ENZYMATIC DEHALOGENATION OF 2,2-DICHLOROPROPIONATE

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The herbicide 2,2-dichloropropionate is an effective inhibitor of panothenic acid production (van Oorschot and Hilton, 1963) and pyruvate utilization in microorganisms (Redemann and Meikle, 1955). Plants do not readily decompose 2,2-dichloropropionate or trichloroacetate, although chloride ion liberation in plants treated with monochloroacetate has been reported (Mayer, 1957; Foy, 1961). Microorganisms can be adapted to metabolize monochlorinated and dichlorinated aliphatic acids (Jensen, 1957; Hirsch and Alexander, 1960). Certain species of soil microorganisms can readily attack 2,2-dichloropropionate and use this acid partly or wholly as a carbon source with rapid liberation of quantitative amounts of chloride ion. Some properties of a cell free preparation of an Arthrobacter sp. capable of dehalogenating 2,2-dichloropropionate are examined for the first time in the present paper.

Pure culture isolation of adapted microorganisms responsible for the decomposition of 2,2-dichloropropionate was accomplished via the dilution-plate technique. Representatives of the following microbial genera were effective in liberating chloride ion from 2,2-dichloropropionate: Pseudomonas, Agrobacterium, Bacillus, Alcaligenes, Arthrobacter, and Nocardia. Several of these genera had been reported previously (Jensen, 1957; Hirsch and Alexander, 1960). Arthrobacter sp.

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was selected for study because of its high utilization rate of the chlorinated aliphatic acid. Routinely, cells were mass-cultured in a modification of the medium described by Hirsch and Alexander (1960). The medium contained 1 gram of 2,2-dichloropropionate* (Na salt) per liter as a carbon source. In addition 50 mls of 0.2 M phosphate buffer pH 7.0 was added to each liter of nutrient solution because the pH of unbuffered solutions dropped below 4.5 with complete cessation of growth.

Samples were removed periodically and growth determined by measuring turbidity at 650 mμ and chloride ion liberation (Iwasaki et al., 1952). Harvested cells were washed twice with ice cold distilled water to remove any excess chloride ion. Resuspended cells were ruptured in a sonic oscillator for 15 minutes. Sonicated cells were centrifuged at 10,000 x g for 10 minutes and the cell debris discarded. All manipulations with the cell-free system were carried out at 0°C.

Protein concentration was determined by the biuret procedure (Gornall et al., 1949) with serum albumin as a standard. Crude and purified enzyme preparations were assayed in a mixture containing 1 ml of enzyme (1-3 mg protein), 1 ml 0.2 M phosphate buffer, and 1 ml 2,2-dichloropropionate (10 μ moles in final volume of 3 mls). The reaction was initiated by adding the substrate to the enzyme and buffer maintained in a water bath at 30°C. The reaction was terminated by pipetting 1 ml aliquots into 2 mls of 9 M nitric acid and then the solution was diluted further with 2 mls of Hg(SCN) for the measurement of chloride ion. Specific activity is defined as the number of μg chloride liberated per mg protein per 10 minutes.

Phosphate, vernol, or tris buffer was used to ascertain the pH optimum for the cell-free system. Since phosphate buffer gave slightly higher specific activities it was used in all subsequent studies. A sharp optimum was noted at pH 8.0 with little or no activity in the acid

*Purified samples of 2,2-dichloropropionic acid were generously donated by the Dow Chemical Company, Midland, Michigan.

region. All values are corrected against boiled enzyme controls, where no chloride liberation above reagent levels was noted. The enzymatic nature of the dehalogenation was indicated by a linear increase in velocity as the enzyme concentration was increased from 0.5 to 3.0 mg of protein. The specific activity of the crude enzyme was 28 ug Cl per mg protein per 10 minutes. The crude enzyme was completely inactivated in less than 15 minutes at 60°C. Cell-free preparations and partially purified fractions were stable when stored in a freezer at -5°C.

The enzyme was partially purified by ammonium sulfate precipitation. The precipitate collected from 30 and 60 per cent saturation and the supernatant were desalted by gel filtration on a column of Sephadex G-50 equilibrated and developed with 0.001M potassium phosphate buffer, pH 8.0. The fraction precipitating between 30 and 60 per cent saturation contained over 90 per cent of the activity. No increase in activity was noted when Co^{++}, Mg^{++}, or Mn^{++} ions or glutathione were added at concentrations up to 10^{-3}M.

The substrate specificity of the partially purified enzyme with nine chlorinated aliphatic acids is shown in Table 1. The enzyme shows greatest activity on 2,2-dichloropropionate with less activity on 2-chloropropionate, dichloroacetate, and 2,2-dichlorobutryate. The enzyme was inactive on any beta chloro substituted aliphatic acid.

In the partially purified enzyme system only two compounds, the substrate and pyruvate, were detectable on paper chromatograms of enzyme incubated with 2,2 dichloropropionate-1-C^{14}. Pyruvate was identified by eluting the radioactivity and cochromatography in n-butanol saturated with 1.5N NH_4OH (Rf.18) and amyl alcohol-5N formic acid (Rf.62). The 2,4 dinitrophenylhydrazone of the enzyme product was prepared, and the melting point (213-216°C) compared with authenic pyruvate (216°C). A stoichiometric relationship was demonstrated between μ moles of 2,2 dichloropropionate disappearing as measured by chloride liber-

ation and μ moles of pyruvate formed as measured by the salicaldehyde method of Berntsson (1955).

TABLE 1.

Substrate specificity of the partially purified enzyme

Substrate	Specific activity
Dichloroacetate	14
Trichloroacetate	0
2-Chloropropionate	14
3-Chloropropionate	0
2,2-Dichloropropionate	85
2,2,3-Trichloropropionate	0
2,3-Dichloroisobutyrate	0
2,2-Dichlorobutyrate	10
2,3,3-Trichlorobutyrate	0

The precursor of pyruvate in this system is probably alpha chloro alpha hydroxypropionate. Smith (1963) working on a similar problem could show this compound was converted rapidly to pyruvate. Two alternatives are possible for the formation of alpha chloro alpha hydroxy propionate from 2,2 dichloropropionate. The first involves beta elimination; some basic group on the enzyme surface could abstract a proton from the beta hydrogen with a subsequent shift in electrons to form the olifinic compound alpha chloroacrylate. Addition of water across the double bond would yield alpha chloro alpha hydroxypropionate followed by pyruvate.

The above mechanism would be inconsistent with the observed activity on dichloroacetate, but this activity appears to be unrelated to

the enzyme under study since a decrease in chloride liberation from this substrate was noted after partial purification.

The other alternative would involve a direct substitution reaction yielding alpha chloro alpha hydroxypropionate from 2,2 dichloropropionate. The classical SN_2 reaction, however, appears to be rare in enzyme chemistry.

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