HALOACETOL PHOSPHATES AS AFFINITY LABELS FOR METHYLGLYOXAL SYNTHASE

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Received December 17,1976

SUMMARY: 3-Bromo- and 3-iodoacetol phosphates irreversibly inactivate methylglyoxal synthase. The substrate, dihydroxyacetone phosphate, and inorganic phosphate protect against the inhibition. Although the 3-chloro derivative does not inactivate the enzyme, it is a competitive inhibitor. Reduction of the enzyme-inactivator complex with [^{3}H]-$NaBH_4$ indicates the incorporation of four haloacetol phosphates per mole of enzyme. These studies suggest the bromo- and iodoacetol phosphates inactivate the enzyme by reacting with a nucleophilic group located in the active center.

Methylglyoxal synthase (EC 4.2.99.11), which catalyzes the conversion of dihydroxyacetone phosphate to methylglyoxal and inorganic phosphate, has been isolated in crystalline form from Proteus vulgaris (1). The overall course of reaction involves the oxidation of carbon 3 of the substrate to the aldehyde with the dephosphorylation and reduction of carbon 1 to the methyl group of the product (2).

Haloacetol phosphates, i.e., 3-chloro, 3-bromo-, and 3-iodoacetol phosphate, are reactive derivatives of dihydroxyacetone phosphate, which have been utilized as active-site reagents for aldolase and triosephosphate isomerase (3-7).
In this communication, we show that bromo- and iodoacetol phosphate may also serve as potential active-site reagents of methylglyoxal synthase.

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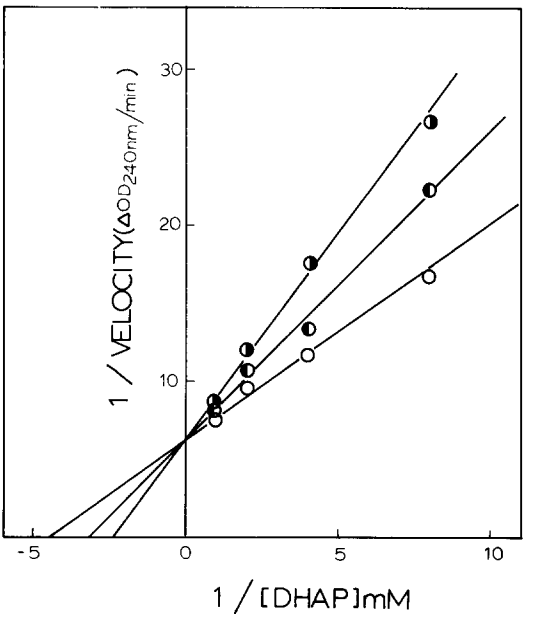

Figure 1. Inhibition of methylglyoxal synthase by chloroacetol phosphate. Initial rates were measured at 30° in 50 mM imidazole buffer, pH 7.0, at various dihydroxyacetone phosphate concentrations in the presence and absence of the analogue. Results are plotted in the double-reciprocal form: O = no inhibitor; ◐ = 0.35 mM chloroacetol phosphate; ● = 0.70 mM chloroacetol phosphate.

MATERIALS AND METHODS

Methylglyoxal synthase was isolated from Proteus vulgaris according to the procedure of Tsai and Gracy (1). Enzyme assays were carried out either by the coupled spectrophotometric assay or the colorimetric stop assay as described by Hopper and Cooper (8). Under conditions of excess substrate and glutathione in the assay mixture, the addition of haloacetol phosphates did not interfere with the enzyme activity assay. Inorganic phosphate and 2-mercaptoethanol were removed by dialysis prior to the inactivation studies. Haloacetol phosphates were synthesized and assayed as previously described (3). The following materials were purchased from Sigma Chemical Co.: dihydroxyacetone phosphate, yeast glyoxalase I, reduced glutathione, iodoacetic acid (recrystallized from petroleum ether), and p-hydroxymercuribenzoate. [^{3}H]-$NaBH_4$ (706 mCi/mmole) was obtained from Amersham Co.

RESULTS

Chloroacetol Phosphate: Reaction of 5 mM chloroacetol phosphate with methylglyoxal synthase (0.1 mg/ml) at 37° for 4 hours resulted in no detectable inactivation of the enzyme.

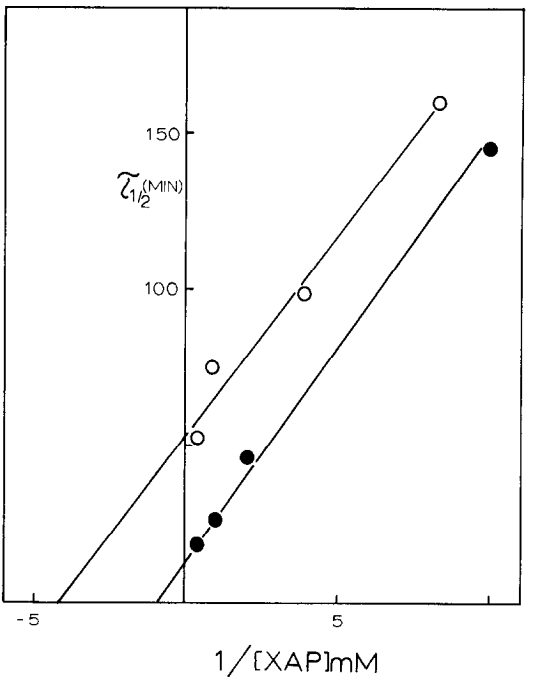

Figure 2. Inhibition half-life (τ) determined from initial rates of inactivation as a function of the reciprocal of concentrations of bromoacetol phosphate (O) or iodoacetol phosphate (●). Reaction mixtures, buffered with 50 mM imidazole (pH 7.0), contained methylglyoxal synthase (0.05 mg/ml), and various concentrations of bromo- and iodoacetol phosphate at 37°C.

However, when enzyme assays were carried out in the presence of chloroacetol phosphate, a typical competitive inhibition pattern was observed (Fig 1). A dissociation constant (Ki) value of 0.93 mM was obtained. This compares with a Km value of 0.22 mM for dihydroxyacetone phosphate.

Bromo- and Iodoacetol Phosphate: Incubation of methylglyoxal synthase with bromo- or iodoacetol phosphate at 37° resulted in the inactivation of methylglyoxal synthase. The initial loss of 40-50% of the activity followed pseudo first-order kinetics. Thereafter, the rate decreased, probably due to the hydrolysis of the haloacetol phosphates during longer periods of incubation (3) or due to the protection of the

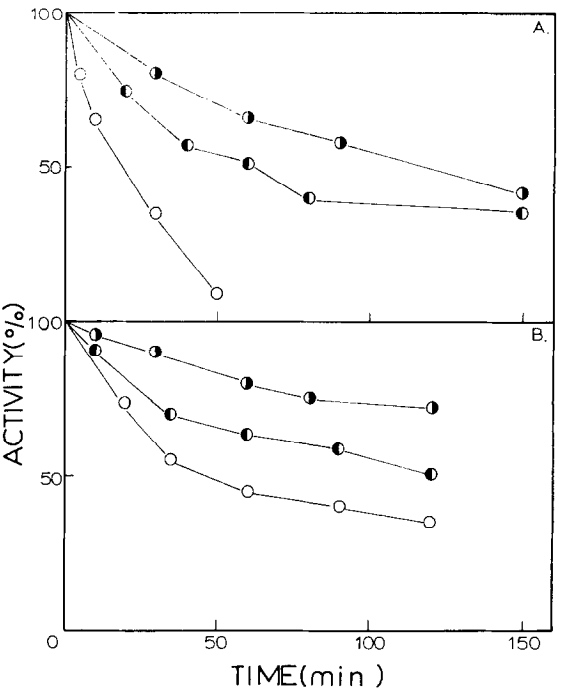

Figure 3. Protective effects of dihydroxyacetone phosphate and inorganic phosphate on the inactivaton of methylglyoxal synthase by haloacetol phosphates.

A. Reaction mixtures contained methylglyoxal synthase (0.05 mg/ml) and 1 mM iodoacetol phosphate with either no further addition (O), 0.15 mM dihydroxyacetone phosphate (◐), or 0.1 mM inorganic phosphate (●). Aliquots were incubated as in Fig 2 and assayed as a function of time for remaining methylglyoxal synthase activity.

B. Reaction mixtures contained methylglyoxal synthase (0.05 mg/ml) and 1.2 mM bromoacetol phosphate with either no other additions (O), 0.15 mM dihydroxyacetone phosphate (◐), or 0.1 mM inorganic phosphate (●). All reactions were carried out at 37°C, pH 7.0.

enzyme by the decomposition product. Fig 2 shows the inactivation half-life (τ), determined from initial rates, as a function of the reciprocal of the haloacetol phosphate concentration. Assuming the formation of a dissociable enzyme-inactivator complex prior to the irreversible covalent bond formation (9), limiting finite half-life values of 52 and 13 min were obtained for the bromo- and iodoacetol phosphates, respectively. Fig 3 shows that the substrate, dihydroxyacetone

phosphate, and the product, inorganic phosphate, protect against the haloacetol phosphate-induced inactivations. The inactivated enzyme could not be reactivated by reducing agents such as cysteine or by exhaustive dialysis against buffer containing 0.1% v/v 2-mercaptoethanol. When 0.1 mg of iodoacetol phosphate-inactivated enzyme (100% activity loss) was treated with [^{3}H]-$NaBH_4$, followed by exhaustive dialysis, a net incorporation (assuming one ^{3}H equivalent per haloacetol phosphate) of 4.1 equivalents per mole of enzyme (132,000 daltons) was obtained. While the number of active centers per mole of enzyme has not yet been established, the enzyme is believed to be composed of two subunits (1). Therefore, these data are consistent with some nonselective modification or the selective modification of a nonessential group, in addition to the active-site residue.

p-Mercuribenzoate and Iodoacetate: Incubation of the enzyme (0.1 mg/ml) for four hours with 5 mM p-mercuribenzoate or iodoacetate in 50 mM imidazole, pH 7.0 or 50 mM triethanolamine, pH 8.0, at 37°, resulted in no loss of enzyme activity. These data suggest the absence of reactive sulfhydryl groups in the enzyme.

DISCUSSION

The haloacetol phosphates have been shown to exhibit different activities with respect to the inactivation of triosephosphate isomerase (6,7), rabbit muscle (4), or yeast aldolase (5). The inactivation of methylglyoxal synthase by these substrate analogs also exhibits a distinct pattern, i.e., the activity of iodoacetol phosphate is greater than that of the bromoacetol phosphate. Furthermore, no inactivation of methyl-

glyoxal synthase was observed by chloroacetol phosphate. These studies suggest that the bromoacetol and iodoacetol phosphate are potential active-site reagents based on the following observations: (a) the initial loss of enzyme activity is a time-dependent pseudo first-order process; (b) dihydroxyacetone phosphate and inorganic phosphate protect against the inactivation process; (c) the incorporation of four equivalents of haloacetol phosphate per mole of enzyme; (d) the enzyme inactivation is irreversible, and does not appear to be caused by the oxidation of sulfhydryl groups; (e) the dissociation constant (Ki) values of these analogs are comparable with the Km value for the substrate; and (f) the different activities of these analogs for inactivation can be explained according to the relative rates of displacement of halogen by an Sn2 mechanism, i.e., I > Br > Cl (10), and suggest a reactive nucleophile may be located in or near the haloacetol phosphate binding site.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the skillful technical assistance of John E. Adelmann. This work was supported by grants from the National Institute of Arthritis, Metabolic and Digestive Diseases (AM14638), the Robert A. Welch Foundation (B-502), and North Texas State University Faculty Research Funds. RWG is the recipient of a National Institutes of Health Research Career Development Award (KO4 AM70198). Research of one of the authors (FCH) was sponsored by the Energy Research and Development Administration under contract with the Union Carbide Corporation.

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