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INHIBITION OF GLYCOLLATE OXIDASE FROM PARSLEY LEAVES BY HCO_3^-

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SUMMARY: HCO_3^- is shown to be a noncompetitive inhibitor of glycollate oxidase from parsley leaves with respect ot glycolic acid. The K_i is found to be 20 mM at pH 7,7.

Glycollate oxidase (glycollate : oxygen oxidoreductase E.C.1.1.3.1.) is a flavoprotein that catalyses the oxidation of glycolic acid to glyoxylic acid (1). It is supposed to be one of the key enzymes in photorespiration in green plants (2). The concentration of the enzyme has also been shown to be higher in the photosynthetic cells of C_3 plants than in the cells of C_4 plants (3).

Many efforts have been made to find specific inhibitors of the enzyme in order to decrease photorespiration and thereby increase plant productivity (4,5). α-Hydroxysulphonic acids have been shown to be powerful inhibitors of glycollate oxidase and in the presence of such inhibitors an increased net CO_2 assimilation has been found in vivo (3). However, these inhibitors are not specific for glycollate oxidase and they will also influence other essential cell reactions.

Here we show that HCO_3^- is an inhibitor of glycollate oxidase in vitro. This might be important in determining the rate of photorespiration.

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MATERIALS AND METHODS: Sephadex DEAE A 50 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Horseradish peroxidase and FMN were purchased from Sigma Chemical Co, Darmstadt. All other chemicals were of analytic grade.

Spectrophotometric measurements were made on a Beckman Acta M IV spectrophotometer. The anaerobic technique has been described earlier (6).

Glycollate oxidase was prepared from commercially available parsley [Petroselinum crispum (Mill.) Airy Shaw.] Parsley leaves (500 g) and 500 ml of deionised distilled water were blended in a Waring blendor at highest speed for 1 minute. The homogenate was strained through cheesecloth and the pH was carefully adjusted to 5.2 with 1 M acetic acid. After 15 minutes of stirring the precipitate was removed by centrifugation at 20 000 x g for 10 minutes. The pH of the supernatant was raised to 7.7 with 1 M NaOH and after that 2.5 g of dry Sephadex DEAD A 50 was added. The pH was adjusted to 7.7 and the solution was stirred for 30 minutes at 25°C, after which the Sephadex was removed by filtration. The solution was concentrated approximately 100 times in an Amicon utlrafiltration cell. This protein fraction was used without further purification since most of the activity otherwise was lost. About 80 % of the total original activity was maintained in this crude fraction.

Tris-HCl buffers (50 mM) made from freshly boiled deionised distilled water was used for measurements of glycollate oxidase activity according to Duley et al. (7). Different oxygen concentrations were prepared by mixing an assay equilibrated with air with an anaerobic assay. Assays saturated with oxygen were prepared by bubbling oxygen through the solution for ten minutes and solutions saturated with CO_2 were made by bubbling CO_2 through deionised distilled water for 30 minutes.

The inhibitory studies were made at pH 7.7. The enzyme was added to the flavin containing assay five minutes before the appropriate amount of glycolic acid at pH 7.7 was added. After 2 minutes a specified amount of HCO_3^- was injected into the cuvette. All kinetic experiments were performed at 25°C and the solutions were kept in gastight vessels before use.

RESULTS AND DISCUSSION: Glycollate oxidase from parsley was found to have a K_m of 0.27 mM for glycolic acid. This is similar to the K_m published for the pea enzyme, 0.25 mM (8) and 0.26 mM (9). The K_m for oxygen was shown to be 0.09 mM (Fig. 1) which is slightly lower than that for the pea enzyme (1).

Dixon plots of the inhibition of glycollate oxidase by HCO_3^- are shown in Fig. 2 for two different oxygen

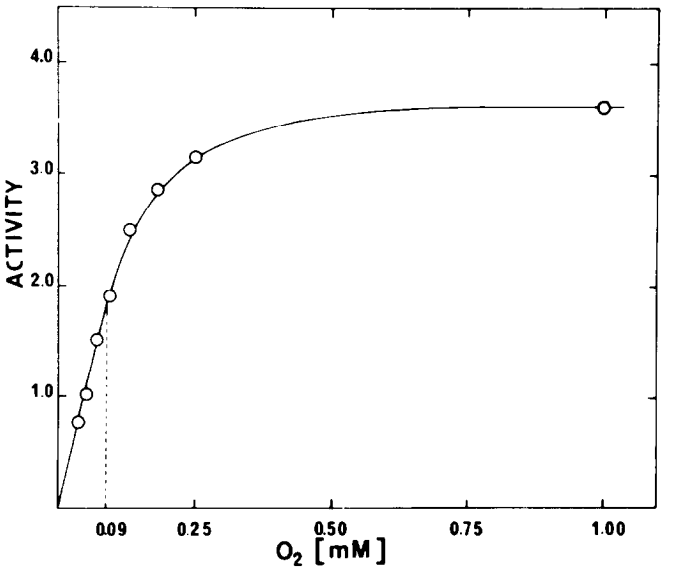

Fig. 1. The effect of the oxygen concentration on the glycollate oxidase activity in 50 mM Tris-HCl buffer at pH 7.7. The glycolic acid concentration was 2.0 mM. Activity is expressed in arbitrary units.

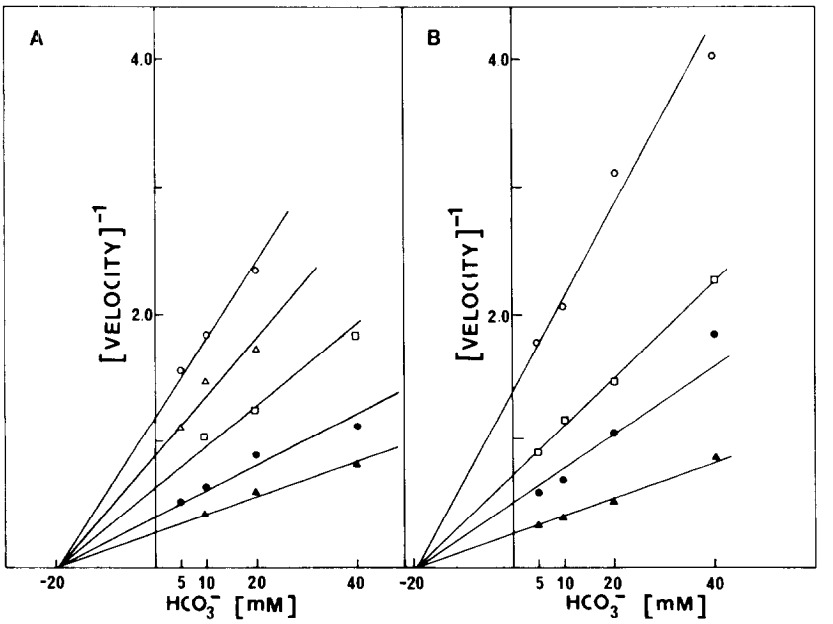

Fig. 2. The Dixon plots of the inhibition of glycollate oxidase by HCO_3^- at different concentrations of glycolic acid : ○ = 0.050 mM, △ = 0.075 mM, □ = 0.1000 mM, ● = 0.150 mM, ▲ = 0.300 mM. The oxygen concentrations were 0.25 mM (A) and 1.25 mM (B).

concentrations. These plots give a K_i of 20 mM for the inhibition by HCO_3^-. They furthermore suggest that the inhibition is noncompetitive with respect to glycolic acid.

Experiments were made to determine whether HCO_3^- or CO_2 is the inhibiting substance. Saturated CO_2 solution (0.1 ml) was added to 1.0 ml of an assay made from freshly boiled distilled deionised water. No inhibition of glycollate oxidase was observed, which indicates that HCO_3^- is the inhibitor.

The steady state kinetics of the inhibition of glycollate oxidase by HCO_3^- is probably more complex than the simple noncompetitive pattern indicated by Fig. 2. The fact that the enzyme is inhibited by HCO_3^- is interesting even if the K_i for the inhibition is 20 mM. This is much higher than the HCO_3^- concentration found in photosynthetic cells (10). However, our experimental conditions are quite different from those found in vivo. Therefore the elucidation of the steady state kinetics of the inhibition of glycollate oxidase with respect to both substrates, oxygen and glycolic acid, might be fruitful for further studies of photorespiration. The high-resolution x-ray structure of glycollate oxidase from spinach will probably be available in the near future (11) and specific, strongly binding inhibitors can then hopefully be developed. In vivo experiments on various plants, including C_4 plants, with glycollate oxidase inhibited by HCO_3^- or a highly specific inhibitor will then certainly give very valuable information about photorespiration.

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