10. Toxicity of a Phytoalexin, Pisatin, to Mammalian Cells

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Phytoalexins, post infectional anti-fungal compounds, have been isolated from various diseased plant tissues and discussed in relation to defense reaction of plants against fungal infection. However, their role in disease specificity and the mode of their fungistatic or fungicidal action remained undetermined, although recent findings in our laboratory indicate that phytoalexins play a significant part in host-parasite specificity at least in some powdery mildew diseases.1)-3) Pisatin, a phytoalexin of pea (Pisum sativum L.), was found to injure the plasma membrane of cells of pea plants at a concentration of 300 ppm which is about the concentration that accumulates in leaves 4 days after infection by powdery mildew, and is enough to cause wilting of the infected leaves.4) No report seems to have appeared on the toxicity of phytoalexins to animal cells other than the one report on the haemolytic activity of phaseollin, a phytoalexin of bean (Phaseolus vulgaris L.).5)

This communication deals with results of experiments on the toxicity of pisatin to human red blood cells and on uncoupling of oxidative phosphorylation by rat liver mitochondria.

A solution of pisatin was added to human blood diluted with physiological saline to give final concentrations of 100 or 300 ppm and morphological changes of erythrocytes were observed under a light or interference phase contrast microscope. The shape of erythrocytes became undetectable by light microscopy within a few minutes when treated with 300 ppm of pisatin. Interference phase contrast microscopy, however, showed that erythrocytes were replaced by ghost-like spherical structures. The detailed study of this haemolytic process is under way. Erythrocytes were deformed to crenated sphere (echinocytes), or cup-shape (stomatocytes) immediately after treatment at 100 ppm (Fig. 1).

The effect of pisatin on the function of erythrocyte membrane
was demonstrated by measuring leakage of potassium ion from cells with a potassium sensitive electrode (Electronic Instruments Limited, type GKN 33B) (Fig. 2). Almost all potassium ions were released from red blood cells within 8 minutes after treatment with 200 ppm pisatin or higher concentrations.

These results, together with previous findings, indicate that

Fig. 1. Effect of pisatin at 100 ppm on human red blood cells observed by interference phase contrast microscopy.
(a) Non-treated cells in buffered physiological saline (10 mM phosphate, pH 7.4) containing 0.3% ethanol.
(b) Echinocytic change 3 min after treatment.
(c) Stomatocytic change 3 min after treatment.
(a), (b) and (c) ×700

Fig. 2. Effect of pisatin on K⁺ compartmentation of human red blood cells. Red blood cells (RBC) (1.6 × 10⁷ cells/ml) incubated at 37°C in 3 ml 0.15 M choline-chloride and 10 mM Tris-HCl buffer (pH 7.4). Pisatin (30,000 ppm ethanol solution) added to incubation mixture to final concentrations of 100, 200, or 300 ppm (final concentration of ethanol <1%). After 7-9 min incubation, 0.05% triton X-100 added to give complete leakage of K⁺.
pisatin injures plasma membranes both plant cells and erythrocytes.

Another interesting finding is that pisatin uncouples oxidative phosphorylation by rat liver mitochondria. Namely, as seen in Fig. 3, pisatin activated oxygen uptake by mitochondria. The stimulation of succinate oxidation by ADP was completely inhibited when pisatin was added to reaction mixture, and the expected increase of oxygen uptake following addition of Ca** was also inhibited by pisatin.

Uncoupling has also been reported for ipomeamarone, a phytoalexin from sweet potato (Ipomoea batatas Poir.). It prevented oxidative phosphorylation by mitochondria from mung bean and sweet potato.5) The results presented in this paper and in others4,6 suggests that toxicity of all phytoalexins to mammalian cells or tissues should be examined because these substances may be present in horticultural and agricultural produce.

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