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THE KINETIC MECHANISM OF INHIBITION OF LIVER PYRIDOXAL KINASE WITH TRYPTOPHAN METABOLITES

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Summary

The activity of purified liver pyridoxal kinase (ATP:pyridoxal 5-phosphotransferase, EC 2.7.1.35) was determined in the presence of 13 different tryptophan metabolites. Only 3-hydroxykynurenine, 3-hydroxyanthranilic acid, xanthurenic acid and quinolinic acid were found to inhibit the enzyme with I_{50} values of 0.1, 0.12, 0.36 and 0.42 mM, respectively. The inhibition was not related to the presence of pyridine nucleus in the metabolite molecule as was proved from the patterns of inhibition.

Pyridoxal kinase (ATP:pyridoxal 5-phosphotransferase, EC 2.7.1.35) catalyzes the formation of the coenzyme pyridoxal 5′-phosphate [1]. The importance of pyridoxal phosphate as a cofactor in the metabolism of amino acids is well established [2]. Also, this coenzyme is involved at stages in the metabolism of proteins, carbohydrates and lipids and in the synthesis of many biogenic amines [3,4].

At present, relatively little is known about the factors regulating tissue levels of pyridoxal phosphate. The precise role of pyridoxal phosphate in human disease is also meager. In laboratory animals, dietary restriction of vitamin B-6 has a wide range of effects [5—7], in addition, B-6 deficiency leads to altered metabolism of the amino acid, L-tryptophan. The most important pathway for tryptophan leads to niacin formation (kynurenine-niacin pathway), using this route most of the carcinogenic metabolites such as 3-hydroxykynurenine, 3-hydroxyanthranilic acid and xanthurenic acid, are formed [8]. Since many enzymes involved in this pathway indispensably require pyridoxal phosphate as

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cofactor, increased excretion of these metabolites is believed to be related to low cellular pyridoxal phosphate levels [8]. Recent evidence indicates that the absence of pyridoxal phosphate may be a factor in determining the breakdown of the apoenzyme proteins [9]. Increased excretion of tryptophan metabolites after a 2 g loading dose is thought to be a clinical indication of pyridoxal phosphate deficiency [10]; this was confirmed in patients with Hodgkin's disease [11—14] and carcinoma of breast and bladder [15—17]. The low plasma pyridoxal phosphate levels could result from several factors, including restricted dietary intake or malabsorption of vitamin B-6, impaired conversion to pyridoxal phosphate or increased catabolism of pyridoxal phosphate. Although liver is known to be a principle organ for pyridoxal phosphate synthesis, nothing is known about the regulation of liver pyridoxal kinase. Therefore, the present study was undertaken to investigate the in vitro effect of tryptophan metabolites on the major step leading to pyridoxal phosphate formation, that is, the efficiency of liver pyridoxal kinase to synthesize the required pyridoxal phosphate for niacin formation as well as several other biological processes.

Materials and Methods

L-Tryptophan, L-kynurenine sulfate, kynurenic acid, 3-hydroxykynurenine, anthranilic acid, 3-hydroxyanthranilic acid, quinolinic acid, nicotinic acid, nicotinamide, N_1-methylnicotinamide, N'-methylnicotinamide, quinaldic acid and xanthurenic acid were Sigma products. Stock solutions were made in 0.5 M potassium phosphate, pH 7.4.

Pyridoxal kinase was purified from sheep liver as previously described [18]. The enzyme activity was determined with a new radiometric assay method using [^{3}H]pyridoxine as substrate [18]. All assays were performed in duplicate. Data points in the double-reciprocal plots and secondary plots were fitted by linear regression analysis.

Results and Discussion

Table I shows pyridoxal kinase activity in the presence of tryptophan and 12 of its metabolites in comparison to a control assay. For comparison of the I_{50} values of the inhibitory metabolites we measured the enzyme activity in the presence of different concentrations of 3-hydroxykynurenine, 3-hydroxyanthranilic acid, xanthurenic acid and quinolinic acid (Table II).

Data in Table I indicate that the inhibition of liver pyridoxal kinase with tryptophan metabolites was not related to the presence of the pyridine ring in their structure, as we first suspected. The metabolites nicotinic acid, nicotinamide, N_1-methylnicotinamide and N'-methylnicotinamide all contain pyridine nucleus but have no inhibitory effect. Quinolinic acid, however, was found to inhibit liver pyridoxal kinase with a rather high I_{50} value (Table II). Therefore, it was interesting to determine the kinetic mechanism of inhibition with these four metabolites. Figs. 1—4 show the double-reciprocal plots of the relationship between the initial velocity of the kinase-catalyzed reaction and [^{3}H]-pyridoxine, as variable substrate, in the presence of different concentrations of

TABLE I

EFFECT OF TRYPTOPHAN METABOLITES ON LIVER PYRIDOXAL KINASE

The reaction mixture was 20 mM potassium phosphate (pH 5.75), 60 mM KCl, 1 mM ATP, 40 μM Zn^{2+}, 100 μM [^{3}H]pyridoxine, 2 μg enzyme (60 I.U./mg) and 0.5 mM of the metabolites listed in a total volume of 125 μl. The incubations were done at 37°C for 30 min.

Added metabolite (0.5 mM)	Enzyme activity (cpm)
None	2100
Tryptophan	2050
Kynurenine	2080
Kynurenic acid	2200
Nicotinic acid	2085
Nicotinamide	1995
N_1-Methylnicotinamide	2090
N'-Methylnicotinamide	2150
Quinaldic acid	2150
Anthranilic acid	1950
3-Hydroxyanthranilic acid	650
3-Hydroxykynurenine	600
Xanthurenic acid	850
Quinolinic acid	920

tryptophan metabolites. Data in Figs. 1—3 show that 3-hydroxykynurenine, 3-hydroxyanthranilic acid and xanthurenic acid had a noncompetitive type of inhibition with K_i values of 108, 137 and 460 μM, respectively [19]. Quinolinic acid, however, showed an uncompetitive type of inhibition (Fig. 4) with a K_i value of 520 μM. The noncompetitive inhibition of liver pyridoxal kinase with 3-hydroxykynurenine, 3-hydroxyanthranilic acid and xanthurenic acid indicates that these compounds interact with the enzyme or the enzyme-substrate complex [19]. This interaction seems to take place away from the substrate binding site, since the inhibitors and the substrate have different chemical structures. The inhibitor binding, however, could induce conformational changes to the enzyme molecule that lead to the noncompetitive inhibition. The presence of the second hydroxyl group in xanthurenic acid molecules seems to be essential for inhibition, since neither kynurenic acid nor quinaldic acid inhibited the enzyme (Table I). Quinolinic acid, on the other hand,

TABLE II

I_{50} VALUES, THE CONCENTRATION OF THE METABOLITE REQUIRED TO PRODUCE 50% INHIBITION OF LIVER PYRIDOXAL KINASE ACTIVITY

The reaction mixture was the same as in Table I. Tryptophan metabolites (0—1 mM) were added to the reaction mixtures.

Metabolite	I_{50} (mM)
3-Hydroxykynurenine	0.10
3-Hydroxyanthranilic acid	0.12
Xanthurenic acid	0.36
Quinolinic acid	0.42

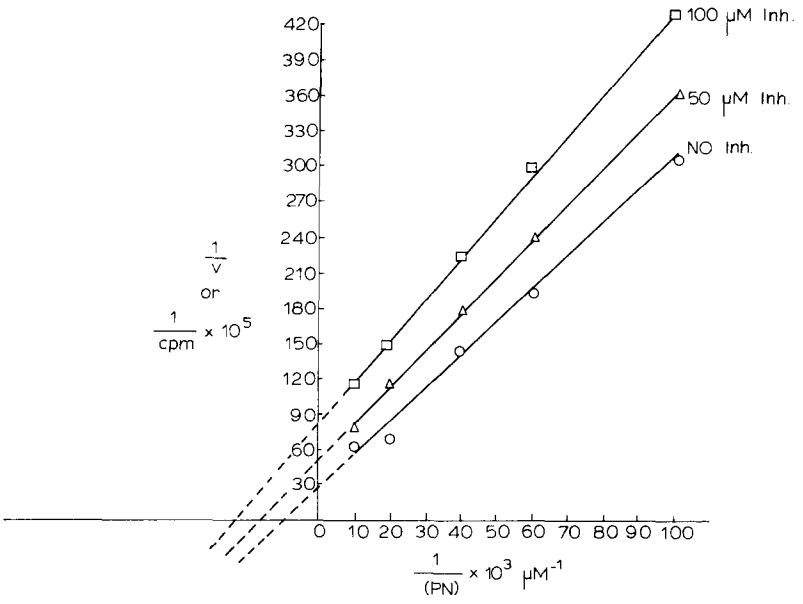

Fig. 1. Double-reciprocal plot of the initial velocity in the presence of 3-hydroxykynurenine as inhibitor and [^{3}H]pyridoxine as variable substrate. The reaction mixture consisted of 20 mM potassium phosphate (pH 5.75)/60 mM KCl/1 mM ATP/40 μM Zn^{2+}/2 μg enzyme (60 I.U./mg). [^{3}H]Pyridoxine was varied from 10 to 100 μM. 3-Hydroxykynurenine concentrations were; ⊙, 0 μM; △, 50 μM and ⊡, 100 μM. The reaction mixtures, 125 μl, were incubated at 37° C for 30 min.

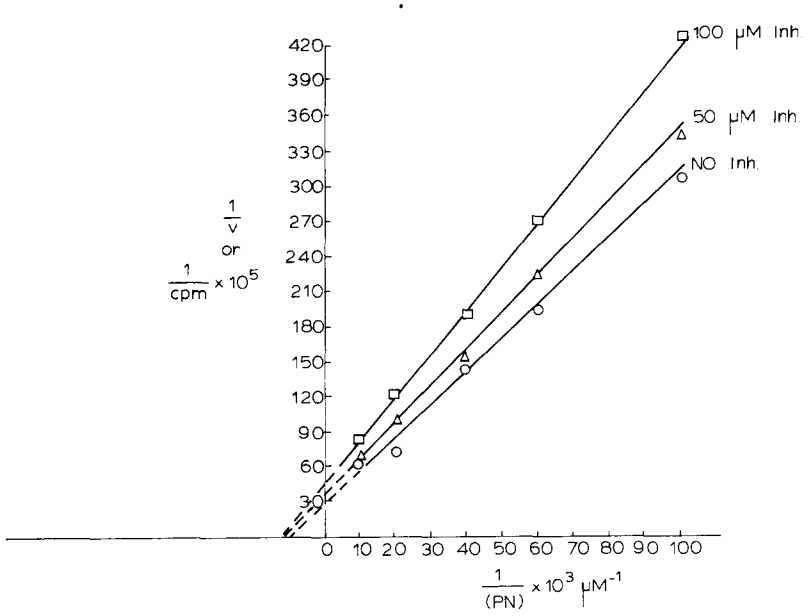

Fig. 2. Double-reciprocal plot of the initial velocity in the presence of 3-hydroxyanthranilic acid as inhibitor and [^{3}H]pyridoxine as variable substrate. The assay conditions were the same as in Fig. 1.

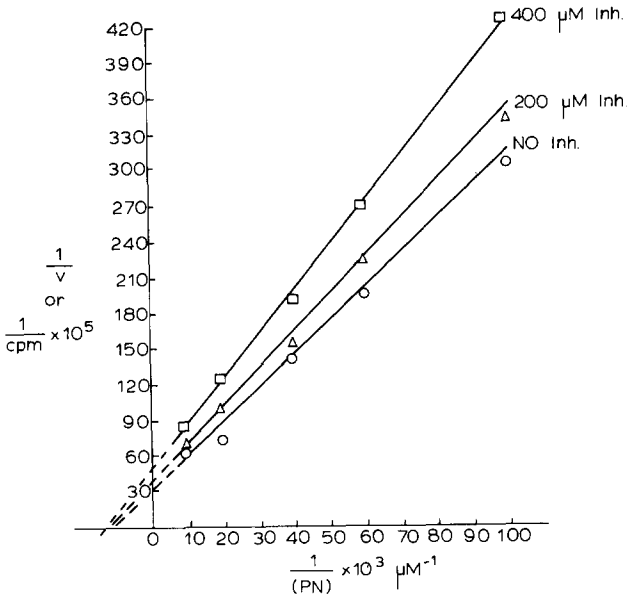

Fig. 3. Double-reciprocal plot of the initial velocity in presence of xanthurenic acid as inhibitor and [^{3}H]-pyridoxine as variable substrate. The assay conditions were the same as in Fig. 1 except the inhibitor concentrations were; ⊙, 0 μM; △, 200 μM and ⊡, 400 μM.

showed an uncompetitive type of inhibition (Fig. 4), that means it binds to the enzyme-substrate complex [19]. The presence of the second carboxylic group, in the structure of quinolinic acid molecule, seems to be essential for its bind-

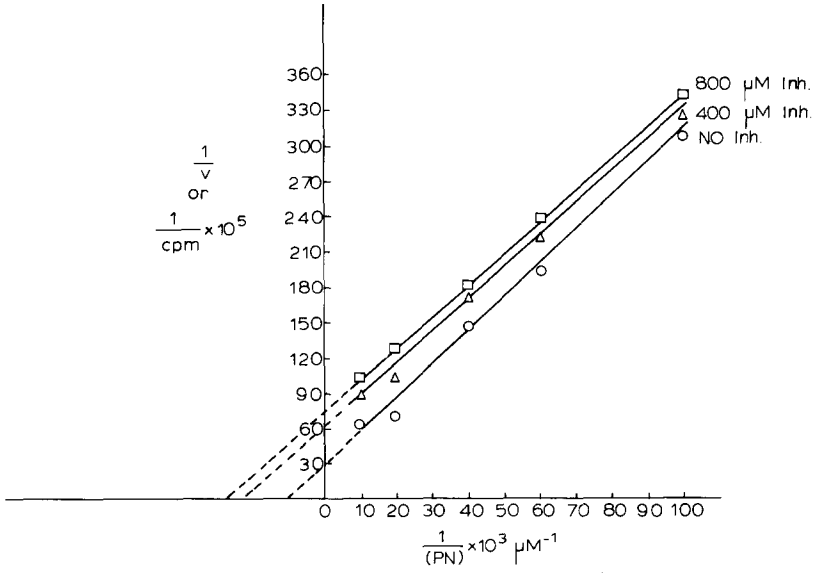

Fig. 4. Double-reciprocal plot of the initial velocity in presence of quinolinic acid as inhibitor and [^{3}H]-pyridoxine as variable substrate. The assay conditions were the same as in Fig. 1 except the inhibitor concentrations were; ⊙, 0 μM; △, 400 μM and ⊡, 800 μM.

ing to the enzyme, since nicotinic acid did not have an inhibitory effect (Table I).

Conclusion

3-Hydroxykynurenine, 3-hydroxyanthranilic acid, xanthurenic acid and quinolinic acid were found to inhibit liver pyridoxal kinase in vitro. Elevated intracellular levels of these metabolites may be harmful, not only because some of them are believed to be carcinogenic, but also because they inhibit the main supply of pyridoxal phosphate by inhibiting liver pyridoxal kinase.

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