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# RAPID ELICITOR-INDUCED CHEMILUMINESCENCE IN SOYBEAN CELL SUSPENSION CULTURES*

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**Key Word Index**—*Glycine max*; Leguminosae; soybean; elicitor; *Phytophthora megasperma* f.sp. *glycinea*; chemiluminescence; peroxidase.

**Abstract**—Between 20 and 30 min after exposure to an elicitor from *Phytophthora megasperma* f.sp. *glycinea*, soybean cell suspensions exhibit enhanced chemiluminescence in the presence of luminol. The enhanced chemiluminescence is inhibited by added catalase (EC 1.11.1.6) and superoxide dismutase (EC 1.15.1.1) as well as by the peroxidase inhibitors salicylhydroxamic acid and cyanide. It is apparent that cells produce hydrogen peroxide and $O_2^-$ as an early reponse to elicitor, and likely that peroxidase (EC 1.11.1.7) is involved in the light generating reactions.

## INTRODUCTION

Within minutes after exposure to elicitor molecules, plant cells exhibit several different responses. These include plasmalemma depolarisation [1], cessation of phosphate uptake [2], reduction of amino acid uptake [3, 4] and an increased permeability to ion-selective and membrane potential sensitive dyes [5]. In this communication we report on still another early response, namely, elicitor induced chemiluminescence in soybean cell suspensions in the presence of luminol.

## RESULTS AND DISCUSSION

Between 20 and 30 min after exposure to elicitor, luminol-mediated chemiluminescence of cell suspensions rises to a value *ca* 10-fold greater than that of unelicited cell suspensions, and continues to rise for at least a further 60 min, Fig. 1 shows the results of three representative experiments. The effects of various additives on elicitor-induced, luminol-mediated chemiluminescence (ILCL) are summarized in Table 1. The observed inhibitory effects of added superoxide dismutase and catalase make it apparent that both $O_2^-$ and hydrogen peroxide ($H_2O_2$) participate in ILCL generation. However, since catalase alone almost completely abolishes ILCL, it is probable that the $O_2^-$ is derived from the $H_2O_2$.

Superoxide could be generated from $H_2O_2$, through peroxidase (POD) action. Soybean cell suspensions exhibit POD activity as they catalyse the polymerization of guaiacol in the presence of $H_2O_2$ (data not shown). In addition, the POD inhibitors salicylhydroxamic acid [6] and cyanide [7] both severely inhibit ILCL. It is proposed that $O_2^-$ arises either during POD-catalysed NAD(P)H oxidation [8, 9] or during POD-catalysed oxidation by $H_2O_2$ of luminol [10]. The sensitivity of ILCL to cyanide inhibition rules out the possibility of $O_2^-$ arising through the action of a cyanide sensitive NADPH oxidase such as occurs in phagocytes [11].

$H_2O_2$, like $O_2^-$, could arise from the POD-catalysed oxidation of NAD(P)H; however, the inhibition studies (Table 1) shed no light on this matter. If POD is responsible for both $O_2^-$ production and the chemiluminescent oxidation, by $H_2O_2$, of luminol (see previous paragraph), inhibition of POD activity will cause inhibition of chemi-

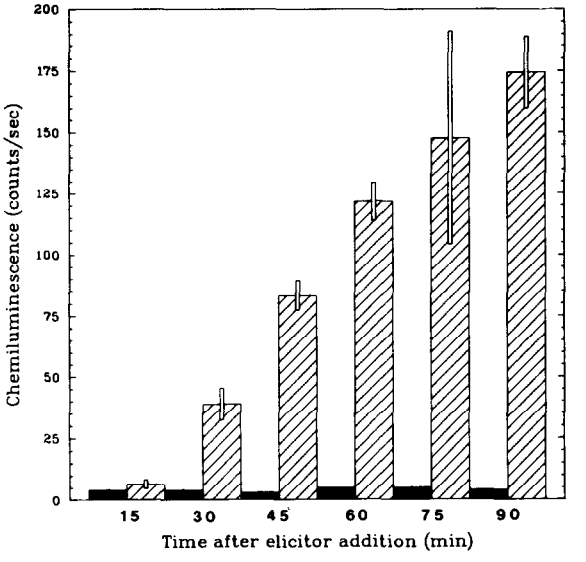


Fig. 1. Luminol-mediated chemiluminescence in the absence (black columns) and presence (shaded columns) of elicitor. Elicitor-induced chemiluminescence was determined in two at 15 min and in three independent experiments at all later times. The temperature was 22°. Bars represent standard deviation of the mean.

*Dedicated to the memory of Tony Swain, 1922–1987.

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Table 1. Inhibition of elicitor-induced chemiluminescence of soybean cell suspensions in the presence of luminol

| Additive | Final activity or concentration | % Inhibition* |
|---|---|---|
| None | | 0 |
| Superoxidase dismutase† | 8 μkat/ml | 78 |
| Boiled superoxidase dismutase† | 8 μkat/ml‡ | 27 |
| Catalase§ | 95 μkat/ml | 92 |
| Boiled catalase§ | 95 μkat/ml‡ | 0 |
| Catalase‖ | *ca* 33 μkat/ml | 96 |
| Boiled catalase‖ | *ca* 33 μkat/ml‡ | 0 |
| Catalase‖ | *ca* 14 μkat/ml | 84 |
| KCN | 2 mM | 94 |
| Salicylhydroxamic acid | 0.2 mM | 100 |
| | 1.0 mM | 100 |
| NaF | 10.5 mM | 90 |
| Malonate | 14.5 mM | 55 |
| Iodoacetamide | 0.11 mM | 0 |
| | 0.55 mM | 0 |
| Citrate | 20 mM | 93 |
| DL-Malate | 19 mM | 82 |
| Guaiacol | 5 mM | 100 |

*Average of at least two determinations.
†Sigma, cat. no. S-2515, from bovine erythrocytes.
‡Before boiling for 10 min.
§ Sigma, cat. no. C-30, from bovine liver.
‖ Boehringer, cat. no. 106810, from bovine liver.

luminescence, even in the presence of $H_2O_2$. Therefore no distinction can be made between the inhibition of POD-catalysed $H_2O_2$ production and the inhibition of the POD-dependent light-generating reactions.

Several other additives inhibit ILCL (Table 1). The inhibition brought about by guaiacol is possibly a result of competition between guaiacol and luminol for POD. The inhibitory effects of malate, citrate and malonate suggest that the Krebs cycle is involved in ILCL. Both citrate and malate also inhibit elicitor-induced phytoalexin synthesis in soybean cells [12]. The disparate effects of two glycolysis inhibitors, NaF and iodoacetamide appear, at first glance, to be contradictory. One explanation is that glycolysis is not involved in ILCL, hence iodoacetate has no effect. The inhibitory action of NaF could then be ascribed wholly to its ability to inhibit POD [7].

ILCL manifests itself rapidly and is easy to detect. It might be possible to screen suspected elicitors rapidly and easily by testing their ability to induce enhanced luminol-mediated chemiluminescence.

## EXPERIMENTAL

*Cells and elicitor.* Soybean cells var. Harosoy 63 were cultured and maintained as described before [13]. Seven-day-old cells were used in all experiments. The elicitor was prepared from cell walls of *Phytophthora megasperma* f.sp. *glycinea* as previously described [14].

*Incubation conditions.* The incubation mixtures in all experiments contained in a total volume of 1 ml 800 μl cell suspension, 100 μmol Na-Pi buffer (pH 7.5) and 200 μg of elicitor.

Mixtures containing elicitor and inhibitor were incubated for 25 min at room temp. Two controls were incubated concurrently with each inhibitor-containing mixture. One control contained neither elicitor nor inhibitor and was used to correct for endogenous chemiluminescence. The other contained elicitor but no inhibitor and was assigned an arbitrary zero percent inhibition.

*Chemiluminescence measurement.* In all cases chemiluminescence was measured over a 10 sec period in a Lumac 3M Biocounter (M 2010) immediately after injection of 100 μl of a saturated solution of Luminol (Sigma) in $H_2O$.

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