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In Vitro Effects of Pure Polychlorinated Biphenyl Isomers on the Rabbit Muscle Lactate Dehydrogenase

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Research work concerning the toxicity of polychlorinated biphenyls (PCBs) which are widely recognized as environmental pollutants has been extensively published. It has been shown that there are marked differences in the absorption (WEBB and MCCALL 1972), metabolism, (HUTZINGER et al. 1972) and excretion (JAN 1975) of PCB isomers. ECOBICHON and COMEAU (1975) reported in detail on the influence of position and degree of chlorination of the biphenyl on hepatic function in the rat.

Because the toxicological assessment of PCBs is complicated by the heterogeneity of the isomers, it is necessary to estimate individually the toxicity of pure PCB isomers having various chlorine substitutions. The present paper describes the in vitro effects of five pure PCB isomers on rabbit muscle lactate dehydrogenase and suggests that there are selective effects on this enzyme due to the degree of chlorination of the biphenyl.

MATERIALS AND METHODS

Lactate dehydrogenase (EC:1,1,1,27;LDH) and nicotinamide adenine dinucleotide, reduced form,(NADH) were obtained from Boehringer-Mannheim. 4-Chlorobiphenyl (MCB), 4,4'-dichlorobiphenyl (DCB), 3,4,3',4'-tetrachlorobiphenyl and (TCB) and 2,4,5,2',4',5'-hexachlorobiphenyl (HCB) were obtained from Gaschro Kogyo Co. Other chemicals were reagent grade.

The activity of LDH was measured with an Hitachi Model 200 recording spectrophotometer, at 25°C by following the initial rate of change in absorbance at 340 nm. The reaction mixture (final volume 3.0 ml) containing 0.1M phosphate buffer (pH 7.6), 0.1 mM NADH, 0.4 µg of LDH and various concentrations of PCB isomers, was incubated for 30 min and the reaction was started by the addition of 1 mM of pyruvate to the solution. In control experiments, the same volume of ethanol was added instead of an ethanol solution of PCB isomers.

PCB isomers were dissolved in alcohol and the solution injected into the reaction mixture with a micro-

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TABLE 1

The Concentration of PCB Isomers Giving 50 % Inhibition (I_{50})

Inhibitor	I_{50} (M)	Water Solubility (μg/ml)
Biphenyl	1.6×10^{-4}	....
4-MCB	3.1×10^{-5}	1.18
4,4'-DCB	5.6×10^{-6}	0.075
3,4,3',4'-TCB	2.8×10^{-6}	0.045
2,4,5,2',4',5'-HCB	2.1×10^{-6}	0.0088

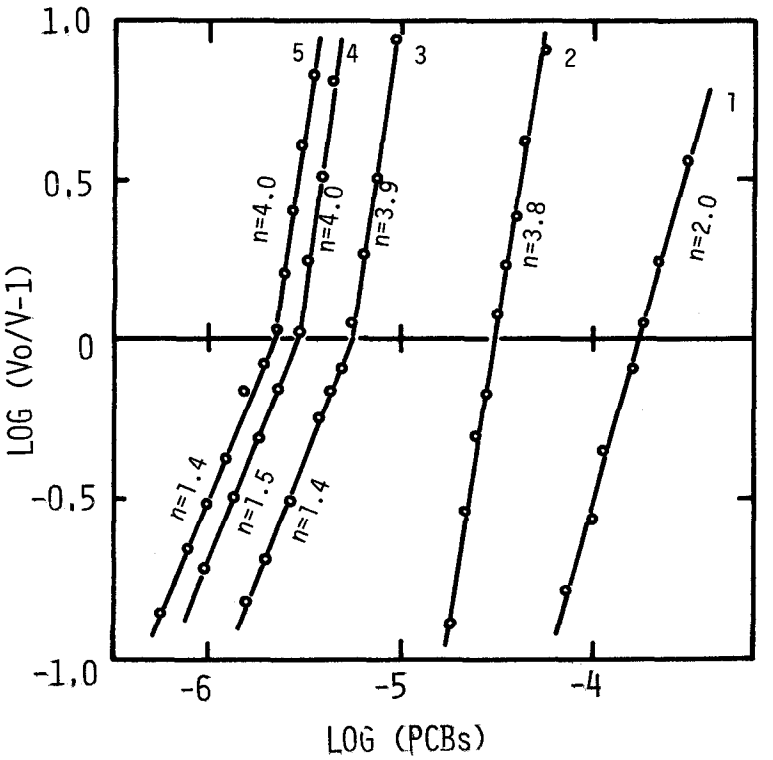

Fig. 1. Inhibition of LDH by PCB Isomers and Their Modified Hill Plots

(1) Biphenyl, (2) 4-MCB, (3) 4,4'-DCB, (4) 3,4,3',4'-TCB, (5) 2,4,5,2',4',5'-HCB

syringe to give a final concentration of 0.5% ethanol solution. PCB solutions of high concentration formed an emulsion with the reaction mixture.

RESULTS AND DISCUSSION

Table 1 shows the concentrations of the PCB isomers required for 50% inhibition (I_{50}) of LDH and also the water solubility of the isomers (JOHNSTONE et al. 1974). The data indicated that as the number of chlorine atoms increased from zero (biphenyl) to six (HCB), the concentration of these compounds which gave 50% inhibition decreased. It was demonstrated that there was an inverse correlation between water solubility and the extent of inhibition. The data would indicate that hydrophobic bonding is of major importance in the affinity of PCB isomers for this enzyme. A similar correlation has been reported by ROGERS and YUSKO (1972) in the reaction between detergents and bovine liver glutamate dehydrogenase, and by OHYAMA (1977) in the reaction between phthalate esters and yeast glucose-6-phosphate dehydrogenase. They found that the extent of inhibition depended on the number of carbons in the alkyl groups of the alkylbenzenesulfonates or phthalate esters. However, JOHNSTONE et al. (1974) found that drug metabolizing enzymes were strongly activated by biphenyls with chlorine substitution at the 4,4' positions, and that this activation was independent of the total chlorine contentof the PCB isomers.

The modified Hill plots (LOFTFIELD and EIGNER 1969) with five PCB isomers are shown in Fig. 1. Interaction between the enzyme and inhibitors was suggested from the observation that a small change in PCB isomer concentration produced a large change in Vmax. The Hill plots of biphenyl and MCB showed linear relationships and their Hill constants, calculated from the slopes, were 2.0 and 3.8 respectively. However, the Hill plots for isomers with two or more chlorine atoms exhibited bilinearity and the Hill constants at concentrations above I_{50} were approximately 4 whereas at concentrations below I_{50} the constants were approximately 1.4. This may reflect the alteration of the inhibitory site or a change in the number of inhibitory sites on the enzyme depending on the concentration of the inhibitor. A similar effect has been found in the interactions of alkylbenzenesulfonates with glutamic dehydrogenase (ROGERS and YUSKO 1972) and of phthalate esters with glucose-6-phosphate dehydrogenase (OHYAMA 1977).

The concentrations of DCB which reduced the activity in half in the presence of 0.1 mM NADH or 1 mM pyruvate during incubation with enzyme are presented in Table 2. The concentration (I_{50}) in the presence of NADH was

TABLE 2

Effect of NADH and Pyruvate on the Inhibition of LDH by 4,4'-Dichlorobiphenyl (DCB)

Incubation	Starting Material	I_{50} (M)
Enzyme + DCB	NADH + Pyr	4.7×10^{-6}
Enzyme + Pyr + DCB	NADH	6.3×10^{-6}
Enzyme + NADH + DCB	Pyr	1.0×10^{-5}

TABLE 3

The Relation Between the Concentration of the Enzyme and Inhibition Degree by 4,4'-DCB

Concentration of Enzyme Protein (μg/ml)	I_{50} (M)
0.067	3.3×10^{-6}
0.133	5.6×10^{-6}
0.267	7.2×10^{-6}
0.400	1.1×10^{-5}

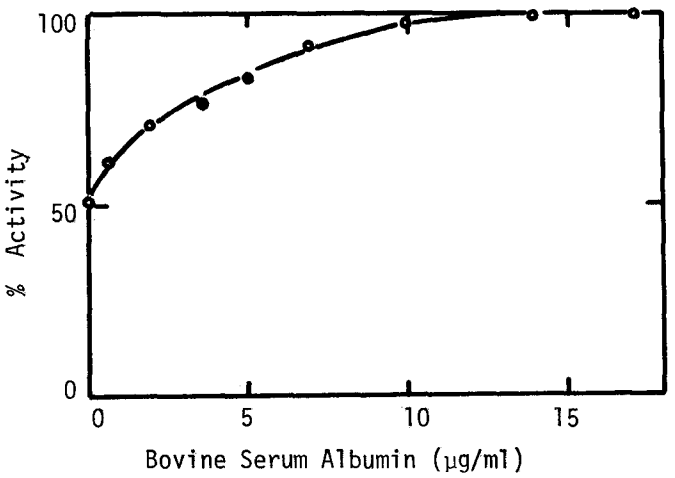

Fig. 3. Protective Effect of Bovine Serum Albumin on the Inhibition of LDH by 4,4'-DCB 5.0×10^{-6} M.

two times higher than the corresponding concentration in the absence of NADH. Table 3 shows the relationship between the concentration of enzyme protein and the extent of inhibition by DCB.When the concentration of protein was increased the concentration of DCB for I_{50} was increased. Therefore, it seems likely that PCBs cause a conformational change in the enzyme protein while various factors,including coenzymes,stabilize the conformation. It has been shown that the association and dissociation of lactate dehydrogenase is affected by the presence of coenzyme or the concentration of enzyme protein (SCHWERT 1963).

The inhibition of LDH by DCB could be prevented by bovine serum albumin. As shown in Fig. 3, when 0.13 µg/ml of LDH was used, the addition of 15 µg/ml of albumin completely protected the enzyme from inhibition by 5×10^{-6} M DCB. The results show that the protective effect was observed at a concentration (0.3 µM) lower than the concentration of DCB. A possible explanation for this effect would be that the binding of DCB was diminished in a region of the enzyme by preliminary binding of bovine serum albumin. However, the addition of albumin to the inactivated enzyme did not restore the activity. It may be that the enzyme undergoes irreversible denaturation by DCB during incubation. The prevention of inhibition by bovine serum albumin points out the importance of protein concentration which is many fold higher *in vivo* than it is in the *in vitro* assay system. A likely explanation for the mechanism of the inhibition caused by PCB isomers may be that the hydrophobic properties of these compounds produce conformational changes in the enzyme.

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