Binding of the Chlorinated Hydrocarbon Bis(p-Chlorophenyl) Acetic Acid with the Enzyme Carbonic Anhydrase

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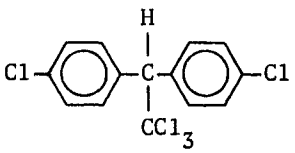
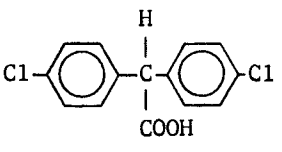
INTRODUCTION

Controversies over the role of chlorinated hydrocarbon insecticides such as DDT (I) in eggshell thinning problems still exist. There are two schools of thought, one that DDT-type insecticides inhibit the activity of the enzyme carbonic anhydrase (PEAKALL 1970), and the other that such chemicals have no effect on the enzymatic activity (POCKER et al. 1971). However in discussing the toxicity of DDT-type compounds, one point that is more or less ignored by research investigators is the consideration of the role of metabolites. It is generally believed that the metabolite DDA (II) of the insecticide DDT is less hydrophobic and possesses higher water solubility. Earlier studies (ROSS and BIROS 1974) have shown that DDA binds with the protein bovine serum albumin. In this manuscript we present evidence for the binding of DDA with the enzyme carbonic anhydrase using nuclear magnetic resonance technique. Since DDA is the final metabolite of DDT, such binding evidence may be of significance in discussing the toxicity and eggshell thinning problems associated with DDT.



(I) DDT



(II) DDA

EXPERIMENTAL

Bis-p-chlorophenyl acetic acid (DDA) was obtained from Aldrich Chemical Company, and carbonic anhydrase was obtained from Sigma Chemical Company as a purified powder from bovine erythrocytes. Solutions of DDA were prepared by dissolving a known amount in D_2O and adjusting the pH by adding DCl or NaOD. A fresh protein solution was made for each series of runs by dissolving the carbonic anhydrase in D_2O and using $E^{1\%}_{280} = 18$ to determine the final protein

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concentration. Proton magnetic resonance (pmr) spectra were recorded on a Varian HA-100 high resolution nmr spectrometer. All the experiments were carried out at slightly above pH 7. This is because the solubility of DDA below pH 7 is extremely low.

RESULTS AND DISCUSSION

A typical pmr spectrum of DDA in slightly alkaline aqueous solution shows a singlet (δ = 5.3 ppm) corresponding to the benzylic proton and an AA'BB' type multiplet pattern ($\delta = \sim 7.6$ ppm) for the ring protons. Earlier studies (HAQUE et al.) have shown that the pmr spectrum of DDA in aqueous alkaline solution shows concentration dependence in the chemical shifts of the aromatic and benzylic proton peaks, and these changes have been explained on the basis of self association of DDA in water. As the concentration of DDA was increased, the ring resonance peak multiplet collapsed into a singlet.

The addition of carbonic anhydrase to a dilute solution of DDA produced line broadening in both the benzylic and the ring proton resonance peaks. However at lower concentrations of DDA the ring proton multiplet collapsed into a broad peak, thus precluding the measurement of line widths. Since at higher concentrations of DDA the ring proton spectrum of DDA showed a single peak, an opportunity was provided to measure the line width and relaxation time of DDA ring protons in the presence of carbonic anhydrase.

The addition of carbonic anhydrase to a D_2O solution of DDA at such concentration where the ring protons show a singlet produced changes in the line widths of both the ring proton and benzylic proton resonance peaks. The line widths of the ring proton peak and the benzylic proton peak increased as the carbonic anhydrase concentration increased. The line width also increased with increasing time. However, the HOD peak under similar conditions showed negligible increase in the line width, indicating that the increased viscosity of the solution was not responsible for the line broadening.

The changes in the line widths of the benzylic and the ring protons with increasing concentration of carbonic anhydrase are shown in Figure 1. It is interesting to note that benzylic and ring protons do not show similar broadening and have different slopes. The increases in the line widths of the ring and benzylic protons of DDA with increasing carbonic anhydrase concentration may be explained on the basis of the interaction of DDA with carbonic anhydrase. The binding of DDA to carbonic anhydrase will reduce the spin-spin relaxation time and thus will produce line broadening. Apparently, the rate of exchange between bound and free DDA molecules is rapid, and thus an average line is obtained. No chemical shift changes were noticed, indicating that the binding is probably weak. Since the ring protons are broadened more

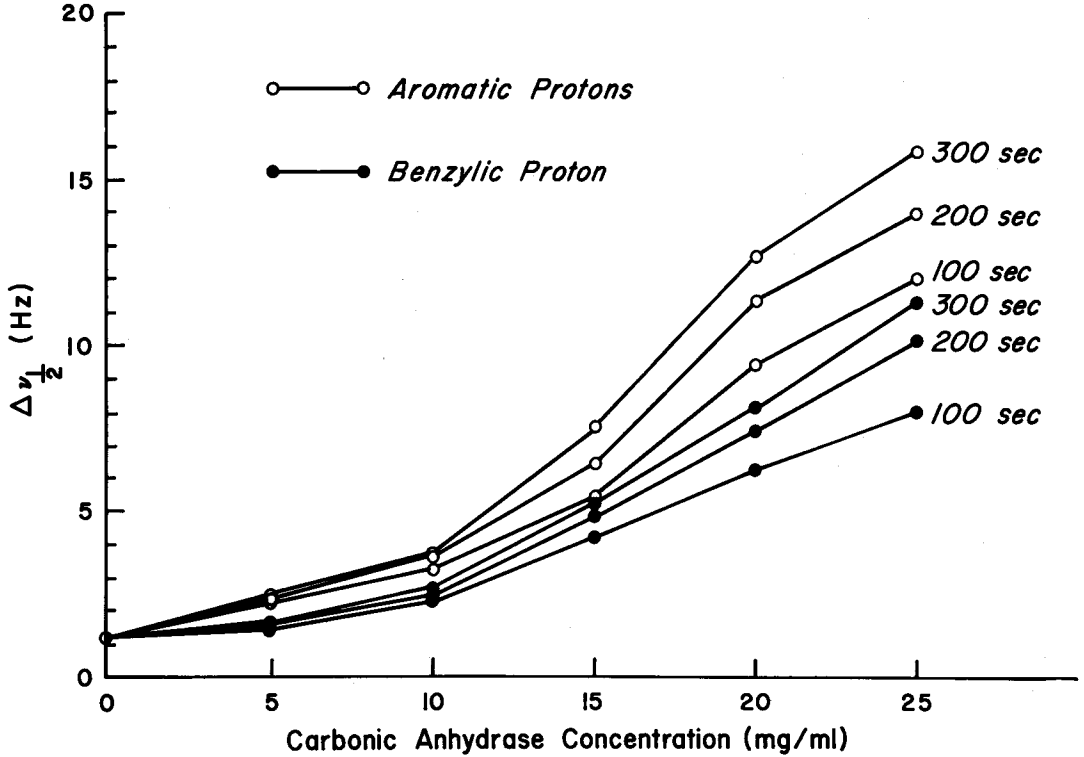

Figure 1 Changes in the line widths of benzylic and ring protons of DDA as a function of carbonic anhydrase concentration at different times after mixing.

than the benzylic proton it is evident that the former is more involved in the binding than the latter. The ring protons probably provide a better site for binding. The nmr technique provides a clear demonstration of the relative role of the benzylic and ring protons in the binding with carbonic anhydrase. This is in contrast with the binding of DDT-type compounds with phospholipids where the benzylic proton is more involved in the binding (HAQUE et al. 1973).

The binding data of DDA with carbonic anhydrase described above are qualitative in nature. Nevertheless, such binding may play an important role in the toxicological problems associated with DDT. These results also indicate a change in the mobility of carbonic anhydrase.

ACKNOWLEDGMENT

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