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Enantioselective Inhibition of Dichlorprop on Catalase

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Abstract The enantioselectivity interaction of 2,4-dichlorprop (DCPP) and catalase were studied, and it was further evaluated with the presence of humus. Both of *rac*-DCPP and *R*-DCPP can inhibit the activity of catalase with the concentrations of 0.05–80 mg L^{-1}, the inhibitory type of *rac*-DCPP was uncompetitive, and of *R*-DCPP was complex. The presence of humic acid has changed the inhibitory ability of DCPP on catalase, the inhibition of *rac*-DCPP disappeared and the inhibition type of *R*-DCPP mainly became uncompetitive. These results suggest that inhibition of chiral DCPP on catalase is enantioselective.

Keywords 2,4-dichlorprop · Chiral herbicide · Catalase · Enantioselective

About 25 % of currently used pesticides are chiral, and this ratio is increasing with the introduction of more structure-complex pesticides (Liu et al. 2005). A pair of enantiomers has identical physical–chemical properties and thus appears as a single compound in common standard analysis. However, they show different activities in biological systems, because most of the identities and structures in nature are chiral, and therefore there are greater chances of the chiral pollutants reacting at different rates. Catalase (EC 1.11.1.6) serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. The reports about the enantioselective effect of pesticide on the activity of catalase were few, because there is no enantiomeric otherness in the catalyze reaction by the catalase. However the enzyme is composed of many chiral amino acid residues, so the enzyme could be treated as the molecule with many chiral cores.

Humus were come from the decompose organic matter, According to the different sources of organic matter, there are many different molecule structures in the humus centre, such as acyclic aromatic, quinone compounds and acyclic structures Schulten (1995). Because of the complex molecule structures, the ecological effect of humus was very contradictorily: one hand, humus could reduce the bioavailability and toxicity of organic contamination by the combining process of hydrophobic and aromatic structure (Giesy et al. 1977; Campbell and Evans 1987; Anisimova et al. 1998); on the other hand, humus also could influence the organism, for example it would influence the biofilm by accumulation (Campbell et al. 1997) or act as surfactant (Wang et al. 1999; Steinberg et al. 2003; Nardi et al. 2002).

Dichlorprop (DCPP) as a phenoxyalkanoic acids pesticide, are intensively used to control broad leaf weeds in pastures, cotton, tobacco, corn, sugar cane and rice among other cultures and also to treat non-agricultural fields that will be used thereafter for planting crops. Their frequent occurrence in groundwater and soil indicated that they may be critical contaminants that deteriorate drinking water resources. DCPP exits two enantiomeric forms, the (*R*)-and (*S*)-forms, but only the (*R*)-forms are active herbicides (Fig. 1). The purpose of this study was to investigate the enantioselective interaction of dichlorprop and catalase, and it was further evaluated in the present of humus.

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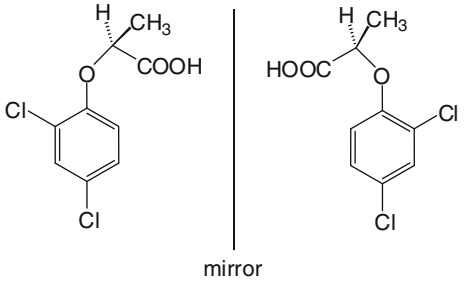

Fig. 1 Chemical structure of the two enantiomers of DCPP

Materials and Methods

Dichlorprop (*rac*-DCPP and *R*-DCPP) was supplied by the organic synthesis lab of our institute, and it was validated by the mass spectrum and NMR with the degree of purity exceeding 99 %; Catalase was purchased from Washington biochemistry corporation. The substrate H_2O_2 was analytical pure, produced by Linan fine chemical reagent plant (Hangzhou, China); other solvents or chemicals used in this study were of analytical grade.

The activity of catalase was calculated by $(E_{240} \times 3)/(0.0436 \times E_w) = \text{U/mg}$, where E_{240} is the decreasing value of the absorbency per min at 240 nm, E_w is the content of enzyme in per 0.10 mL enzyme solution (mg). Inhibitory type of DCPP versus catalasewere described by Michaelis–Menten mechanism: $V=V_{max} \times [S]/(K_m+[S])$, where V is reaction rate, [S] is substrate concentration, V_{max} is maximum rate, K_m is Michaelis–Menten constant (measures enzyme/substrate affinity).

Assay for catalase inhibition: various concentrations of pesticide solution (2.5, 5.0, 10.0, 20.0, 40.0, 60.0 and 80.0 mg L^{-1}) were added into 50 μL enzyme solution, diluted with buffer solution to 2 mL. The mixture was incubated at 20°C for 1 h, followed by the addition of 1 mL H_2O_2 solution, and E_{240} was measured by UV–visible Spectrometer (UV-2401PC, Shimadzu, Japan). Meanwhile, control samples with no pesticide were also prepared. All of the tests were performed in four replicates.

Kinetics of enzymatic reaction: Various concentrations of pesticide solution (2.5, 5.0, 10.0, 20.0, 40.0, 60.0 and 80.0 mg L^{-1}) were added into 50 μl enzyme solution, diluted with buffer solution to 2 mL. The mixture was incubated at 20°C for 1 h, followed by the addition of 1 mL H_2O_2 solution, the H_2O_2 concentration were 10.0, 12.5, 14.3, 16.7, 20.0, 25.0, 33.3 and 50.0 μmol L^{-1}, respectively. Determine the E_{240} for 1 min at once. Every substrate concentration was performed in four replicates.

Assay for humic acid: 50 μL humic acid solution was added into 10-mL tubes before the test, and the following steps were same to assay for catalase.

Results and Discussion

As showed in the Fig. 2a, b, both *rac*-DCPP and *R*-DCPP inhibited catalase activity. With the rising of the inhibitor concentrations, the decrease of enzyme activity was expressed as linear correlation. But the inhibition action was unobvious at lower concentration. When the pesticide concentration reached at 20 mg L^{-1}, the inhibition rates of catalase activity by *rac*-DCPP and *R*-DCPP were 10 % and 20 %, respectively. Between 20 and 60 mg L^{-1}, the inhibition of *R*-DCPP was unobvious, basically keeping the level between 80.86 % and 77.23 %, however the inhibition of *rac*-DCPP was expressed as obvious linear relation, the relative activity of enzyme decreased from 90.4 % to 71.4 %. When the pesticide concentration was between 60 and 80 mg L^{-1}, the inhibition of *R*-DCPP increased obviously, which declined from 77.23 % to 67.70 %, the inhibition of *rac*-DCPP was basically kept similar with 60 mg L^{-1}. There were no significant differences with inhibition on the enzyme (exceed the level of 0.05 mg L^{-1}) by statistical analysis.

As showed in Fig. 2c, d, *R*-DCPP has obvious inhibition effect on catalase with humic acid, however the inhibition of *rac*-DCPP was almost disappeared. With the rising of the *R*-DCPP concentration, the decrease of enzyme activity was also expressed as linear correlation. At low concentrations, the inhibition of *rac*- and *R*-DCPP was different: *R*-DCPP also had the weak inhibition action; but *rac*-DCPP had more active action in some degree. Between 20 and 60 mg L^{-1}, the inhibition of *R*-DCPP was also unobvious, basically kept the level between 85 % and 80 %, however the inhibition of *rac*-DCPP was relatively obvious, the relative activity of enzyme was decreased from 105 % to 95 %. The pesticide concentrations between 60 and 80 mg L^{-1}, the inhibition of *R*-DCPP increased obviously, which declined from 80 % to 65 %, the inhibition of *rac*-DCPP on the relative activity of enzyme was basically kept similar with 60 mg L^{-1}. There were significant differences with inhibition on the enzyme (exceed the level of 0.05) by statistical analysis.

The action of inhibitor on the enzyme could be divided into reversible and irreversible reaction. And the reversible inhibition could be divided into three types: competitive inhibition (K_m has increased, V_{max} has unchanging), non-competitive inhibition (K_m has unchanging, V_{max} has reduced) and uncompetitive inhibition (both K_m has reduced, and V_{max} has also reduced).

The substrate concentration was changed under different concentration of *rac*- and *R*-DCPP, determining the initial velocity of the enzyme reaction. The inhibition types of *rac*- and *R*-DCPP on the catalase were obtained by Fig. 3 which constructed use 1/V versus 1/S according to the method of Lineweaver–Burk double reciprocal. The Table 1 showed

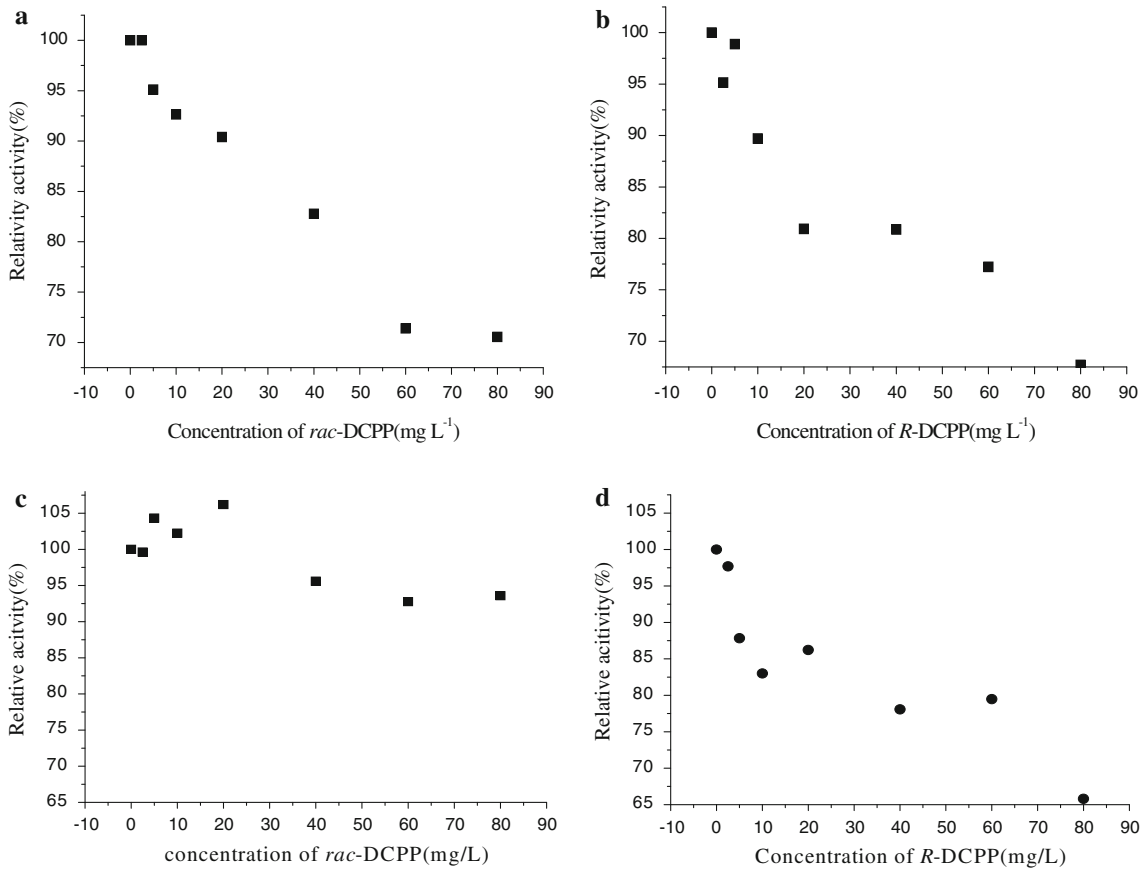

Fig. 2 Effect of *rac*-DCPP and *R*-DCPP on activity of Catalas (**a**, **b** without the treatment of humic acid; **c**, **d** with the treatment of humic acid)

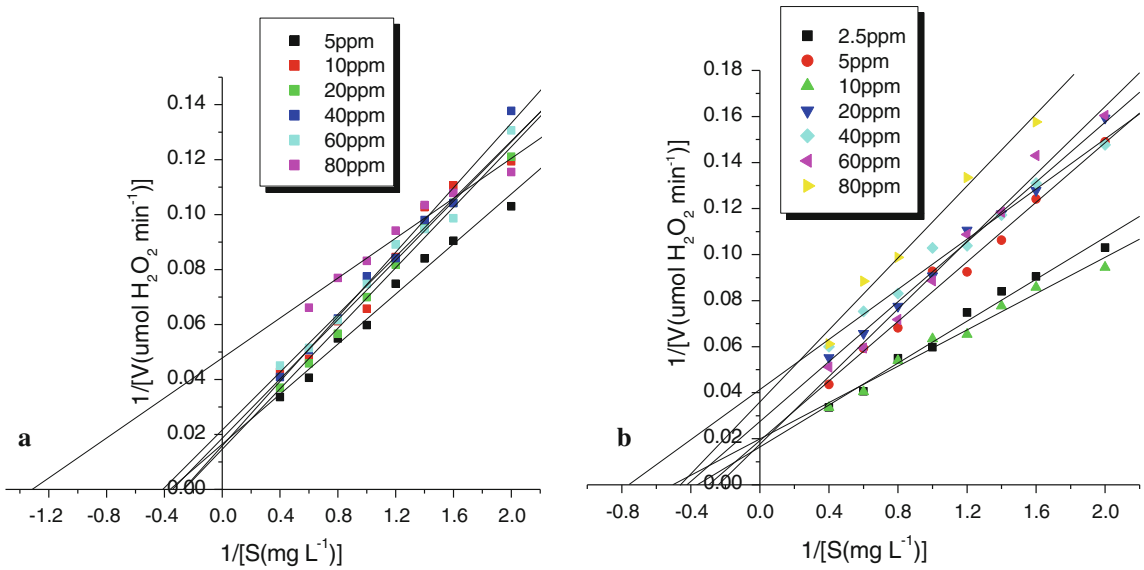

Fig. 3 Inhibitory type of DCPP versus Catalase (**a** *rac*-DCPP; **b** *R*-DCPP)

the kinetics constants of enzymatic reaction with the presence of *rac*-and *R*-DCPP: the apparent maximal reaction velocity: V_{maxapp} and apparent dissociation constant: K_{mapp}.

In the Fig. 3a, double reciprocal curves of the different concentrations of *rac*-DCPP were almost parallel except for 5 and 80 mg·L^{-1}. From the Table 1, $1/V_{maxapp}$ was rose with the increasing of inhibitor concentration, meaning the

Table 1 Kinetics constants of the enzymatic reaction of *rac*- and *R*-DCPP

Inhibitor (mg L^{-1})	K_{mapp}		V_{maxapp}		K_{mapp}/V_{maxapp}		$1/V_{maxapp}$	
	rac	*R*	*rac*	*R*	*rac*	*R*	*rac*	*R*
5	3.060	4.559	63.99	63.72	0.0478	0.0715	0.0156	0.0157
10	3.201	1.888	58.29	46.94	0.0549	0.0402	0.0172	0.0213
20	4.010	2.191	74.00	35.62	0.0542	0.0615	0.0135	0.0281
40	3.191	1.432	57.70	26.07	0.0553	0.0550	0.0173	0.0383
60	2.035	5.798	40.70	76.33	0.0500	0.0760	0.0246	0.0131
80	0.712	9.151	20.32	76.12	0.0350	0.1202	0.0492	0.0131

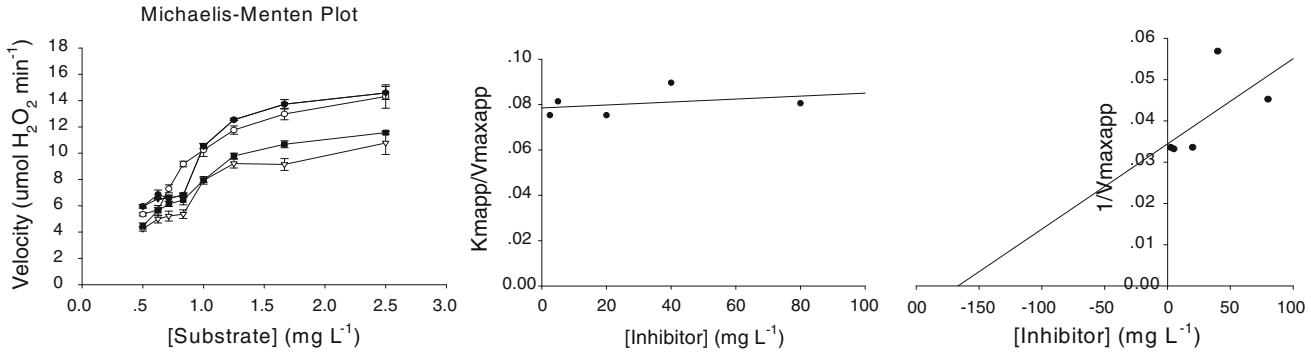

Fig. 4 Velocity, K_{mapp}/V_{maxapp} and $1/V_{maxapp}$ of enzymatic reaction with different concentration of substrate and *R*-DCPP with humic acid

V_{maxapp} incessantly to reduce. In addition, except for the K_{mapp}/V_{maxapp} declined slightly under 80 mg L^{-1}, K_{mapp}/V_{maxapp} was basically kept in line under other concentrations. From the above analysis, the inhibition type of *rac*-DCPP on the catalase was uncompetitive inhibition.

In the Fig. 3b, the inhibition of *R*-DCPP on the catalase was complex. From the Table 1, the $1/V_{maxapp}$ represented the trend with rising firstly, and declined later, according to the increasing concentration of inhibitor. This meant V_{maxapp} declined with the increasing concentrations of inhibitor firstly, then gradually increased. In conclusion, the inhibition kinetics of *R*-DCPP on catalase could be divided into two parts: V_{max}, K_{mapp} and K_{mapp}/V_{maxapp} reduced with increasing concentration from 0 to 40 mg L^{-1}; K_{mapp} and K_{mapp}/V_{maxapp} increased and V_{maxapp} has unchanged with increasing concentrations from 60 to 80 mg L^{-1}.

In the Fig. 4, the inhibition type of *R*-DCPP to catalase with the humic acid was uncompetitive inhibition, it was different from the instance without humic acid, that meant it would be combined with DCPP, or the selectivity maybe exist, which would be determined in the future. The further study was mainly to better explain that which humic acid group was produced effect on DCPP.

Racemic dichlorprop is currently being replaced by *R*-dichlorprop worldwide. This chiral switch is expected to reduce the application amount of this pesticide and the potential side effects on non-target organisms (Poiger et al. 2002). It is demonstrated in this study that the inhibition rate and inhibition type of DCPP to catalase (in vitro) are enantioselective, and this enantioselectivity was also influence by the humic acid.

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