The Effects of Dichlorvos and Trichlorphon on Catalase Induction, Respiration and Growth of Yeast, Saccharomyces Cerevisiae

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INTRODUCTION

The hazards of the use of organophosphate pesticides depend on an ignorance of their mode of action (CASIDA 1973). The available data show that these compounds have an inhibitory effect not only on acetylcholinesterase but also on a number of other enzymes (HEIDKER and PARDINI 1972, SITKIEWICZ and ZALEWSKA 1975, PACHECKA et al. 1975). Several authors have reported that organophosphate pesticides have also cytotoxic (PIEKARSKI et al. 1971) and mutagenic activity (ASWOOD-SMITH et al 1972, VOODG et al. 1972).

In this paper we report a simple and rapid method of determination of the effects of organophosphate pesticides on enzymes induction. This method is based on the determination of the action of the pesticides on catalase induction in yeast during adaptation to aerobic conditions. Yeast cells cultured anaerobically have very low catalase activity (LORENC et al. 1968), but if they are subsequently exposed to oxygen, catalase activity appears and increases during a few hours (CHANTRENNE and COURTOIS 1954). This process known as an oxygen induction is connected with de novo catalase synthesis (PACHECKA and SITKIEWICZ 1971).

The purpose of the present work was to study the effects of Dichlorvos and Trichlorphon on catalase induction, respiration and growth of yeast Saccharomyces cerevisiae.

MATERIALS AND METHODS

Strains and growth conditions

In all experiments respiratory-sufficient i.e., standard Yeast Foam and respiratory-deficient mutant Yeast Foam-A from our own museum were used.

For anaerobic cultures the following medium was used: glucose, 50 g; peptone Difco, 20 g; yeast extract Difco, 2 g; potassium monophosphate, 1 g; ammonium sulphate, 1 g; calcium pantothenate, 2 mg; nicotinic acid, 0.2 mg; biotin, 0.25 mg; ergosterol, 0.5 mg; Tween 80, 0.5 ml; and distilled water, 1 liter.

The basic semisynthetic medium for aerobic cultures contained: yeast extract Difco, 2 g; ammonium sulphate, 2 g; potassium monophosphate, 1 g; magnesium sulphate, 0.5 g; sodium chloride,

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0.5 g; and distilled water, 1 liter. It was supplemented with glucose, 10 g, in YG-1 medium; glucose, 50 g, in YG-5 medium or with glycerol, 30 g, in YGlyc-3 medium, and if necessary, solidified with agar Difco, 20 g.

Aerobic or anaerobic cultures incubated at 28°C were inoculated with stationary phase cells grown aerobically in YG-1 medium. The inoculum, which represented about 2 mg of dry weight of cells, was added to 1 liter of growth medium. Aerobic or anaerobic conditions of growth were secured by continuous flow of oxygen or argon by the media, respectively.

The growth rate was followed by measuring the turbidity of cells suspension at 570 nm. For dry weight measurements the cells were harvested and washed by centrifugation and dried at 110°C. There was a constant relationship between turbidity and dry weight during all period of growth.

Induction of catalase

Induction of catalase by oxygen in anaerobically grown yeast was carried out in the medium containing: phosphate buffer, pH 5.0, 67 mM; magnesium sulphate, 0.2 mM; and glucose, 10 mM. The stationary phase anaerobically grown cells were washed and resuspended in above medium to a final concentration of 1.5 - 2.5 mg of dry weight per ml. This suspension was aerated at 28°C. Induction was stopped by cooling and centrifugation of cells. In cells harvested at different times of aeration the catalase activity was determined.

Catalase activity determination

The quantitative method of SUMNER and DOUNCE (1955) was applied for determination of catalase activity in yeast cells (PACHECKA and SITKIEWICZ 1971). The cell samples harvested at different times of aeration were resuspended in water to a final concentration of about 2 mg per ml. To 3 ml of the cells suspension 1 ml of chloroform was added and the mixture was shaken vigorously for 1 min. After partition, the catalase activity was determined in the aqueous phase. The activity is expressed as a coefficient of the first order reaction per mg of dry weight of yeast cells.

Respiration measurements

Oxygen uptake by aerobically grown yeast cells was determined manometrically in Warburg apparatus at 28°C in the atmosphere of air. The final volume of the reaction mixture was 3 ml. It contained: phosphate buffer, pH 5.0, 2.5 mM; glucose, 10 mM or ethanol, 100 mM; and about 2 mg of dry weight cells. Oxygen consumption is expressed as Q_{O_2} (μl O_2/hr/mg dry weight).

Chemicals

Dichlorvos and Trichlorphon were obtained from Zaklady Chemiczne "Azot" Jaworzno, Poland. Other reagents had been of analytical grade

and were obtained from local commercial sources.

RESULTS

Data presented in Table 1 show that Dichlorvos but not Trichlorphon had an inhibitory effect on catalase induction in yeast adapting to aerobic atmosphere. In the control conditions, induction of catalase proceeds during about 5 hrs of aeration of anaerobically grown yeast. During this period manifold increase of catalase activity was observed. Dichlorvos at the concentrations 0.1 mg/ml, 0.5 mg/ml and 1 mg/ml inhibited catalase induction after 1 hr of aeration by about 75%, 80% and 85% and after 5 hrs by 51%, 64% and 70% respectively.

The effects of both pesticides on oxygen uptake by yeast grown aerobically was studied in the presence of glucose or ethanol as substrates. As seen from data presented in Table 2 Dichlorvos at concentrations of 0.1 mg/ml, 0.5 mg/ml and 1 mg/ml caused a marked decrease of oxygen uptake in the presence of both substrates. Trichlorphon at all concentrations tested had no effect on respiration of yeast.

TABLE 1 Effects of Dichlorvos and Trichlorphon on catalase induction

Pesticide added mg/ml	Catalase activity		
	Induction time (hr)		
	1	3	5
Dichlorvos			
0	3.9 ± 0.01	5.0 ± 0.01	9.4 ± 0.01
0.01	3.9 ± 0.01	5.0 ± 0.01	0.4 ± 0.01
0.10	0.9 ± 0.01[a]	2.5 ± 0.02[a]	4.6 ± 0.02[a]
0.50	0.8 ± 0.01[a]	2.4 ± 0.03[a]	3.4 ± 0.01[a]
1.00	0.6 ± 0.02[a]	1.3 ± 0.02[a]	2.8 ± 0.02[a]
Trichlorphon			
0	2.5 ± 0.02	6.3 ± 0.02	9.8 ± 0.02
0.10	2.5 ± 0.02	6.3 ± 0.02	9.8 ± 0.02
1.00	2.5 ± 0.03	6.7 ± 0.02	9.8 ± 0.02

Here and in other Tables the numbers represent mean values from 5 experiments ± standard error of mean
a/ statistically significant diference $p \leq 0.01$

TABLE 2 Effects of Dichlorvos and Trichlorphon on oxygen uptake by aerobically grown yeast

Pesticide added mg/ml	Oxygen uptake(µl 0_2/hr/mg) Substrates	
	Glucose	Ethanol
None	108 ± 2.8	135 ± 4.0
Dichlorvos		
0.01	110 ± 2.6	97 ± 3.9[a]
0.10	84 ± 2.7[a]	78 ± 3.9[a]
1.00	65 ± 3.0[a]	61 ± 3.9[a]
Trichlorphon		
1.00	105 ± 3.0	145 ± 4.0

For explanation see Table 1.

For the study of the influence of the pesticides on the growth, we used standard strain Yeast Foam and cytoplasmic respiratory deficient mutant Yeast Foam - A. The standard strain is able to grow on the media containing fermentable, as well as nonfermentable carbon sources, whereas respiratory-deficient mutant can utilize only fermentable compounds.

In Table 3 are presented the effects of both pesticides on the yield of yeast cells expressed in mg of dry weight per ml of medium after 30 hrs of growth under identical conditions. Dichlorvos at concentration 1 mg/ml completely inhibited growth of standard strain on the glycerol containing medium.

In the case of glucose containing media, the inhibition level depended on the concentrations of Dichlorvos and glucose in the medium, in YG - 1 medium it was 67% but in YG - 5 medium only 27% at maximum concentrations of Dichlorvos used in these experiments. In contrast, Dichlorvos had no effect on the growth of respiratory-deficient mutant. Trichlorphon had no effect on the growth of both strains in all media tested.

It was possible that the inhibition of catalase induction, respiration and growth of yeast by Dichlorvos could be due to killing of cells during the incubation with the pesticide. To test this possibility the samples of cells, which had been incubated with Dichlorvos for 5 hrs were after proper dilution plated on YG-1 solid medium. The colony counts were carried out after 5 days of incubation at 28°C. The numbers of colonies obtained were exactly the same in control and treated samples. This indicates that Dichlorvos had no cytotoxic action on yeast cells.

TABLE 3

Growth yields (mg/ml) of standard and respiratory - deficient mutant in different media in the presence of Dichlorvos and Trichlorphon

Pesticide added mg/ml	Standard strain			Respiratory mutant
	YG-1	YG-5	YGlyc-3	YG-1
Dichlorvos				
0	1.30 ± 0.07	1.45 ± 0.07	0.90 ± 0.07	0.83 ± 0.07
0.01	1.30 ± 0.06	1.45 ± 0.07	0.90 ± 0.07	0.83 ± 0.07
0.10	0.87 ± 0.07[a]	1.45 ± 0.08	0.70 ± 0.07[a]	0.83 ± 0.07
0.50	0.75 ± 0.08[a]	1.45 ± 0.08	0.54 ± 0.08[a]	0.81 ± 0.07
1.00	0.58 ± 0.08[a]	1.45 ± 0.08	0.00 ± 0.00[a]	0.78 + 0.08
Trichlorphon				
0	1.30 ± 0.08	1.45 ± 0.08	0.90 ± 0.07	0.78 ± 0.07
1.00	1.25 ± 0.07	1.45 ± 0.08	0.85 ± 0.08	0.79 ± 0.08

Growth yields was determined after 30 hrs of incubation at 28°C:
YG-1 and YG-5 media contained 1% and 5% glucose respectively,
YGlyc-3 medium contained 3% glycerol.
For other explanations see Table 1.

DISCUSSION

The data presented in this paper indicate that Dichlorvos inhibited catalase induction, respiration and growth of the standard yeast strain in glycerol containing medium. On the other hand, it had no effect on growth of the standard strain in YG-5 medium and on the growth of the respiratory-deficient mutant. These results suggest strongly that this pesticide disturbs the oxidative processes in yeast cells. This suggestion is confirmed by the literature data concerning the effects of organophosphate pesticides on the respiratory chain enzymes (SITKIEWICZ and ZALEWSKA 1975).

In contrast to Dichlorvos, Trichlorphon did not change catalase induction and other processes tested. The differences in the action of these pesticides could presumably be a result of their various capability to penetration into yeast cells or different sensitivity of tested processes to the action of the pesticides tested. No effect of Trichlorphon could be due to impermeability of yeast cells walls for this substance. However, if it penetrated into cells, the lack of its effect could indicate that, in contrast to other organism (ARTHUR and CASIDA 1957), in yeast Trichlorphon is not converted to Dichlorvos.

Catalase induction depends on a metabolic energy supply, therefore factors influencing oxidative processes can affect this process. Thus inhibition of catalase induction and respiration by Dichlorvos can suggest that the decrease of synthesis of this enzyme during aeration could be due to the disturbance of oxidative processes. On the other hand, the influence of Dichlorvos on catalase induction in the first period of aeration, when the yeast cells have not yet efficient respiratory chain enzymes can indicate that not only the disturbance of oxidative processes is responsible for this effect.

No effects of Dichlorvos and Trichlorphon on growth of yeast in YG-5 medium, as well as on growth of respiratory-deficient mutant Yeast-Foam-A lead to the conclusion that these pesticides have no effect on fermentation in yeast.

In this paper we report a simple system for the study of the effects of organophosphate pesticides on enzymes induction, oxidative and fermentative processes. This system is based on the determination of the effects of these compounds on the oxygen induced catalase synthesis in yeast, as well as their effects on respiration and growth on the media containing various carbon sources. The yeast cells growth in the presence of glycerol produce energy only from oxidative phosphorylation, whereas cells grown in anaerobiosis or in the media containing high concentration of glucose, i.e. 5% produce energy only from fermentation. Similarly, the respiratory-deficient mutants utilize only fermentable compounds as energy sources. Thus the use of different growth conditions for standard strain, as well as respiratory-deficient mutants allows to study the effects of the pesticides and other

environmental agents on catalase induction, respiration and fermentation in yeast cells.

It could be concluded, that yeast may be a particularly suitable organism for screening studies of the effects of environmental contaminations on a number of biochemical processes. The methods of these studies are simple, rapid and inexpensive.

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