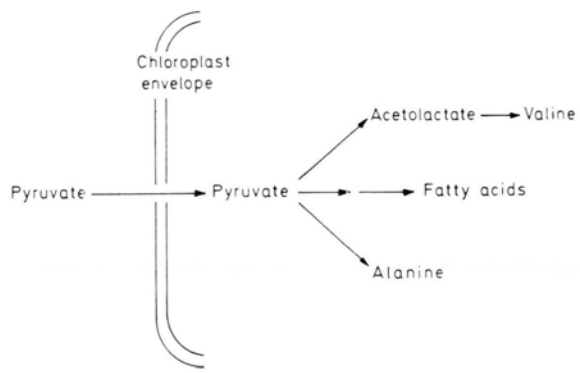


Fig. 3. Effect of increasing concentrations of Chlorsulfuron on fatty acid synthesis. Fatty acids were fractionated into 16:0/18:0 and 18:1 acids. (Note, only rates of fatty acid synthesis in chloroplasts supplied by pyruvate were measured. For further details see Results and Discussion.)

(Fig. 3) (A considerable delay in the regulation was not observed).

It should be noted, that the normally more intensive fatty acid formation *via* acetate → acetyl-CoA in spinach chloroplasts [10] was abolished by using pyruvate which was nearly free from acetate contamination [6].

The results again confirm the specific action of Chlorsulfuron on the synthesis of branched chain amino acids – tested here for valine. On the other hand, the increase of the synthesis of fatty acids as well as of alanine might be deduced on an accumulation of pyruvate because of the diminished consumption by the inhibited enzyme acetolactate synthase.



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