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A Degradation Product of Fenitrothion, 3-Methyl-4-nitrophenol, Is an Inhibitor of Mammalian Ribonucleotide Reductase

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Fenitrothion (0,0-dimethyl 0-(3-methyl-4-phenyl) phosphorothioate is a broad-spectrum insecticide used extensively throughout the world for the control of agricultural and forest pests. As with other members of the phosphorothioate class of organophosphates, fenitrothion exerts its toxic potential through inhibition of acetylcholinesterase when converted to the phosphate or "oxon" form (IVERSON & MARSHALL 1977). Other mechanisms of action may exist as well (e.g. LEHOTZKY & UNGVARY 1976).

As degradation of fenitrothion can occur rapidly through various physical and biological pathways (SYMONS 1977), it is reasonable to assume that the biological impact of the insecticide is modified by the presence of decomposition products. 3-methyl-4-nitrophenol is found as a contaminant of fenitrothion and is a breakdown product of the insecticide in water and soil; decomposition of fenitrothion by microorganisms, plants and animals normally occurs rapidly with 3-methyl-4-nitrophenol as a major metabolite (MIYAMOTO 1977).

Ribonucleotide reductase is the enzyme solely responsible for the conversion of the four ribonucleotides to their corresponding deoxyribonucleotides required for DNA synthesis. Many independent investigations have uncovered a critical association between the reductase and cell growth. This has led to the suggestion that ribonucleotide reduction is a rate-limiting step in DNA synthesis and cell division (WRIGHT et al. 1981). In this report we show that 3-methyl-4-nitrophenol inhibits the activity of this key regulatory enzyme.

METHODS

Chinese hamster ovary (CHO) cells were grown in culture as previously described (WRIGHT 1973; HARDS & WRIGHT 1981). To determine plating efficiencies, cells were added to tissue culture plates at cell numbers ranging from 10^2 to 10^4 cells/100 mm plate. After a suitable period of incubation at 37°C, the plates were drained and colonies were stained with a saturated solution of methylene blue in 50% ethanol and counted (HARDS & WRIGHT 1981). The plating efficiency was estimated by dividing the number of colonies counted by the number of cells plated. The relative plating efficiency was defined as the plating efficiency in the presence of 3-methyl-4-nitrophenol (Aldrich Chemical Co.) divided

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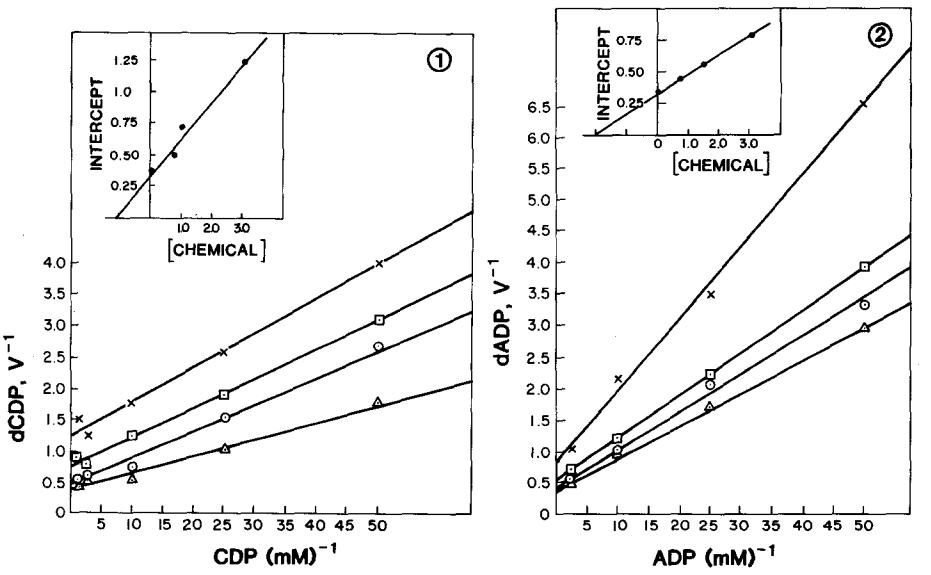
by the plating efficiency in the absence of the chemical.

Enzyme preparations were prepared from suspension grown cells and the procedures used to determine CDP and ADP reductions were slight modifications of those described earlier by LEWIS & WRIGHT (1978). In brief, CDP reductase was assayed in the presence of 50 mm Hepes buffer, pH 7.2, 4 mM ATP, 8 mM $MgCl_2$, 6 mM dithiothreitol and various concentrations of (^{14}C)-CDP in a total volume of 150 µL. After 30 min at 37°C the amount of dCDP formed was estimated by the procedure of STEEPER & STEUART (1970) with the use of Dowex 1-borate columns. ADP reductase was assayed in 50 mM Pipes buffer, pH 6.8, 0.5 mM dGTP, 6 mM dithiothreitol and various concentrations of (^{14}C)-ADP in a total of 150 µL. The amount of dADP formed during a 30 min assay at 37°C was determined with the use of Dowex-1-borate chromatography as described by CORY et al. (1973). One unit of enzyme activity is defined as the amount that reduces 1 nmole of ribonucleotide in 1 h.

Stock solutions of 3-methyl-4-nitrophenol were prepared in acetone/water (1:1) and control experiments were performed with the solvent alone in plating efficiency and enzyme activity studies. Chemicals used to assay ribonucleotide reductase were obtained from sources previously described (WRIGHT et al. 1981; HARDS & WRIGHT 1981; LEWIS & WRIGHT 1978). A sample of fenitrothion was kindly provided by Dr. W.L. Lockhart of the Freshwater Institute, Winnipeg, Manitoba, Canada.

RESULTS AND DISCUSSION



Figures 1 and 2. Double reciprocal plots of velocity against CDP and ADP at several fixed concentrations of 3-methyl-4-nitrophenol. Assays were performed in the absence (Δ) or presence of 1 mM (O), 1.5 mM (□) and 3 mM (X) 3-methyl-4-nitrophenol.

Since ribonucleotide reductase catalyzes the reduction of both pyrimidine and purine ribonucleotides (WRIGHT et al. 1981), we examined enzyme activity in the presence of a pyrimidine (CDP) and a purine (ADP) substrate. Figure 1 shows double reciprocal plots of velocity of mammalian ribonucleotide reductase against CDP concentrations at several fixed levels of 3-methyl-4-nitrophenol. Clearly, the chemical is a good inhibitor of pyrimidine nucleotide reduction. The lines were linear and the pattern of inhibition appeared to be noncompetitive. A replot (inset, figure 1) of velocity intercepts against inhibitor concentrations showed a Ki value of 1.1 mM. Figure 2 contains double reciprocal plots of velocity against ADP at various constant levels of 3-methyl-4-nitrophenol. Purine nucleotide reduction was also inhibited in a noncompetitive fashion and replots (inset, figure 2) of velocity intercepts versus inhibitor concentrations yielded a Ki value of 2.0 mM. Fenitrothion, on the other hand, was not an effective inhibitor of ribonucleotide reduction (results not shown). The Ki values obtained for the inhibition of CDP and ADP reduction by 3-methyl-4-nitrophenol closely resembled those reported for the inhibition of CDP and ADP reductase by various antitumor agents like hydroxyurea, guanazole and N-carbamoyloxyurea (WRIGHT et al. 1981). Hydroxyurea inhibits enzyme activity by interacting with one of the two enzyme subunits and interfering with the formation of a tyrosine free radical required for the reductive process. Moreover, there are structural similarities between 3-methyl-4-nitrophenol and a series of polysubstituted benzene derivatives recently described by ELFORD et al. (1981), which are inhibitors of ribonucleotide reductase and appear to have a mode of action similar to hydroxyurea. These observations suggest that 3-methyl-4-nitrophenol may also interfere with enzyme activity by a

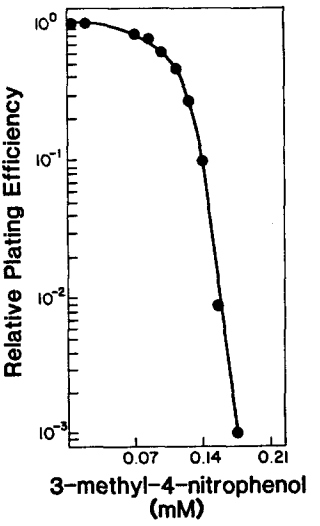


Figure 3. Relative plating efficiency of CHO cells in the presence of various concentrations of 3-methyl-4-nitrophenol.

mechanism involving tyrosine free radical formation. It is known that ribonucleotide reductase inhibitors such as hydroxyurea, guanzole and N-carbamoyloxyurea are cytotoxic for cells in culture (WRIGHT et al. 1980, 1981). Figure 3 shows that similar to these other enzyme inhibitors, 3-methyl-4-nitrophenol is a potent cytotoxic agent for cultured animal cells.

In conclusion, the results reported here indicate for the first time that inhibition of ribonucleotide reductase by 3-methyl-4-nitrophenol, which is a common metabolite and breakdown product of fenitrothion, should be taken into account when considering the overall biological activity of this organophosphate insecticide.

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