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Inhibition of pyridoxal phosphokinase by aminooxyacetic acid

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AMINOOXYACETIC ACID (AOAA) has been shown to inhibit several pyridoxal-dependent enzymes *in vitro*. Among these are γ-aminobutyric-α-ketoglutaric transaminase,[1] glutamic decarboxylase,[2] and glutamic-pyruvic transaminase.[3] In addition it inhibits pyridoxal phosphokinase, the enzyme that catalyzes the phosphorylation of pyridoxal, pyridoxine, and pyridoxamine.[4] Large doses of AOAA result in convulsions in several species of animals, and toxicity with the compound has been associated with the production of a vitamin B_6 deficiency.[5, 6] Chemically, AOAA readily forms an oxime with the aldehydic form of the vitamin. It was of interest to us therefore to attempt to pinpoint the exact locus at which AOAA interferes with vitamin B_6 metabolism. Included herein are preliminary results dealing with the mechanism by which AOAA inhibits pyridoxal phosphokinase. This enzyme was selected for study because it controls the formation of the cofactor involved in a number of metabolic reactions.

MATERIAL AND METHODS

Pyridoxal phosphate, reduced nicotinamide adenine dinucleotide (NADH), α-ketoglutaric acid, aspartic acid, and pyridoxamine dihydrochloride were purchased from Calbiochem; adenosine triphosphate (ATP), pyridoxal hydrochloride, and malic dehydrogenase (L-malate: NAD oxi-

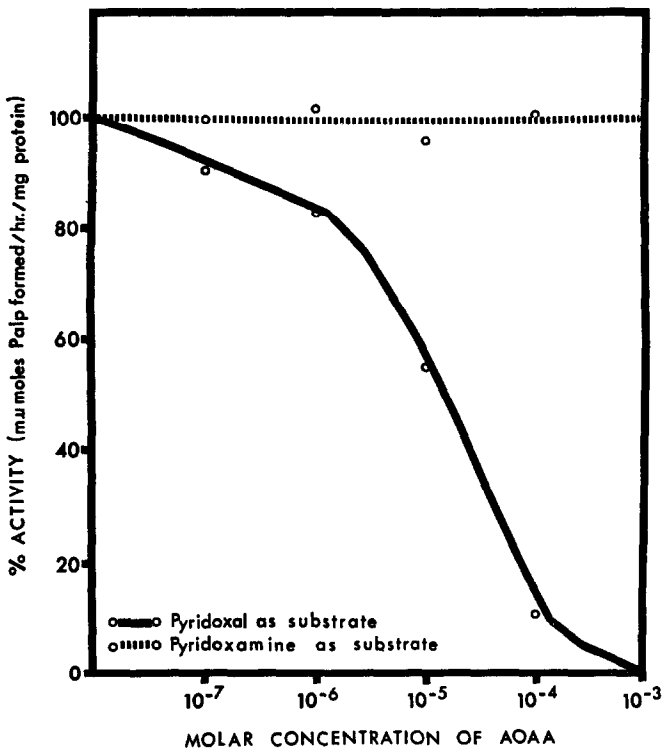

FIG. 1. Effect of various concentrations of aminooxyacetic acid on the phosphorylation of pyridoxal and pyridoxamine. The enzyme activity was assayed at 37° in 0·07 M potassium phosphate buffer, pH 6·5, with 1·0 μmole pyridoxal hydrochloride or pyridoxamine dihydrochloride, 2·5 μmoles $ZnCl_2$ and 0·25 ml enzyme preparation (approximately 0·4 mU, as defined by the International Union of Biochemistry), in 2·5 ml. Incubation was continued for 1 hr; then the flasks were boiled for 5 min and the pyridoxal phosphate or pyridoxamine phosphate formed was assayed as described by Holzer and Gerlach.[8]

doreductase) from General Biochemicals; and pyridoxamine phosphate from Nutritional Biochemicals. Aminooxyacetic acid was obtained through the courtesy of Dr. Paul O'Connel, the Upjohn Co., Kalamazoo, Mich. Brewer's yeast used for the purification of the apotransaminase was a gift of the Home Brewing Co. of Richmond, Va.

Pyridoxal phosphokinase was assayed as described by McCormick *et al.*[7] except that the pyridoxal phosphate formed was determined by the method of Holzer and Gerlach.[8] This method employs an apotransaminase (aspartate: 2-oxoglutarate aminotransferase) from brewer's yeast, and the reaction is coupled with malic dehydrogenase at pH 9. The oxalacetate formed from the transamination of aspartic acid is reduced to malate and is accompanied by the disappearance of NADH. The extinction of the latter at 366 mμ is followed for 6 min. In this assay the rate of NADH disappearance is dependent upon the amount of pyridoxal phosphate present. For each determination involving the inhibitor, appropriate controls were included to show that the inhibition was not due to an effect on the apotransaminase. The supernatant of a 10% rat liver homogenate in 0·07 M phosphate buffer, pH 6·5, after centrifuging at 20,000 *g* for 30 min at 0°, was used as the source of enzyme.

For kinetic studies, extracts of rat liver acetone powder in phosphate buffer were used. Protein determinations were made according to the method of Lowry *et al.*[9]

RESULTS AND DISCUSSION

Under our conditions AOAA inhibits pyridoxal phosphokinase approximately 50% at a concentration of 10^{-5} M. Figure 1 shows that concentrations of AOAA as low as 10^{-7} M inhibited phosphorylation of pyridoxal by rat liver and that over 90% of the enzyme activity was abolished at a concentration of 10^{-4} M AOAA when pyridoxal was used as substrate. The enzyme activity was unaffected by a concentration of AOAA as high as 10^{-3} M when pyridoxamine was substituted

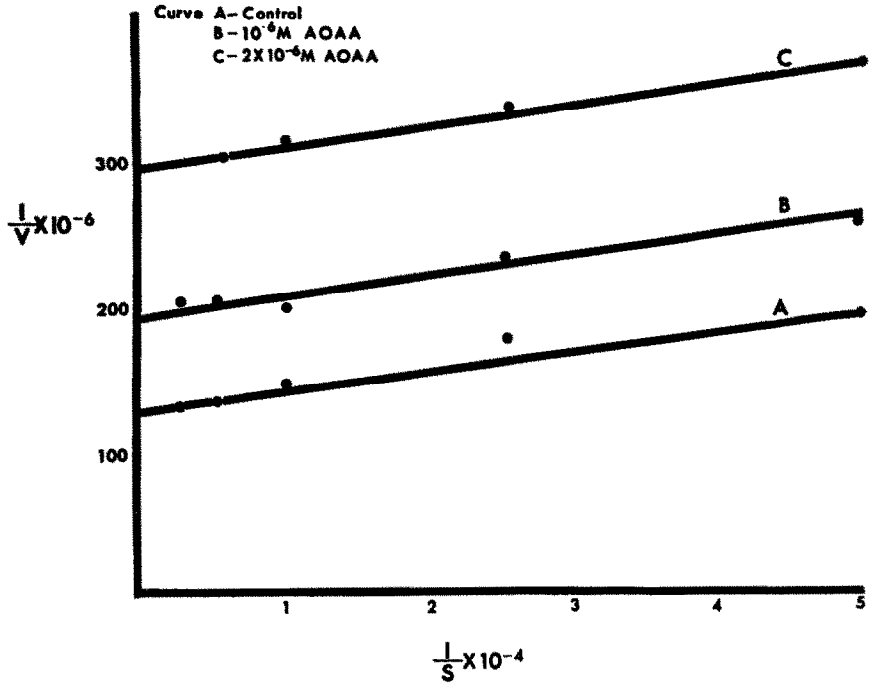

FIG. 2. Lineweaver-Burk plot of rat liver pyridoxal phosphokinase with varying concentrations of pyridoxal and aminooxyacetic acid. Incubation and pyridoxal phosphate determinations were carried out as described in Fig. 1 except that acetone powder prepared from rat liver was used as the source of enzyme. S = molar concentration of pyridoxal, and V = millimoles of pyridoxal phosphate formed per hour per milligram protein.

for pyridoxal. McCormick *et al.*[7] reported an I_{50} of 2×10^{-6} M for the beef brain enzyme and found that the rat liver enzyme was less sensitive to carbonyl reagents than the beef brain preparation.[4] This strongly suggests that the inhibition is mediated through the effect of AOAA on pyridoxal. These results are in agreement with the observation of Hopper and Segal who found that AOAA strongly inhibited glutamic-alanine transaminase by a mechanism involving combination with the aldehydic form of the enzyme only.[3] It is of interest that McCormick *et al.* reported a lack of inhibition of pyridoxal phosphokinase by carbonyl reagents when pyridoxine was employed as substrate.[4]

Kinetic studies (Fig. 2) indicated that the inhibition of pyridoxal phosphokinase by AOAA is of the uncompetitive type, as described by Ebersole *et al.*[10] In this type of inhibition, the inhibitor acts upon the enzyme–substrate complex. In this case the inhibitor apparently has a high affinity for the enzyme–substrate complex, as evidenced by the low K_i value ($K_i = 1{\cdot}75 \times 10^{-6}$ M; $K_s = 1{\cdot}33 \times 10^{-5}$ M). It was reported that inhibition of pyridoxal phosphokinase by pyridoxal dihydrazone was neither competitive nor noncompetitive inhibition.[4]

Carbonyl reagents administered *in vivo* undoubtedly act upon sites in addition to the kinase. Combination with either free or enzyme-bound pyridoxal phosphate would affect many enzymes which utilize it as coenzyme, as pointed out by Roberts and Simonsen.[2] Since AOAA does not combine with pyridoxamine, as indicated by the results of our experiments, and since pyridoxamine phosphate functions as a coenzyme only in transaminations, amino acid metabolism would be expected to be altered by the administration of AOAA. In amino acid metabolism the formyl group of pyridoxal phosphate has been postulated by Snell to be a substrate-binding site.[11] Biochemical changes in the brain after administration of a convulsive dose of AOAA as reported by Roa *et al.* appear to be mediated through its effect on amino acid-metabolizing enzymes.[12] Since pyridoxal phosphokinase is more sensitive to hydrazine (another carbonyl-trapping agent) than is glutamic decarboxylase,[13] the possibility exists that the effect of AOAA *in vivo* is actually more pronounced on the kinase than it is on pyridoxal-dependent enzymes.

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