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Heat production and oxygen consumption of root cells with blocked fatty-acids synthesis[1]

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Abstract

The action of the specific inhibitor of fatty acid synthesis cerulenin has been studied in excised roots of 5–6-day-old wheat seedlings grown in $CaCl_2$ solution (2.5×10^{-4} mol/l). Cerulenin decreases heat production and oxygen consumption. Cerulenin also increases plasma membrane permeability for ions and stops adaptive processes of roots. The decrease of heat production and oxygen consumption by roots is probably caused by reduced energy expenditure on fatty acids and lipid biosynthesis and ion-transporting systems. © 1998 Elsevier Science B.V.

Keywords: Fatty-acids synthesis; Heat production; Ion transport; Oxygen consumption; Wheat roots

1. Introduction

The sources of the heat production in cells long been of interest. Zholkevich [1] distinguishes 'first' and 'second' heat sources. The 'first' heat arises from energy dissipation by oxidative reactions. Some of the energy liberated by oxidative reactions is not immediately dissipated as heat, but passes to energy of ATP bonds. Only after doing biochemical synthesis, electrical work, mechanical work etc. is this energy transformed to heat. The heat production follows the equalization of chemical potentials of cell, and serve as a measure of this work [1]. Thus, alteration of metabolic paths of cell must be accompanied by an increase or decrease in heat production coincident with changes in oxygen consumption.

Previously, we had shown that oxygen consumption of and heat production by wheat roots depends, to some extent, on the intensity of peptide synthesis. Inhibition of peptide synthesis by cycloheximide leads to a decrease of oxygen consumption and heat production [2]. In this connection, we expect that the inhibition of energy-consuming process of fatty-acid synthesis will also be accompanied by a decrease in oxygen consumption and heat production. Current literature does not contain any information on the dependence of heat production of intensity of fatty-acid synthesis. We have previously shown that incubation of excised wheat roots in $CaCl_2$ solution under 30°C allows observation within 6 h of different phases of adaptation of roots to new conditions [3] (alarm phase and adaptation phase, Selye [4]). Lipids contribute significantly to adaptation of excised wheat

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roots [5]. In this connection, the goal of our investigation was to determine the oxygen consumption of and heat production by wheat roots with fatty-acid synthesis blocked by the specific inhibitor cerulenin.

2. Experimental

Excised roots of 5-day-old spring wheat seedlings were studied. Seeds were wetted with tap water and grown on 2.5×10^{-4} mol/l $CaCl_2$ solution. Samples of excised roots were incubated with cerulenin (50 mg/l) [6] or antimycin A (2×10^{-5} mol/l) in 2.5×10^{-4} mol/l $CaCl_2$ at 30°C during 6 h. Roots incubated in 2.5×10^{-4} mol/l $CaCl_2$ solution were used as control.

Heat production rate of roots in 3 cm^3 vessels was measured with LKB-2277 bio-activity monitor (Sweden). Oxygen consumption was measured by the Warburg manometric method [7]. K^+-content in the incubation solution was determined by flame photometer Phlapho-41 (Germany). Results are an average of two different experiments, with three replications each.

3. Results

Cerulenin inhibited the oxygen-consumption (Fig. 1) and the heat-production rates (Fig. 2) compared with the control. Blocking fatty-acids synthesis also led to considerable increase in K^+-ion efflux from the roots (Table 1). The loss of K^+-ions by control roots took place in the initial stage after excision (5,2±0,0 μeqv/g), but all K^+-ions were absorbed back into roots during the adaptation process (Table 1). Cerulenin reduced CR ratio [8] compared with control (Fig. 3).

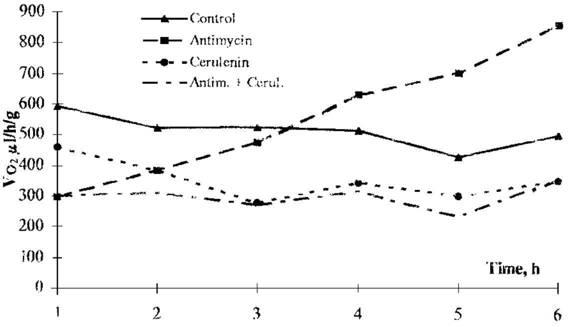

Fig. 1. Oxygen consumption rate of excised roots under the action of cerulenin and antimycin A.

Antimycin A initially suppressed the respiration rate, but then stimulated respiration (Fig. 1). Concurrent action of antimycin A and cerulenin caused suppression of oxygen consumption by roots during the entire period of 6 h. Antimycin A as well as cerulenin retarded the absorption of K^+-ions to 6 h of incubation. Simultaneous action of antimycin A and cerulenin considerably increased the K^+-ion efflux (Table 1).

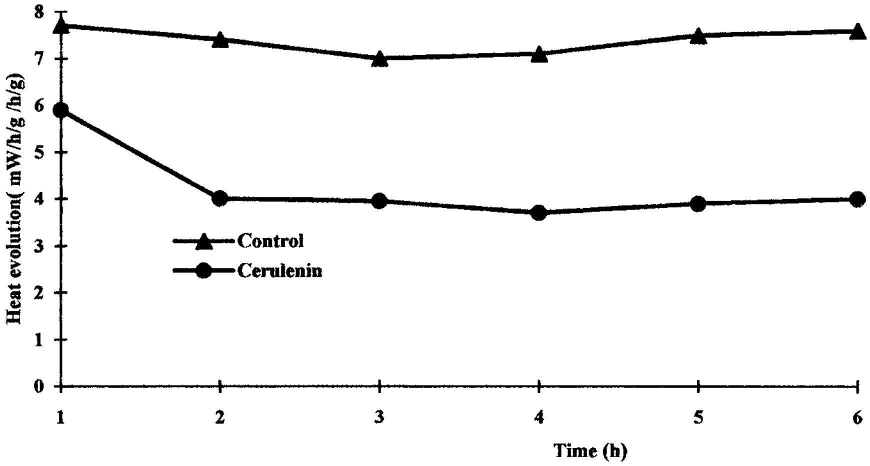

Fig. 2. Heat-production rate of excised roots under the action of cerulenin.

Table 1
K^+-ion efflux from excised roots into the action of incubation medium under antimycin A and cerulenin after 6 h incubation

K^+-efflux	Control	Cerulenin	Antimycin A	Antimycin A+Cerulenin
K^+, μeqv/g	0.7±0.0	8.1±0.5	10.9±1.6	14.9±1.6

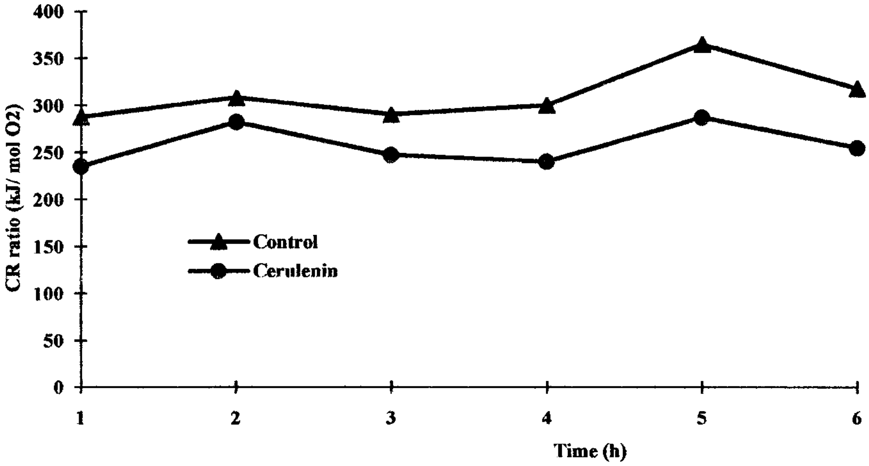

Fig. 3. CR ratio of excised roots under the action of cerulenin.

4. Discussion

Synthesis of fatty acids is energy-consuming process. For instance, synthesis of one molecule of palmitic acid takes 249 molecules of ATP [9] during which only some part of energy of ATP are reserved in bond energy of acid, but the other part dissipates as heat. Blocking fatty-acid synthesis possibly leads to a reduction of P_{in}ADP/ATP ratio and in the synthesis rate of ATP. Decreasing energy expenditures by blocking of fatty-acid synthesis is probably the main reason for the reduction in oxygen consumption and heat production (Figs. 1 and 2), whereas the heat production decreases to a greater extent than the oxygen consumption. As result of this the decrease of CR-ratio takes place under cerulenin action (Fig. 3). It seems likely that blocking fatty-acid synthesis is accompanied with an increase in respiration efficiency.

It is possible that inhibition of oxygen consumption and heat production by cerulenin is determined by reasons other than blocking fatty-acid biosynthesis. For example, the decrease of activity of ion-transport systems in plasma membranes is a result of lipid-deficient root cells [10]. One of the ways lipids influence enzyme activity is by alteration of membrane fluidity.

The excision of roots from wheat seedlings accompanied by K^+-efflux from roots into incubation medium (5.2±0.0 for control and 7.6±1.0 for cerulenin, (eqv/g) after the first 2 h incubation) is a response reaction on the mechanical damage [3]. But during 6-h incubation, the control roots reabsorb all the K^+-ions (Table 1) and transform into a state of 'relative dormancy'. This state is characterized by the recovery of ion homeostasis of root cells, the decrease of plasma-membrane permeability for ions as a result of alteration in structural lipid content [5] and reduction of energetic expense of root cells for ion transport [3]. The action of cerulenin leads to a considerable increase of K^+-efflux, but the lost K^+-ions are not absorbed by the roots (Table 1).

It has been shown that the block of lipid synthesis causes the disruption of barrier properties of plasma membranes [6]. The disruption of barrier properties of plasma membrane with a simultaneous decrease in the activity of ion-transporting systems is one of the reasons for the inability of root cells to restore ion homeostasis and transit to the state of 'relative dormancy' under the action of cerulenin.

Previously, it has been shown that cerulenin suppresses the photosynthetic electron transport in chloroplasts [11]. The authors suppose that the reason for this suppression is a disruption of chloroplast membranes as a result of blocking lipid biosynthesis. This is consistent with our supposition about the role of lipid in determining the activity of ATPases of root cells.

It is interesting to compare actions of cerulenin and mitochondria respiration chain inhibitor, antimycin A. Antimycin A suppresses the cell respiration during the initial incubation stage of excised roots, but this suppression is followed by stimulation of respiration (Fig. 1). Root cells under antimycin A action do not compensate the loss of K^+-ions (Table 1); moreover, antimycin A is protonofore and can carry H^+-ions through membranes along concentration gradients [12]. Like they do under cerulenin action, the root cells do not pass to the state of 'relative dormancy' under antimycin A action. Contrary to the action of cerulenin, antimycin A activates ATPases of plasma membranes [7]. The increase in oxygen consumption rate is thus evidently connected with an increase of energetic expenditures for active transport of ions, in particular K^+-ions. It is likely that lipids take part in activation of ATPases because blocking of fatty-acid synthesis in this condition fully eliminates the stimulation of oxygen consumption by antimycin A (Fig. 1). It also confirms our supposition that one of the reasons of the decrease of oxygen consumption and heat production by root cells under the action of cerulenin is the inhibition of activity of ion-transporting systems of plasma membranes.

5. Conclusion

Cerulenin action leads to a decrease of oxygen consumption and heat production of wheat roots. This is probably connected with the reduction of energy expenditure on fatty acid and lipid biosynthesis and the decrease of activity of plasma membrane ion-transporting systems.

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