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Transformylation of carcinogenic aromatic amines by kynurenine formamidase: a detoxication mechanism

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IN AN earlier paper the purification (over 200-fold) and characterization of guinea pig liver kynurenine formamidase (aryl-formyl-amine amidohydrolase EC 3·5.1.9) was reported.[1] This enzyme is active in tryptophan metabolism catalyzing the deformylation of *N*-formyl-L-kynurenine with simultaneous liberation of formic acid. Our studies on the substrate specificity of this enzyme revealed that the enzyme is capable of readily hydrolyzing except acylamide derivatives of benzene ring also those of naphthalene, biphenylene and fluorene (Table 1). The *N*-formyl group of the substrate can be replaced by acetyl- and even better by chloroacetyl group. This enzyme is activated by aliphatic alcohols suggesting that the enzyme might carry also transacylation reactions.[2–4] It was demonstrated that, esters are formed during the enzymic hydrolysis of the substrate in the presence of aliphatic alcohols and hydroxamic acid was formed in the presence of hydroxylamine.[5] The data presented in this paper demonstrate that kynurenine formamidase can transfer the acyl group (formyl, acetyl) also to aromatic amines, e.g. 1- and 2-naphthylamine.

TABLE 1. HYDROLYSIS RATES OF VARIOUS SUBSTRATES BY KYNURENINE FORMAMIDASE PURIFIED FROM GUINEA PIG LIVER. DATA OF SANTTI AND HOPSU-HAVU

Substrate	Hydrolysis rate mumole/min/mg protein
Formyl-kynurenine	23,200
Formyl-anthranilic acid	8 000
Formanilide	2080
Acetyl-anthranilic acid	1800
Acetanilide	585
Chloroacetyl-anthranilic acid	48,000
Acetyl-1-Naphthylamine	425
Acetyl-2-Naphthylamine	22
Formyl-1-Naphthylamine	620
Chloroacetyl-1-Naphthylamine	28,500
2-Acetamidofluorene	3
4-Acetamidobiphenylene	11

The effect of the presence of 1-naphtylamine and 2-naphthylamine on the rate of the enzymic hydrolysis of formylkynurenine was first studied. The incubation medium was as follows: 0·5 ml of *N*-formyl-L-kynurenine (1 mM, Calbiochem) in aquous solution, 0·5 ml of guinea pig liver kynurenine formamidase preparation purified as presented earlier,[1] 0·5 ml of Tris-HCl buffer (0·2 M, pH 7·0) and 0·5 ml of aquous solution (1 mM) of 1- or 2-naphthylamine hydrochloride (Dr. Theodor Schuchardt, München) neutralized with 0·1 N NaOH. The rate of deformylation of formylkynurenine was followed at 37° by measuring the absorbance at 360 mμ against the control in which the enzyme was replaced by destilled water. The results are seen in Fig. 1. 1-Naphthylamine enhanced the rate of enzymic deformylation of the substrate while the same effect was not obtained with 2-naphthylamine. Similar sets of data on the effect of 1-naphthylamine is presented as a Lineweaver–Burk plot in Fig. 2. It is evident that both K_m and V_{max} had increased in the presence of 1-naphthylamine.

The rate of disappearance of free 1-naphthylamine during the incubation was followed by adding to the incubation tubes 2·0 ml of diazonium salt solution (Garnet GBC 0·25 mg/1·0 ml of 1·0 M acetate buffer pH 4·2 containing 10% Tween 20) after various periods of incubation and by measuring the colour intensity at 525 mμ. The results are seen in Fig. 3. All of the substrate was hydrolyzed and

the major part of free naphthylamine disappeared concomitantly. Direct evidence on the formation of formyl-1-naphthylamine was obtained by analysing the incubated solution using chromatography on thin layer. After incubation the incubation mixture was extracted with 1·0 ml of diethylether, which was then concentrated by evaporation and applicated on a thin layer (Silica gel, G. E. Merck, A.-G. Darmstadt) and chromatographed with chloroform: ethylacetate: acetic acid (6:3:1). The spots were identified in u.v. light.[6] The formation of formyl-1-naphthylamine in the presence of enzyme

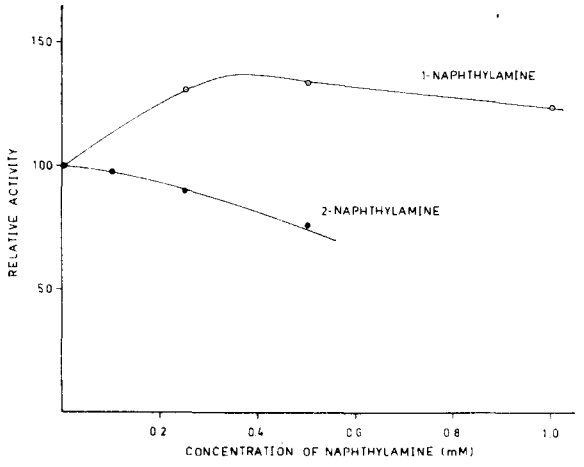

FIG. 1. Effect of 1- and 2-naphthylamine hydrochloride on the rate of deformylation of *N*-formyl-L-kynurenine catalyzed by purified kynurenine formamidase. Details in text.

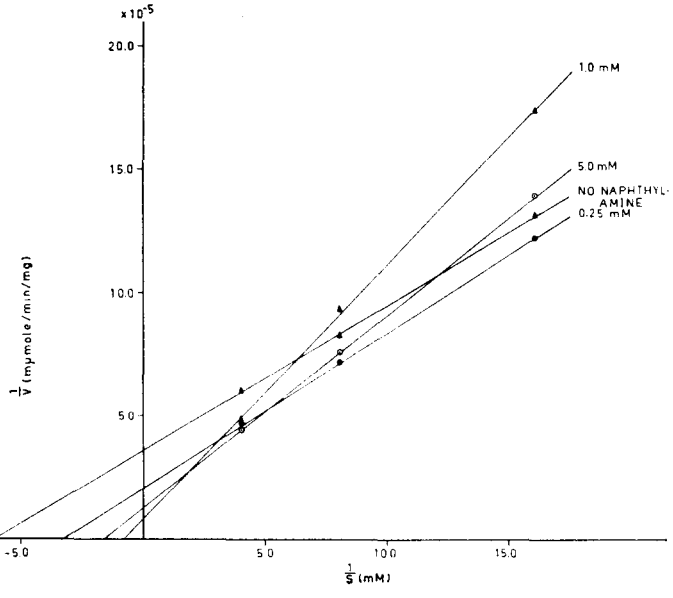

FIG. 2. Double reciprocal plots of the effect of 1-naphthylamine hydrochloride on deformylation of *N*-formyl-L-kynurenine by kynurenine formamidase. Details in text.

(but not in the absence of it) was evident. At a concentration 0·25 mM of *N*-formyl-L-kynurenine and of 1-naphthylamine in the incubation medium, 62 per cent of the formyl groups liberated from the substrate were transferred to 1-naphthylamine, the rest being liberated as formic acid. The corresponding figure in the case of 2-naphthylamine was only 32 per cent.

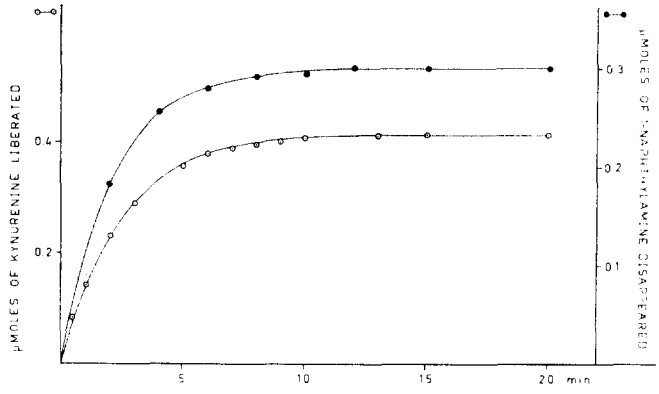

FIG. 3. The rate of disappearance of free 1-naphthylamine during the deformylation of *N*-formyl-L-kynurenine by kynurenine formamidase. Details in text.

Kynurenine formamidase is capable of transferring the formyl group to aniline and anthranilic acid as well. Using a concentration of 0·25 mM of substrate as well as of aniline, 80 per cent of the formyl groups liberated from the substrate were transferred to aniline. The corresponding figure for anthranilic acid was 45 per cent. In these experiments the concentration of free aniline and anthranilic acid was determined using 1.2-naphthoquinone sulphonic acid (E. Merck AG) which gives an intense colour at pH 7·0 with these substancies but does not react with *N*-formyl-L-kynurenine or with L-kynurenine. After incubation 1·0 ml of 1.2-naphthoquinone sulphonic acid (1 mg/ml of Tris-HCl buffer pH 7·0) was added in the test tubes and absorbancies were read at 490 mμ 1 min later.

These data demonstrate that guinea pig liver kynurenine formamidase is capable of catalyzing the transfer of a formyl group from its physiological substrate *N*-formyl-L-kynurenine to 1- and 2-naphthylamine as well as to aniline and anthranilic acid. This suggests that formylation and possibly also acetylation of aromatic amines might be carried out by this enzyme also *in vivo*. In fact, the occurence of 2-formamido-1-naphthyl hydrogen sulphate was recently demonstrated in the urine of several animal species after feeding of 2-naphthylamine or of 2-amino-1-naphthyl hydrogen sulphate.[6] In view of the present data this reaction may have been catalyzed also by kynurenine formamidase. Formylation catalyzed by this enzyme may, therefore, represent a detoxification mechanism of the organism. It had been shown earlier that there is in the liver an enzyme capable of acetylating aromatic amines with acetyl- Co A as substrate or through a transfer reaction from one amine to another.[7, 8] An enzyme has also been described in transferring an acetyl group from one aromatic amine to another but not from acetyl -CoA.[9] Further studies will show whether one or other of these enzymes could be considered as identical with kynurenine formamidase. Since this enzyme is capable of deacylating acyl derivatives of aromatic amines and also acylates aromatic amines in the presence of *N*-formyl-L-kynurenine, it has an important role in the metabolism of those carcinogenic compounds.

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Chlorpromazine: differential effects on membrane-bound enzymes from rat brain*

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THE MECHANISM of action of chlorpromazine (CPZ) remains obscure despite a multitude of experimental studies documenting a variety of pharmacological and biochemical effects. As a unifying hypothesis to relate the many diverse effects of the drug, Guth and Spirtes[1] proposed that a major mode of action was through the modification of membrane properties. In this context we have examined the effects of a pharmacological concentration of CPZ on three membrane-bound enzymes involved with adenine nucleotide metabolism in brain. With a fresh microsomal preparation, CPZ inhibited slightly the Mg^{2+}-dependent adenosine triphosphatase (Mg^{2+}-ATPase), inhibited markedly the $Na^+ + K^+ + Mg^{2+}$-dependent ATPase (Na^+ K^+ Mg^{2+}-ATPase), but stimulated the adenylate kinase 2-fold. Treatment of the membrane preparation with a detergent abolished the ability of CPZ to stimulate adenylate kinase, whereas the ability to inhibit the Mg^{2+}-ATPase was increased.

METHODS

A microsomal fraction was prepared from brains of adult albino rats as previously described.[2] From this fraction the deoxycholate-treated microsomes (DOC-microsomes) were prepared as described by Järnefelt[3] using 1·5 mg deoxycholate/ml in 1·0 M KCl.[4] All incubations were performed within 4 hr after preparation.

For nucleotide phosphatase determinations, the standard incubation medium contained 50 mM Tris-HCl (pH 7·3), 3 mM $MgCl_2$, 3 mM adenosine triphosphate (ATP) or adenosine diphosphate (ADP) as the Tris salt, and about 0·3 mg microsomal protein (in 0·1 ml of 0·25 M sucrose) per ml.

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