ACTA CHEMICA SCANDINAVICA 24 (1970) 791–796

Acylation and Deacylation Reactions Catalyzed by Kynurenine Formamidase

RISTO S. SANTTI and VÄINÖ K. HOPSU-HAVU

Departments of Anatomy and Dermatology, University of Turku, Turku 3, Finland

Kynurenine formamidase (EC 3.5.1.9) of the rat and guinea pig liver was found to be capable of hydrolysing several acylamido derivatives of benzene, naphthalene and biphenylene. 1-Naphthylamine and aniline enhanced the rate of enzymatic deacylation of *N*-formyl-L-kynurenine by the guinea pig liver enzyme but not by the rat liver enzyme. The enzymes of both species were shown to transfer the acyl group from *N*-formyl-L-kynurenine, acetyl anthranilic acid, formyl anthranilic acid and 4-*N*-acetyl aminobiphenylene to aniline, 1- and 2-naphthylamine, 4-aminoazobenzene and anthranilic acid as well as to aliphatic alcohols and hydroxylamine. Formate did not serve as acyl donor and 2-aminofluorene as well as tetrahydrofolic acid did not serve as acyl acceptors. The same hydrolytic and transfer reactions were catalyzed by kynurenine formamidases from the rat and guinea pig liver and the differences between the species were considered quantitative rather than qualitative.

Kynurenine formamidase (formamidase, arylformylamine aminohydrolase EC 3.5.1.9) purified from guinea pig liver was found to catalyze the hydrolysis of *N*-formyl-L-kynurenine as well as of several acylamido derivatives of benzene, naphthalene, and biphenylene.[1] Formyl, acetyl, and propionyl groups and their halogen-substituted derivatives were liberated from the substrates. Chloroacetyl derivatives were hydrolyzed even faster than the corresponding formyl derivatives. Kynurenine formamidase was found to catalyze the formation of esters in the presence of the above substrates and aliphatic alcohols[2] and the transformylation of aromatic amines such as 1- and 2-naphthylamines and aniline.[3] We have suggested that kynurenine formamidase could be the enzyme responsible for the formation of formylamido derivatives of 2-naphthylamine recovered from the urine of rats or dogs fed with 2-naphthylamine. Since no corresponding formyl derivatives were found in the urine of the guinea pig,[4] it was decided to purify kynurenine formamidase from rat liver and to compare the rat and guinea pig liver enzymes more thoroughly with special attention to the transacylation functions of the enzymes. Our preliminary experiments with a partially purified rat liver

enzyme revealed marked differences in the stabilities of the enzymes from different sources as well as in the effects of methanol on the enzymic reactions.[5]

Rat liver kynurenine formamidase was purified by the same method as guinea pig liver enzyme.[1] The specific activity of the final preparation was 128 times that of the initial homogenate and the enzyme yield was 20 %. The preparation of kynurenine formamidase purified from guinea pig liver had a specific activity that was 10 times that of the rat liver enzyme preparation. The sources of the substrates used in this study as well as the assay methods were as described earlier.[1] The enzymic hydrolysis of chloroacetanilide was studied in the same incubation conditions as that of acetanilide.[1] 2-*N*-Hydroxy-acetamidofluorene (2-N-OH-AAF) was synthetized as described earlier [7] from 2-nitrofluorene (Fluka AG) and its hydrolysis tested according to Irving.[8]

Table 1 shows data on the substrate specificity of the kynurenine formamidase preparations purified from rat and guinea pig livers. For comparison, the data of Mehler and Knox for rat liver enzyme have been included.[6] The substrate specificities of the rat and guinea pig liver kynurenine formamidases are very much alike even though there are obvious differences. The

Table 1. Relative hydrolysis rates of several substrates in the presence of kynurenine formamidase preparations purified from guinea pig and rat liver. The hydrolysis rates are relative to that of *N*-formyl-L-kynurenine which was taken as 100. For comparison data of Mehler and Knox for rat liver kynurenine formamidase [6] are also reproduced.

Substrate	Kynurenine formamidase		
	Guinea pig [1]	Rat	
		This work	Mehler and Knox
N-Formyl-L-kynurenine	100	100	100
Formylanthranilic acid	35	55	16
Acetylanthranilic acid	3.9	5.2	1
Formanilide	9.0	3.9	2.6
Acetanilide	2.5	0.1	0.4
Chloroacetanilide	131	4.3	
Trifluoroacetanilide	1.9	1.3	
Chloroacetylanthranilic acid	207	233	
Formyl-1-naphthylamine	2.7	0.5	
Formyl-2-naphthylamine	0.45	0.17	
Acetyl-1-naphthylamine	1.8	0.03	
Acetyl-2-naphthylamine	0.1	0.0	
Chloroacetyl-1-naphtylamine	123	2.0	
4-*N*-Acetamidobiphenylene	0.01	0.01	
2-*N*-Acetamidofluorene	0.01	0.01	
2-*N*-Hydroxy-acetamidofluorene	0.02		
Formylaspartic acid	0	0	
Formylglutamic acid	0	0	
Formylglycine	0		
10-Formyltetrahydrofolic acid	0		

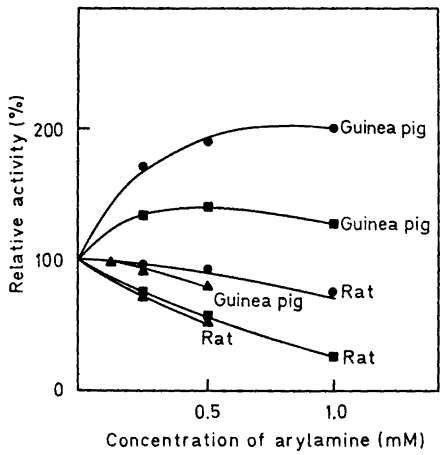

Fig. 1. The effect of aniline and 1- and 2-naphthylamine hydrochlorides on the rate of deformylation of *N*-formyl-L-kynurenine catalyzed by rat and quinea pig liver kynurenine formamidases. For details, see text. Aniline ●, 1-naphthylamine ■, 2-naphthylamine ▲.

rat liver enzyme was capable of hydrolysing several acylamido derivatives of benzene, naphthalene, and biphenylene rings besides *N*-formyl-L-kynurenine. In contrast to the guinea pig liver enzyme, the rat liver enzyme seemed to release the *N*-formyl group more effectively than the *N*-acetyl group. A comparison of the hydrolysis rates of the aniline and 1-naphthylamine derivatives in the presence of the enzymes of both species reveals this clearly. The substitution of a hydrogen in the acetyl group by a chlorine atom led to a 40 to 60-fold increase in deacylation activity regardless of the aromatic amine moiety of the substrate. A carboxyl group in the *ortho* position in the benzene ring of the substrate enhanced the rate of enzymic hydrolysis by the rat liver enzyme much more than that by the guinea pig liver enzyme. The structure of the aromatic amine was found to affect the rate of the enzymic hydrolysis, which decreased in the order: aniline, 1-naphthylamine, 2-naphthylamine, and 4-aminobiphenylene. No clear difference could be demonstrated between the two enzymes in this respect. The enzymes of both species hydrolysed 4-acetamidobiphenylene and 2-acetamidofluorene very slowly and no hydrolysis of acetyl 2-naphthylamide by the rat liver enzyme was detected. *N*-Hydroxylation of 2-acetamidofluorene did not increase the rate of deacetylation by guinea pig liver kynurenine formamidase. This is in contrast to the 100 times greater activity of a guinea pig liver microsomal enzyme in the hydrolysis of N-OH-AAF than in the hydrolysis of the corresponding non-hydroxylated acetyl derivate.[9]

The effects of 1- and 2-naphthylamine hydrochlorides and aniline on the enzymic breakdown of *N*-formyl-L-kynurenine are shown in Fig. 1. The incubation conditions were the same in these experiments as earlier.[3] 1-Naphthylamine and aniline enhanced the deacylation catalyzed by the guinea pig liver enzyme while 2-naphthylamine partly inhibited it in the same experimental conditions. None of these amines enhanced the rates of deacylation of the same substrates catalyzed by the rat liver enzyme. The effects of 1- and 2-naphthylamine hydrochlorides at various concentrations on the deformyla-

tion of *N*-formyl-L-kynurenine (at various concentrations) by the rat and guinea pig liver enzymes were studied. The results were analyzed by plotting $1/v$ *versus* $1/[S]$ (v=reaction rate, [S]=substrate concentration). 1-Naphthylamine increased both K_m and V in the experiments with the guinea pig enzyme. K_m and V rose also in the presence of 2-naphthylamine, but above a certain concentration (0.25 mM), V began to decrease. Both 1- and 2-naphthylamine inhibited the rat enzyme in the same experimental conditions. The final concentrations of the naphthylamines in these experiments were 0.125, 0.25, 0.5, and 1.0 mM. It may be added that preincubation of the enzymes with the aromatic amines at 37°C for 10 min had no effect on the results.

We have found earlier that guinea pig liver kynurenine formamidase transfers a formyl group from the physiological substrate *N*-formyl-L-kynurenine to arylamines such as 1- and 2-naphthylamines and aniline.[3] Using mainly the same methods as earlier, we investigated whether rat liver kynurenine formamidase also is able to act as a transacylase. The various donors and acceptors tested as well as the observed relative transacylation rates are listed in Table 2. Additional methodical details are described below. The rate of transacylation with N-OH-AAF as acetyl donor and 4-aminoazobenzene as acceptor was measured by the method described by Booth [10] except that the pyrophosphate

Table 2. (Trans)acylation reactions catalyzed by rat and guinea pig liver kynurenine formamidases. The numbers are percentages of the acyl groups liberated from the substrates and transferred to the acceptor amines. The numbers are in italics when the breakdown of the substrate was enhanced in the presence of an arylamine.

Acyl donor	Acyl acceptor	(Trans)acylation	
		Guinea pig	Rat
N-Formyl-L-kynurenine (0.25 mM)	Aniline (0.25 mM)	*80*	47
» (0.25 »)	» (0.125 mM)	*67*	29
» (0.25 »)	1-Naphthylamine (0.25 mM)	*62*	13
» (0.25 »)	2-Naphthylamine (0.25 mM)	*32*	17
» (0.25 »)	2-Aminofluorene (0.025–0.1 mM)	0	—
» (0.25 »)	Tetrahydrofolic acid (0.8 mM) [9]	0	—
» (0.25 »)	Anthranilic acid (0.2 mM)	*45*	—
Acetylanthranilic acid (1.5 mM in 2.5 vol.% final conc. of methanol)	1-Naphthylamine (0.5 mM)	+	—
2-*N*-Hydroxy-acetamidofluorene (0.4 mM)	4-Aminoazobenzene (0.1 mM)	0	—
4-*N*-Acetylamidobiphenyl (0.2 mM)	» (0.1 »)	(+)	—
Formylanthranilic acid (7.5 mM)	Ethanol (10 vol.%)	*60*	—
Acetylanthranilic acid (7.5 »)	Methanol (10 »)	*72*	—
Formylanthranilic acid (7.5 »)	Hydroxylamine hydrochloride (0.025–0.25 M)	+	—
Acetylanthranilic acid	Hydroxylamine hydrochloride (0.025–0.25 M)	+	—
Formate, ATP, $MgCl_2$	Tetrahydrofolic acid [9]	0	—
Formate (0.25 mM)	1-Naphthylamine (0.25 mM)	0	0

buffer and cysteine were replaced by a sodium orthophosphate buffer of pH 7.0. The acetyltransferase activity of the rat liver homogenate which served as a control was 0.72 μmole/g of tissue/h. The corresponding activity with 4-(*N*-hydroxyacetamido)biphenylene as acetyl donor was given by Booth as 5.18 μmole/g of tissue/h. The same method was used when testing 4-*N*-acetylamidobiphenylene as acetyl donor. The transfer of the formyl group from *N*-formyl-L-kynurenine to 2-aminofluorene (Nutr. Biochem. Corp.) was tested (after incubation for 10 min at 37°C) by measuring the free 2-aminofluorene by adding 1.0 ml of a 1,2-naphthoquinone sulphonic acid solution (1 mg in 0.5 ml of 0.2 M Tris-HCl buffer pH 7.0, and 0.5 ml of methanol) to the incubation tubes and measuring the colour intensity at 515 nm 1 min later. To dissolve 2-aminofluorene (0.1, 0.05 and 0.025 mM), the final concentration of methanol was 2.5 %. The rate of disappearance of the acceptor amine was taken as the measure of the transacylation rate. The amount of free acceptor amine was found to remain constant during incubation in the absence of substrate or enzyme. Direct evidence for the formation of acylamino derivatives was obtained by analyzing the incubation solutions by chromatography on thin layers or paper. The acyl derivatives of the acceptor amines were extracted with diethyl ether (2×2.0 ml), the extracts evaporated to dryness, and the residues dissolved in acetone and applied to plates for chromatography with chloroform:ethyl acetate:acetic acid (6:3:1) as eluent. After chromatography, the plates were sprayed with *p*-dimethylaminocinnamaldehyde (K. et K. Laboratories Inc., 1 % w/v in 50 % v/v ethanolic 3 N HCl). Free amines reacted immediately and formyl derivatives within a few minutes.[4] Acyl derivatives of anthranilic acid were identified by pipetting a portion (10 μl) of the incubation mixture on a paper (Whatman No. 1) for chromatography with methanol:butanol:benzene:water (2:1:1:1) as eluent. The spots were identified in UV light. Acetylanthranilic acid and formylanthranilic acid (5 mM in 2.5 vol. % *N*,*N*-dimethylformamide) were incubated with guinea pig liver kynurenine formamidase in the presence of hydroxylamine (hydroxylamine hydrochloride, E. Merck AG, in 0.025—0.25 M concentrations, neutralized with NaOH) in 0.05 M Tris-HCl buffer, pH 7.0, in a total volume of 2.0 ml. After an incubation period of 60 min at 37°C, the amount of hydroxamic acid was estimated according to Hestrin.[1] To a 1.0 ml sample was pipetted 1.0 ml of 20 % trichloroacetic acid, followed by 0.5 ml of 0.44 M ferric chloride (in 0.1 N HCl). The colour intensity at 500 nm was measured 30 min later.

The data in Table 2 show that kynurenine formamidase from rat liver as well as that from guinea pig liver are able to transfer the acyl group (acetyl or formyl) from *N*-formyl-L-kynurenine, acetylanthranilic acid, formylanthranilic acid, and *N*-acetylaminobiphenylene to arylamines (aniline, 1- and 2-naphthylamines, 4-aminoazobenzene, and anthranilic acid) or to aliphatic alcohols (methanol and ethanol) to form esters, or to hydroxylamine to form hydroxamic acids. From the point of view of chemical carcinogenesis as well as of the general metabolism of the C_1-moiety, it was of special interest that 2-aminofluorene and tetrahydrofolic acid did not serve as acceptors and formate did not serve as substrate (acyl donor) for kynurenine formamidase. The enzymes were also unable to transfer the acetyl group from 2-N-OH-AAF to 4-aminoazobenzene as did the acetyltransferase described by Booth.[10]

Kynurenine formamidases from the guinea pig and rat livers appear to be basically capable of catalyzing the same hydrolytic and transfer reactions; the differences between species are quantitative rather than qualitative. These differences can partly explain the differences observed in the metabolism of aromatic amines in these species.

REFERENCES

1. Santti, R. S. and Hopsu-Havu, V. K. *Z. physiol. Chem.* **349** (1968) 753.
2. Santti, R. S. and Hopsu-Havu, V. K. *Scand. J. Clin. Lab. Invest.* **19** *Suppl.* **95** (1967) 51.
3. Santti, R. S. and Hopsu-Havu, V. K. *Biochem. Pharmacol.* **17** (1968) 1110.
4. Boyland, E. and Manson, D. *Biochem. J.* **99** (1966) 189.
5. Santti, R. S. *Suomen Kemistilehti* **B 41** (1968) 75.
6. Mehler, A. H. and Knox, W. E. *J. Biol. Chem.* **187** (1950) 431.
7. Poirier, L. A., Miller, J. A. and Miller, E. C. *Cancer. Res.* **23** (1963) 790.
8. Irving, C. C. *Cancer. Res.* **26** (1966) 1390.
9. Santti, R. S., Puisto, J. and Hopsu-Havu, V. K. *Scand. J. Clin. Lab. Invest.* **21** *Suppl.* **101** (1968) 50.
10. Booth, J. *Biochem. J.* **100** (1966) 745.

Received September 1, 1969.