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Heat production of wheat roots induced by the disruption of proton gradient by salicylic acid☆

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Abstract

Salicylic acid (SA) results in an increase in oxygen consumption and heat production by excised wheat roots. Activation of respiration and heat production can be caused by protonophoric abilities of SA, which can acidify cell cytoplasm. This is accompanied by increased H^+-ATPase activity of the plasma membrane. Oxygen consumption and heat production by root cells in the presence of SA are sensitive to KCN, an inhibitor of cytochrome oxidase. As cytoplasm acidification leads to electron transfer via the outer mitochondrial membrane, it is believed that these electrons are transferred to cytochrome oxidase. Malate is a unique substrate that may be oxidized by the outer mitochondrial membrane. In the presence of SA, malate causes an increase in respiration, which is sensitive to KCN. Ascorbate, as the electron donor for cytochrome *c* and cytochrome oxidase, stimulates respiration of roots in the presence of SA as well, and this stimulation is also sensitive to KCN. Unike SA, malate and ascorbate do not combine with protons in the membrane and thus do not disrupt the energy accumulation due to ion translocations, that do not lead to the extra heat production by roots. However, as for SA, respiration of roots treated with malate and ascorbate is stimulated because their ions remain dissociated in the aqueous phase and can be transported through the outer mitochondrial membrane via cytochrome/redox reactions.
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Keywords: Salicylic acid; Proton gradient; Respiration; Heat production

1. Introduction

Salicylic acid (SA) is known to possess a thermogenic effect [1,2] that is related to the activation of the alternative (cyanide resistant) oxidase of mitochondria [3]. However, the SA-induced increase in the heat released by plant cells can be due not only to an activation of an alternative oxidase, but also to the protonophoric properties of SA [3]. SA can uncouple oxidation and phosphorylation [4], and can acidify the cytoplasm [5,6]. Earlier we showed that in wheat roots SA treatment depolarizes the plasma membrane, induces potassium loss and considerably increases oxygen consumption by wheat roots [7]. We suggested that because SA is a protonophore it can increase the inward flux of protons. This leads to the acidification of cytoplasm and activation of H^+-ATPase that eventually increases the oxygen consumption of roots. Support for this view came from experiments in which the inhibition of H^+-ATPase by Ag^+ prevented the stimulation of respiration by SA [7]. Moreover, an inhibition of H^+-ATPase was shown to be accompanied not only by a reduction of oxygen consumption, but also a reduction of heat release by plant cells [8].

The aim of the present research was to study the effect of SA on heat production by wheat roots and the possible involvement of mitochondrial oxidation in this process.

2. Experimental

The excised roots of 5-day old wheat (*Triticum aestivum* L.) seedlings grown hydroponically in solution of 0.25 mM $CaCl_2$ were used as the subject of investigation. In all incu-

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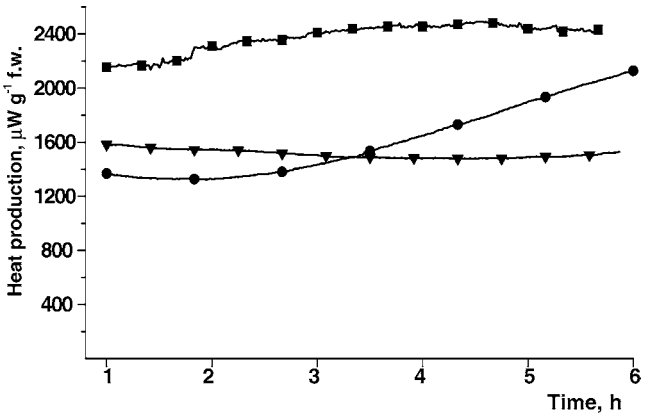

Fig. 1. The effect of SA and carnosine on the heat release by wheat roots: control (0.25 mM $CaCl_2$) (closed circles); 0.25 mM $CaCl_2$ + 1 mM SA (closed squares); 0.25 mM $CaCl_2$ + 1 mM SA + 20 mM carnosine (closed triangles).

bation solutions with effectors and inhibitors 0.25 mM $CaCl_2$ as a membrane-stabilizing compound was present. pH of all solutions used was adjusted to 7.0.

Heat production by root cells was measured using differential microcalorimeter (LKB-2277 Bio Activity Monitor, Sweden) at 30 °C. Glass vials with a total volume of 3 cm^3 were used in our experiments.

Oxygen consumption was measured using manometric method of Warburg. Vessels, 20 ml volume, containing 150 mg of excised roots and 3 ml of incubation solution were shaken for certain period of time with 110 oscillations per min at 30 °C.

In order to add malate, ascorbate and KCN, glass vials where roots were incubated with 0.25 mM $CaCl_2$ or 0.25 mM $CaCl_2$ + 1 mM SA were taken out of calorimeter or Warburg apparatus at certain intervals of time, effectors from liquid stock solutions to give appropriate final concentrations were added and then vials were reloaded back to the calorimeter or Warburg apparatus.

All experiments were performed 4 or 5 times with three replicates.

3. Results

Fig. 1 illustrates the effect of SA on heat production by wheat roots. SA progressively increased heat release by cells during exposure for 5 h (Fig. 1), and this was almost completely prevented by KCN (Fig. 2), an inhibitor of cytochrome oxidase. Treatment of the roots with dipeptide carnosine reduced the stimulation of heat production by SA (Fig. 1).

Fig. 3 illustrates the effect of malate on the respiration of wheat roots in the presence of SA. Treatment of roots with malate caused a sharp increase in KCN sensitive respiration. However, the thermogenic effect of SA was not changed by malate (Fig. 4).

In further experiments, we used ascorbate as an electron donor for cytochrome oxidase. In the presence of SA ascor-

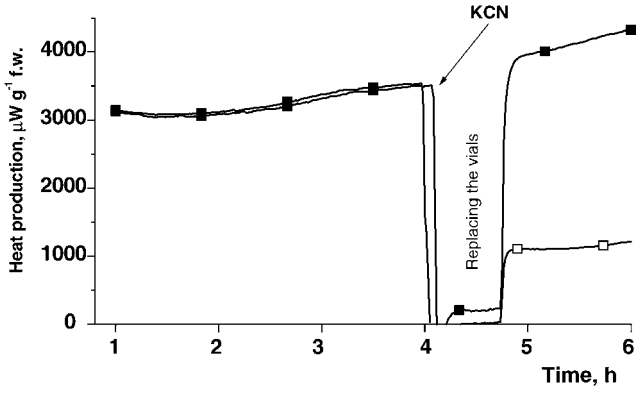

Fig. 2. Heat release by wheat roots treated with SA and KCN: 0.25 mM $CaCl_2$ + 1 mM SA (closed squares); 0.25 mM $CaCl_2$ + 1 mM SA + 3 mM KCN (open squares). After 4 h measuring the rates of heat release of roots in solution of 0.25 mM $CaCl_2$ + 1 mM SA, both vials were removed and KCN from stock solution with final concentration of 3 mM was added to one vial. Appropriate amount of 0.25 mM $CaCl_2$ was added to other vial.

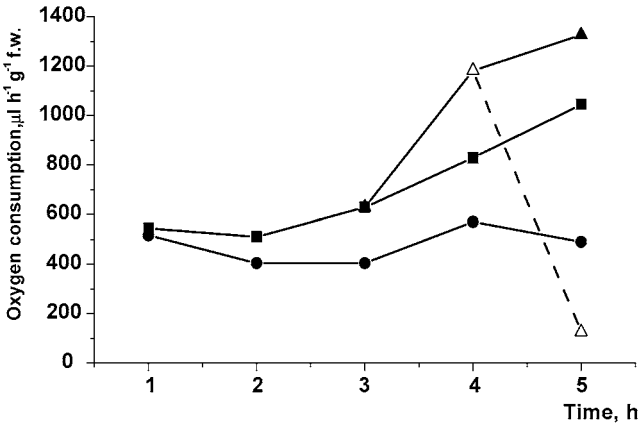

Fig. 3. The effect of malate and KCN on the respiration of wheat roots, where control (0.25 mM $CaCl_2$) (closed circles); 0.25 mM $CaCl_2$ + 1 mM SA (closed squares); 0.25 mM $CaCl_2$ + 1 mM SA + 5 mM malate (closed triangles); 0.25 mM $CaCl_2$ + 1 mM SA + 5 mM malate + 3 mM KCN (open triangles). After 3 h measuring the rates of oxygen consumption by roots in solution of 0.25 mM $CaCl_2$ + 1 mM SA, malate from stock solution with final concentration of 5 mM was added. After following 1 h KCN from stock solution with final concentration of 3 mM was added to incubation solution containing 0.25 mM $CaCl_2$ + 1 mM SA + 5 mM malate. Appropriate amount of 0.25 mM $CaCl_2$ was added to other vials.

bate stimulated oxygen consumption by roots, and this stimulation was prevented by KCN (Fig. 5). As for malate, ascorbate did not change the increase in heat production of roots caused by SA (Fig. 6).

4. Discussion

Results obtained here with plant material support previous findings made using animal tissues that SA treatment increases heat production.

We suggest that one reason for the thermogenic effect of SA could be that SA disrupts the proton gradient of the

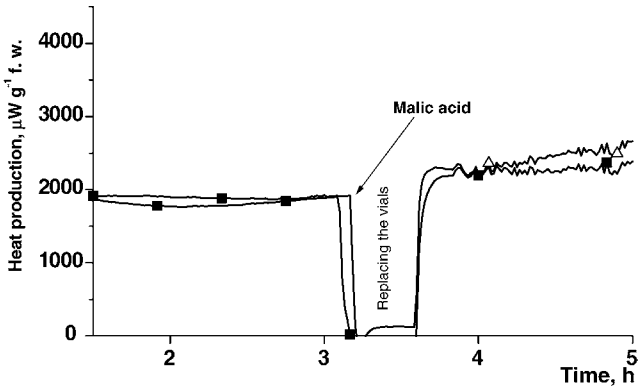

Fig. 4. The effect of SA and malate on the heat production of wheat roots: 0.25 mM $CaCl_2$ + 1 mM SA (closed squares); 0.25 mM $CaCl_2$ + 1 mM SA + 5 mM malate (open triangles). After 3 h measuring the rates of heat release of roots in solution of 0.25 mM $CaCl_2$ + 1 mM SA, both vials were removed and malate from stock solution with final concentration of 5 mM was added to one vial. Appropriate amount of 0.25 mM $CaCl_2$ was added to other vial.

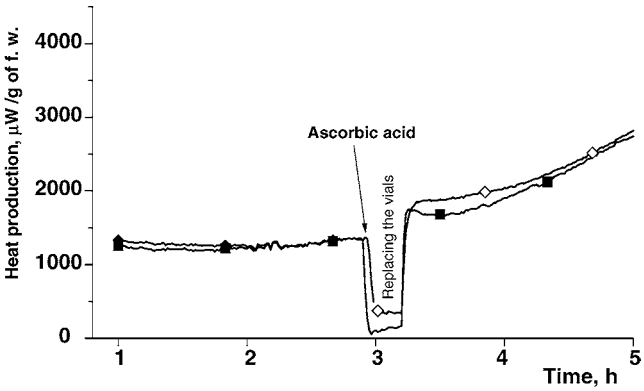

Fig. 6. The effect of SA and ascorbate on the heat production of wheat roots: 1 mM SA (closed squares); 1 mM SA + 5 mM ascorbate (closed diamonds). After 3 h measuring the rates of heat release of roots in solution of 0.25 mM $CaCl_2$ + 1 mM SA, both vials were removed and ascorbate from stock solution with final concentration of 5 mM was added to one vial. Appropriate amount of 0.25 mM $CaCl_2$ was added to other vial.

plasma membrane resulting in an influx of protons, acidification of cytoplasm and a consequent activation of proton pumping. Activation of the proton pump increases energy expenditure and therefore mitochondrial oxidation. Prevention of SA-induced acidification of cytoplasm by carnosine decreased the level of heat production. Carnosine, possessing a strong pH buffering capacity [9], can bind a large amount of protons transported to the cytoplasm by SA. This, in turn, can decrease the activity of plasma membrane H^+-ATPase and the rate of mitochondrial oxidation and, as consequence, lower the level of heat production by cells.

Since it is known that an acidification of cytoplasm leads to an intensification of electron transfer via outer mitochondrial membrane to cytochrome oxidase involving cytochrome *c* and avoiding the first two segments of the respiratory chain [10], we performed an experiment using malate as an electron donor. Previously it was shown that malate can be oxidized by means of electron transport chain of external mitochondrial membrane [4]. The oxidation path via the outer mitochondrial membrane including cytochrome *c* and cytochrome oxidase is economically more beneficial [10]. In our experiments, malate caused a great stimulation of the respiration of roots in the presence of SA, supporting our suggestion of the possibility of electron transfer via the outer mitochondrial membrane. Further experiments showed an intensification of oxygen consumption by root cells when ascorbate was added in the presence of SA. Ascorbate is known to be oxidized by cytochrome *c* and cytochrome oxidase [11]. Similar to malate, ascorbate caused an increase in the respiration by cells which was sensitive to KCN.

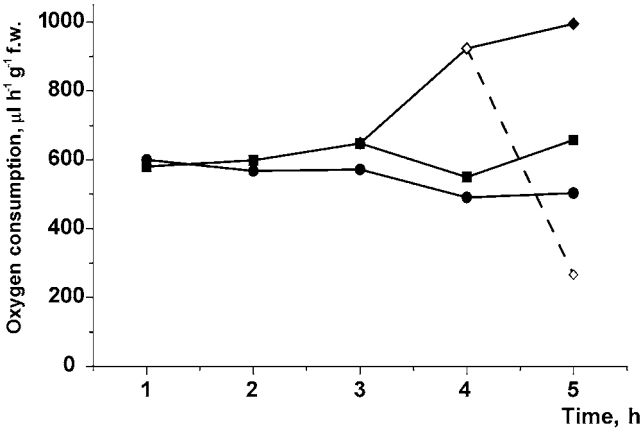

Fig. 5. The effect of ascorbate and KCN on the respiration of wheat roots: control (0.25 mM $CaCl_2$) (closed circles); 0.25 mM $CaCl_2$ + 1 mM SA (closed squares); 0.25 mM $CaCl_2$ + 1 mM SA + 5 mM ascorbate (closed diamonds); 0.25 mM $CaCl_2$ + 1 mM SA +5 mM ascorbate + 3 mM KCN (open diamonds). After 3 h measuring the rates of oxygen consumption by roots in solution of 0.25 mM $CaCl_2$ + 1 mM SA, ascorbate from stock solution with final concentration of 5 mM was added. After following 1 h, KCN from stock solution with final concentration of 3 mM was added to incubation solution containing 0.25 mM $CaCl_2$ + 1 mM SA + 5 mM ascorbate. Appropriate amount of 0.25 mM $CaCl_2$ was added to other vials.

Our results can be explained by the uncoupling mechanisms proposed within the Torsional mechanism of energy transduction and ATP Synthesis [12–14]. The uncoupling process involves the entry of U^- (anion) and H^+ from one bulk aqueous phase (e.g. salicylate and proton) through specific access channels of the H^+-ATPase, their recombination (UH) in the vicinity of the proton and anion-binding sites in the membrane due to the lipid solubility of the uncoupler, their exit as a single, neutral species, thus disrupting the accumulation of energy of the ion translocations, which is converted to heat and is measured in our calorimetric experiments. Dissociation of UH into U^- and H^+ occurs in the aqueous phase, and these dissociated ions are transported to the other side of the membrane by the cytochromes/redox reactions. This leads to the observed stimulation of respiration seen in our experiments. If one of these processes is inhibited (e.g. using KCN or Ag^+), the other coupled process also gets inhibited, thus explaining all our observations in this context with SA. On the other hand, malate and ascorbate do not combine with

H^+ in the membrane and thus do not disrupt the energy accumulation due to ion translocations described [12,13]. Thus no extra heat is produced in these cases. However, respiration is stimulated because the ions remain dissociated in the bulk aqueous phase and the situation is similar in this aspect with the case of SA.

Thus, the results obtained support the suggestion that an effect of SA on the heat production of root cells can be caused by protonophoric features of SA leading to an activation of the plasma membrane proton pump, an increase in energy expenditure and an intensification of mitochondrial oxidation. An increase in the rate of mitochondrial oxidation is related to the intensification of the activity of outer mitochondrial membrane-bound enzymes and cytochrome oxidase. However, the SA-induced activation of respiration did not occur along with an increase in the thermogenic effect of SA when malate and ascorbate were added.

Acknowledgments

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