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ACTIVATION OF THE *O*-GLUCURONIDE OF THE CARCINOGEN *N*-HYDROXY-*N*-2-FLUORENYLACETAMIDE BY ENZYMATIC DEACETYLATION *IN VITRO*: FORMATION OF FLUORENYLAMINE-tRNA ADDUCTS

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Abstract—The *O*-glucuronide of *N*-hydroxy-*N*-2-fluorenylacetamide (N-GlO-FAA) was deacetylated by guinea pig liver. tRNA reacted with the product of this deacetylation, the *O*-glucuronide of *N*-2-fluorenylhydroxylamine (N-GlO-FA), to give fluorenylamine-substituted nucleic acid adducts. The quantity of adduct formation was used to determine deacetylase activity.

Of the various guinea pig tissues assayed, only the liver contained enzyme activity, and this activity was confined to the microsomal fraction of the cell. Guinea pig liver microsomes were about four times as active as rabbit liver microsomes and about fourteen times as active as rat liver microsomes in promoting fluorenylamine–tRNA adduct formation. Adduct formation induced by guinea pig microsomes was about seven times greater at pH 8.5 than at pH 7.0.

The aglycone of the *O*-glucuronide, *N*-hydroxy-*N*-2-fluorenylacetamide (*N*-hydroxy-FAA) also yielded fluorenylamine-substituted nucleic acid adducts following deacetylation at pH 8.5 by guinea pig liver microsomes in the presence of tRNA. In contrast to results obtained with N-GlO-FAA, adduct formation with *N*-hydroxy-FAA was not as efficient, and it was independent of pH over the range 7.0–8.5. Rabbit and rat liver microsomes were more active in promoting adduct formation of tRNA with *N*-hydroxy-FAA than with N-GlO-FAA.

The differential inhibition of the microsome-induced formation of adducts of N-GlO-FAA and *N*-hydroxy-FAA with tRNA affirms that the first step in the binding mechanism of N-GlO-FAA with tRNA is enzymatic deacetylation and not hydrolysis to the aglycone *N*-hydroxy-FAA.

N-Hydroxylation is considered to be the primary step in the metabolic activation of carcinogenic aromatic amines and amides. Further metabolism of the *N*-hydroxylated metabolites is required to initiate their reaction with tissue macromolecules [1, 2].

The *O*-glucuronide of *N*-hydroxy-*N*-2-fluorenylacetamide (N-GlO-FAA) is a metabolite of *N*-2-fluorenylacetamide (FAA) in species which are capable of *N*-hydroxylation [3]. Along with *N*-acetoxy-*N*-2-fluorenylamine, a reactive intermediate which is believed to be formed as a product of the reaction between the enzyme acyltransferase and *N*-hydroxy-*N*-2-fluorenylacetamide (*N*-hydroxy-FAA) [4–6], the *O*-glucuronide has been considered to be responsible for the binding of fluorenylamine residues to nucleic acids *in vivo* [3]. In the case of N-GlO-FAA, this modification of nucleic acids is thought to involve prior deacetylation to the unstable and more reactive *O*-glucuronide of *N*-2-fluorenylhydroxylamine (N-GlO-FA) [7].

In vitro studies have shown that, with increasing pH, N-GlO-FAA is readily deacetylated to N-GlO-FA. This product reacts with the guanine residues of RNA and DNA at a rate much faster than its acetylated analog, N-GlO-FAA [7]. While it has been suggested that N-GlO-FA may be formed *in vivo* by conjugation of *N*-2-fluorenylhydroxylamine (*N*-hydroxy-FA) [8], the deacetylation of N-GlO-FAA would also yield the reactive conjugate of the hydroxylamine.

The present investigation was undertaken to determine if N-GlO-FAA could be converted to the more reactive N-GlO-FA by enzymatic deacetylation. Since *N*-hydroxy-FAA and FAA are rapidly deacetylated by guinea pig liver, as compared to rabbit and rat liver [9], initial experiments designed to detect the presence of a deacetylase capable of acting on N-GlO-FAA were carried out with guinea pig tissues. tRNA was used to trap the reactive product of this deacetylation, N-GlO-FA [7]. Consequently, the demonstration of deacetylase activity was a function of the rate of formation of fluorenylamine–tRNA adducts.

MATERIALS AND METHODS

Chemicals. *N*-Hydroxy-FAA-9-[^{14}C] (12.7 mCi/m-mole) was purchased from International Chemical

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and Nuclear Corp., Cleveland, Ohio. *N*-Hydroxy-FAA-2′-[^{3}H] (41.5 mCi/m-mole) was prepared by the reaction of *N*-hydroxy-FA [10] with acetic anhydride-[^{3}H] (Amersham/Searle Corp., Arlington Heights, Ill.) (1.5:1 mole ratio) in benzene. The hydroxamic acid was purified by conversion to its sodium salt, extraction with ether, and precipitation with acid. The yield was 90%, based on acetic anhydride. Double-labeled *N*-hydroxy-FAA-[9-^{14}C]-[2′-^{3}H] was prepared to give final specific radioactivities of 6.3 mCi ^{14}C/m-mole and 19.1 mCi ^{3}H/m-mole. Radiochemical purity was determined by t.l.c. on Eastman 6060 Silica gel using chloroform–methanol (97:3) as a solvent for *N*-hydroxy-FAA (R_f 0.54) [6]. Radioautography with Kodak Medical No-Screen X-ray film (Eastman Kodak Co., Rochester, N.Y.) gave a single radioactive spot coincident with the fluorescent-quenching spot detected by t.l.c.

The *O*-glucuronide of *N*-hydroxy-FAA-[9-^{14}C]-[2′-^{3}H] (0.99 mCi ^{14}C/m-mole and 4.9 mCi ^{3}H/m-mole) was prepared biosynthetically. The bile ducts of 3 female Sprague–Dawley rats were cannulated and a solution of 10 mg of *N*-hydroxy-FAA-[9-^{14}C]-[2′-^{3}H] in 150 μl of dimethylsulfoxide was administered i.p. to each rat. The bile was collected and the *O*-glucuronide was isolated according to the method of Irving, Veazey and Russell [8]. A sample of the *O*-glucuronide was treated with β-glucuronidase (bacterial, Type I, Sigma Chemical, Co., St. Louis, Mo.) in Sorensen's phosphate buffer, pH 7.0, at 37° for 1.5 hr. Ether extraction of the incubation mixture and subsequent t.l.c. and radioautographic analysis of the ether extract gave one fluorescent-quenching radioactive spot due to the aglycone, *N*-hydroxy-FAA.

The following were obtained from the commercial sources indicated: yeast tRNA, *p*-chloromercuribenzoate, *N*-ethylmaleimide, dithiothreitol, saccharo-1,4-lactone, and *p*-nitrophenyl-β-glucuronide (Calbiochem, Los Angeles, Calif.); diethyl *p*-nitrophenyl phosphate (K & K Laboratories, Inc., Plainview, N.Y.); trisodium pentacyanoamine ferrate (Fisher Scientific Co., Pittsburg, Pa.); sodium fluoride (Merck and Co., Inc., Rahway, N.J.).

Animals and tissue preparation. Male animals were used in all experiments. Albino guinea pigs (340–450 g) (Camm Research Institute, Wayne, N.J.), albino rabbits (2–3 kg) (Thompson Research Foundation, Monee, Ill.) Syrian golden hamsters (100–125 g) (Charles Rivers Lakeview Hamster Colony, Newfield, N.J.), albino rats (180–230 g) and mice (20–25 g) (ARS/Sprague–Dawley, Madison, Wis.) were purchased from the commercial sources indicated.

The rabbits were sacrificed by injection of air into the heart. The other animals were anesthetized with ether prior to removal of tissue samples. The tissues were minced and homogenized in the cold with 9 ml of 0.25 M sucrose/g of tissue in a glass and Teflon homogenizer [10]. The homogenates were centrifuged at 8000 *g* for 20 min. The sediments were discarded and the supernatant centrifuged at 105,000 *g* for 1 hr. The pellets were suspended in the original volume of 0.25 M sucrose and recentrifuged at 105,000 *g* for 30 min. These pellets were homogenized in the cold in 0.05 M Tris HCl buffer, pH 8.5 (1 g equiv tissue wt/2 ml buffer) and used as the microsomal preparation. The washed guinea pig liver microsomal pellets were stable at −20° for several days. For assay of urinary bladder epithelium the mucosa was stripped from rabbit tissue or from a surgically-removed human specimen, and homogenized in 0.05 M Tris HCl buffer, pH 8.5.

Protein determination. Protein concentrations were determined by use of a modified Folin method using bovine serum albumin as a standard [11].

Assay method for enzyme-induced adduct formation of the O-glucuronide of N-hydroxy-FAA with tRNA. The standard assay system contained 1.0 ml of 0.05 M Tris HCl buffer, pH 8.5, 3 mg of yeast tRNA, 0.2 ml of whole homogenate (equivalent to 20 mg tissue wt) or 0.2 ml of microsomal homogenate (equivalent to 100 mg tissue wt, usually 0.5–0.7 mg protein), and the *O*-glucuronide of *N*-hydroxy-FAA-[9-^{14}C]-[2′-^{3}H] (0.028 μmoles) in water (10 μl). The mixture was incubated with shaking at 37° for 20 min in air. The reaction was stopped by addition of buffer-saturated phenol (1.0 ml). The incorporation of fluorenylamine residues into RNA was determined as previously described [6] by precipitating the nucleic acid from 0.6-ml aliquots of the aqueous phase with 6 ml of 2% potassium acetate in 95% ethanol. The precipitate was collected on glass-fiber filters and washed successively with 70 and 95% ethanol, acetone, and diethyl ether. The dry filters were placed in counting vials and moistened with 0.15 ml of water. A solubilizer (1 ml, NCS, Amersham/Searle Corp., Arlington Heights, Ill.) and toluene-based scintillator (10 ml) were added, and the vials were counted in a liquid scintillation counter. Enzyme activity was expressed as nmoles of fluorenylamine bound to tRNA.

Controls used in this study included incubations containing boiled microsomes, no enzyme or the aglycone of the *O*-glucuronide, *N*-hydroxy-FAA-[9-^{14}C]-[2′-^{3}H] (0.028 μmole 10 μl dimethylsulfoxide). Single assays were carried out routinely; duplicate assays using the glucuronide as substrate usually agreed within 10 per cent, but replicates using *N*-hydroxy-FAA were subject to slightly greater variation. With the exception of Table 1, which is a composite of experiments carried out on 5 different microsomal preparations, data from analysis of single tissue preparations are shown. Most experiments were carried out at least twice with different microsomal preparations.

RESULTS

Deacetylation of N-GlO-FAA by guinea pig liver and formation of fluorenylamine-tRNA adducts. Incubations of N-GlO-FAA with tRNA and guinea pig liver microsomes resulted in substantially greater levels of nucleic acid adduct formation as compared with incubations containing either no enzyme or boiled microsomes. Since the nucleic acid adducts contained less than 1 per cent incorporation of the acetyl group their formation was most likely due to enzymatic deacetylation of N-GlO-FAA. The product of this deacetylation, N-GlO-FA, is unstable and known to combine spontaneously with nucleic acids to yield fluorenylamine nucleic acid adducts [7].

Incubation of the aglycone, *N*-hydroxy-FAA, with tRNA and guinea pig liver microsomes also gave fluorenylamine-tRNA adducts. As with the *O*-glucuronide incubations, only slight incorporation of the

Table 1. Effect of modifying substances on the formation of fluorenylamine–nucleic acid adducts on incubation of N-GlO-FAA or its aglycone, *N*-hydroxy-FAA, with tRNA and guinea pig liver microsomes

Modifier	Concentration	% of control activity*	
		N-GlO-FAA as substrate	*N*-Hydroxy-FAA as substrate
None (Control)		100	100
p-Chloromercuribenzoate	10^{-3}	96	45
	10^{-4}	100	105
N-Ethylmaleimide	10^{-2}	8	105
	10^{-3}	40	92
	10^{-4}	100	165
Diethyl *p*-nitrophenyl phosphate	10^{-4}	0	0
	10^{-5}	0	0
	10^{-6}	52	107
Sodium fluoride	10^{-2}	62	74
	10^{-3}	92	115
	10^{-4}	100	95
Trisodium pentacyanoamine ferrate	10^{-2}	0	106
	10^{-3}	25	179
	10^{-4}	99	112
Dithiothreitol	10^{-3}	77	148
Saccharo-1,4-lactone	10^{-2}	100	87
	10^{-3}	99	138

* Enzyme activity was determined by the amount of fluorenylamine-tRNA adducts formed and is expressed as per cent of activity without modifiers for each substrate. Adduct formation by 5 different microsomal preparations ranged from 0.25 to 0.45 (average value = 0.35) nmoles fluorenylamine bound to tRNA/mg protein using N-GlO-FAA as substrate, and 0.18–0.35 (average value = 0.25) using *N*-hydroxy-FAA as substrate.

The enzyme-induced adduct formation by guinea pig liver microsomes was determined in 0.05 M Tris–HCl buffer, pH 8.5, as described in Materials and Methods.

acetyl group (2 per cent) of *N*-hydroxy-FAA was shown to be present in these adducts.

The effect of pH on adduct formation using either N-GlO-FAA or its aglycone as substrate in 0.05 M Tris–HCl buffer is shown in Fig. 1. Whereas adduct formation with *N*-hydroxy-FAA as substrate was essentially independent of pH over the range 7–8.5, approximately a 7-fold increase was found with N-GlO-FAA as substrate on increasing the pH of the incubation mixture from 7.0 to 8.5. At pH 9.0 the N-GlO-FAA is rapidly deacetylated and no difference in the yield of adduct formation by enzymatic or chemical deacetylation could be shown.

In incubation mixtures containing N-GlO-FAA, tRNA and guinea pig liver microsomes at pH 8.5, the

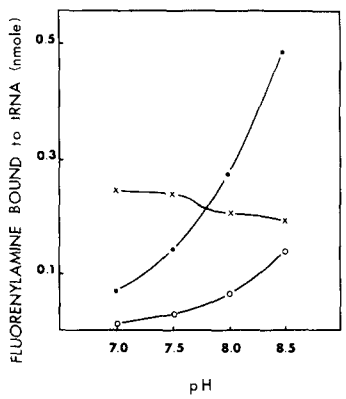

Fig. 1. Effect of pH on the guinea pig liver microsome-induced activation of N-GlO-FAA and *N*-hydroxy-FAA. The formation of fluorenylamine–nucleic acid adducts as a function of pH on incubation of N-GlO-FAA or *N*-hydroxy-FAA with tRNA and guinea pig liver microsomes was determined in 0.05 M Tris–HCl buffer as described in Materials and Methods. Data shown are from incubation of: ●, N-GlO-FAA and microsomes; ○, N-GlO-FAA and boiled microsomes; ×, *N*-hydroxy-FAA and microsomes.

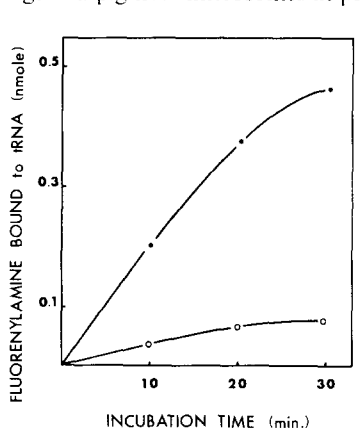

Fig. 2. Time-dependent formation of fluorenylamine–nucleic acid adducts on incubation of N-GlO-FAA with tRNA and guinea pig liver microsomes. The formation of fluorenylamine-nucleic acid adducts as a function of the period of incubation of N-GlO-FAA with tRNA and guinea pig liver microsomes (●) or boiled microsomes (○), in 0.05 M Tris–HCl buffer, pH 8.5, was determined as described in Materials and Methods.

amount of fluorenylamine residues bound to the nucleic acid was proportional to incubation times of up to ~ 20 min (Fig. 2), and to the concentration of microsomes (Fig. 3) and substrate (Fig. 4). The results of control incubations which contained no microsomes or boiled microsomes are also shown in Fig. 1–4. Adduct formation in control incubations was ~ 1/5–1/10 of that obtained in incubations with guinea pig liver microsomes.

Effect of modifying substances on adduct formation. The effect of various modifying substances on the formation of fluorenylamine nucleic acid adducts obtained on incubation of N-GlO-FAA or its aglycone, *N*-hydroxy-FAA, with tRNA and guinea pig liver microsomes at pH 8.5 is shown in Table 1. Whereas nucleic acid-adduct formation with *N*-hydroxy-FAA was reduced by 55 per cent with *p*-chlororomercuribenzoate at a concentration of 10^{-3} M, it was essentially unchanged with N-GlO-FAA. In contrast to this, *N*-ethylmaleimide almost completely inhibited adduct formation with N-GlO-FAA at a concentration of 10^{-2} M, but had no effect on adduct formation with *N*-hydroxy-FAA.

Diethyl *p*-nitrophenyl phosphate at a concentration of 10^{-5} M completely inhibited adduct formation with both substrates. However, at a concentration of 10^{-6} M it had no effect on adduct formation with *N*-hydroxy-FAA, but it reduced adduct formation with N-GlO-FAA by approximately one half.

In the presence of 10^{-3} M dithiothreitol, adduct formation was enhanced by about one and a half times with *N*-hydroxy-FAA as substrate, but it was reduced by about 1/4 when N-GlO-FAA was used as substrate. Sodium fluoride at a concentration of 10^{-2} M inhibited adduct formation to approximately the same degree with both substrates, while 10^{-2} M saccharo-1,4-lactone, a known β-glucuronidase inhibitor [12], did not inhibit adduct formation with either substrate.

Trisodium pentacyanoamine ferrate, which rapidly forms a stable colored complex with *N*-hydroxy-FA

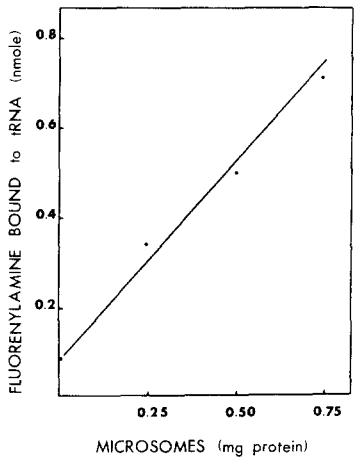

Fig. 3. Fluorenylamine-substitution of nucleic acid as a function of guinea pig liver microsomes incubated with N-GlO-FAA and tRNA. The quantity of fluorenylamine nucleic acid adducts formed on incubation of varying amounts of guinea pig liver microsomes with N-GlO-FAA and tRNA was determined in 0.05 M Tris HCl buffer, pH 8.5, as described in Materials and Methods.

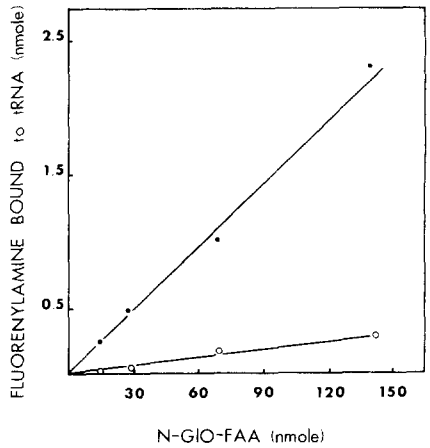

Fig. 4. Substrate-dependent formation of fluorenylamine-nucleic acid adducts on incubation of N-GlO-FAA with tRNA and guinea pig liver microsomes. The quantities of fluorenylamine-substituted nucleic acid formed on incubation of varying amounts of N-GlO-FAA with tRNA in the presence (●) or absence (○) of guinea pig liver microsomes was determined using 0.05 M Tris HCl buffer, pH 8.5, as described in Materials and Methods.

[13], did not inhibit adduct formation with *N*-hydroxy-FAA at concentrations of 10^{-2}–10^{-4} M. However adduct formation with N-GlO-FAA was inhibited by 75 per cent at 10^{-3} M trisodium pentacyanoamine ferrate.

Intracellular distribution of enzyme activity. Adduct formation resulting from incubation of N-GlO-FAA or its aglycone, *N*-hydroxy-FAA, with tRNA and subcellular fractions of guinea pig liver is shown in Table 2. When N-GlO-FAA was used as the substrate, only the microsomal fraction deacetylated the *O*-glucuronide and produced fluorenylamine tRNA adducts. In contrast, each intracellular fraction was capable of adduct formation with *N*-hydroxy-FAA as substrate. In other experiments the microsomal fraction was shown to be relatively more effective in the formation of adducts using *N*-hydroxy-FAA as substrate.

Distribution of enzyme activity in tissues of various species. Table 3 shows a comparison of the formation of nucleic acid adducts on incubation of N-GlO-FAA or its aglycone with tRNA and the liver microsomes of several species. With N-GlO-FAA as substrate, guinea pig liver microsomes were about four times as active as rabbit liver microsomes in inducing combination of fluorenylamine residues with tRNA. Rat liver microsomes were only marginally active and no activity was detected in either hamster or mouse microsomes.

In contrast to these results, when the aglycone *N*-hydroxy-FAA was used as substrate, rabbit liver microsomes were about one and a half times as active as guinea pig liver microsomes in forming fluorenylamine tRNA adducts. Also, rat liver microsomes were about three times as active in promoting nucleic acid adducts with *N*-hydroxy-FAA as substrate as they were with N-GlO-FAA as substrate.

The amounts of fluorenylamine nucleic acid adducts formed on incubation at pH 8.5 of N-GlO-FAA, tRNA and microsomes isolated from the spleen,

Table 2. Fluorenylamine-nucleic acid adduct formation on incubation of N-GlO-FAA or its aglycone, *N*-hydroxy-FAA, with tRNA and subcellular fractions of guinea pig liver

	Fluorenylamine bound to tRNA nmoles × 10^{1}*			
	N-GlO-FAA		*N*-hydroxy-FAA	
Liver cell fraction†	Per 100 mg tissue	Per mg protein	Per 100 mg tissue	Per mg protein
Whole homogenate	7.8	1.0	7.3	0.9
Nuclei	<0.1	<0.1	1.3	1.3
Mitochondria	0.2	0.3	0.7	0.9
Cytosol	0.3	0.1	2.0	0.5
Microsomes	4.9	4.9	1.0	1.0

* Values given for adduct formation were corrected by subtracting the binding obtained in control incubations with boiled microsomes.

† Fractionation of guinea pig liver was accomplished according to the method of Lotlikar, *et al.* [10].

The enzyme-induced adduct formation by guinea pig liver fractions was determined in 0.05 M Tris HCl buffer, pH 8.5, as described in Materials and Methods.

small intestine, colon, kidney, lung, stomach or bladder of the guinea pig or rat, or homogenates of human or rabbit bladder mucosa were not significantly different from control incubations containing boiled microsomes.

Partially purified arylhydroxamic acid acyltransferase [6], an enzyme which is present in the cytosol of tissues of a variety of species and is known to promote fluorenylamine-RNA adduct formation on incubation with *N*-hydroxy-FAA [14] did not induce adduct formation when incubated with N-GlO-FAA and tRNA at pH 7.0. Similarly, homogenates of hamster liver which contain high levels of acyltransferase [14] were also ineffective in promoting nucleic acid adduct formation when incubated with the *O*-glucuronide and tRNA at pH 8.5.

DISCUSSION

This study has demonstrated the enzymatic deacetylation of an *O*-glucuronide conjugate, N-GlO-FAA. The possibility that hydrolysis of this *O*-glucuronide conjugate to its aglycone, *N*-hydroxy-FAA, was occurring prior to enzymatic deacetylation and binding with tRNA was not supported by the data.

Table 3. Comparative formation of fluorenylamine nucleic acid adducts on incubation of N-GlO-FAA or its aglycone, *N*-hydroxy-FAA, with tRNA and the liver microsomes of several species

	Fluorenylamine bound to tRNA mg Protein nmoles × 10^{1}*	
Species	N-GlO-FAA	*N*-hydroxy-FAA
Guinea pig	5.6	4.2
Rabbit	1.3	6.6
Rat	<0.4	1.2
Mouse	<0.1	
Hamster	0.1	

* Values given for adduct formation were corrected by subtracting the binding obtained in control incubations with boiled microsomes.

The enzyme-induced adduct formation by liver microsomes was determined in 0.05 M Tris HCl buffer, pH 8.5, as described in Materials and Methods.

The dependence of enzyme-induced fluorenylamine nucleic acid adduct formation on pH was critical with N-GlO-FAA as substrate. The optimum pH for adduct formation was 8.5, and while incubations at pH 7.0 gave little adduct formation, incubations at pH 9.0 rapidly deacetylated the *O*-glucuronide and enhanced the yield of fluorenylamine-nucleic acid adducts even in enzyme-free controls. However, adduct formation with *N*-hydroxy-FAA as substrate was essentially independent of pH between 7.0 and 8.5. The pH optimum of β-glucuronidase in mammalian tissues is about 5 [12], and the possibility that enzyme-induced nucleic acid-adduct formation with N-GlO-FAA as substrate resulted from initial cleavage of the *O*-glucuronide to its aglycone by β-glucuronidase was unlikely. If this were the case, enzyme-induced nucleic acid adduct formation with the *O*-glucuronide would have been expected to be greater at pH 7.0, where β-glucuronidase activity would be higher, than at pH 8.5. Indeed, independent measurement in our laboratory of the ability of guinea pig liver microsomes to hydrolyze *p*-nitrophenyl-β-glucuronide [12] has shown that the β-glucuronidase activity was essentially abolished as the pH was increased from 7.0 to 8.5. Also, saccharo-1,4-lactone, an inhibitor of β-glucuronidase [12], did not decrease the amount of adduct formation when it was added at a concentration of 10^{-2} M to a mixture of N-GlO-FAA, tRNA and guinea pig microsomes at pH 8.5. Although it has previously been demonstrated that the efficiency with which saccharo-1,4-lactone inhibited β-glucuronidase activity decreased as the pH was increased, the net effect of the inhibitor was to inhibit the hydrolysis of the glucuronide conjugates in all cases [15].

The possibility of cleavage of N-GlO-FAA to *N*-hydroxy-FAA prior to enzyme-induced nucleic acid adduct formation was also diminished by the fact that 10^{-3} M *p*-chloromercuribenzoate inhibited adduct formation by 55 per cent when *N*-hydroxy-FAA was used as substrate, but had essentially no effect on nucleic acid adduct formation with the

O-glucuronide. If hydrolysis of N-GlO-FAA to *N*-hydroxy-FAA were an obligatory event for the activation of the *O*-glucuronide, then *p*-chloromercuribenzoate should also have inhibited adduct formation when the *O*-glucuronide was used as substrate.

In view of the preceding discussion, and the observation that *N*-ethylmaleimide and trisodium pentacyanoamine ferrate, at concentrations of 10^{-2} M, inhibited adduct formation only when the *O*-glucuronide was used as substrate, it appears that two distinct enzymes in guinea pig liver microsomes may be responsible for the activation of N-GlO-FAA and *N*-hydroxy-FAA. Irving [9] has demonstrated that trisodium pentacyanoamine ferrate slightly stimulated the deacetylation of *N*-hydroxy-FAA by guinea pig liver microsomes at pH 7.0. In our study trisodium pentacyanoamine ferrate was used in an attempt to inhibit adduct formation of *N*-hydroxy-FAA and tRNA by reaction with the deacetylated product, *N*-hydroxy-FA, if indeed it was formed. Since adduct formation was not inhibited, either the trisodium pentacyanoamine ferrate did not trap the *N*-hydroxy-FA at a rate sufficient to prevent its reaction with tRNA or some other intermediate was responsible for the introduction of fluorenylamine residues into tRNA when *N*-hydroxy-FAA was used as substrate.

Enzyme-induced nucleic acid–adduct formation with both substrates was inhibited by low concentrations of diethyl *p*-nitrophenyl phosphate. The enzymes in guinea pig liver microsomes which deacetylate *N*-hydroxy-FAA and FAA have been shown to be inhibited by low concentrations of this organic phosphorous compound [9, 16]. This suggests that the enzyme for the deacetylation of the *O*-glucuronide of *N*-hydroxy-FAA may be an esterase-type enzyme [9, 16].

Although steroid glucuronides have been shown to undergo metabolic transformation without hydrolysis of the carbohydrate moiety [17], analogous metabolic transformations of xenobiotic conjugates are less well known. The present study indicates that enzymatic action on the aglycone moiety of so-called detoxified conjugates, such as glucuronides, may have important pharmacological and toxicological implications.

The role of the enzymatic deacetylation of N-GlO-FAA in carcinogenesis is at present difficult to assess. The tissue distribution of the enzyme appears to be limited inasmuch as deacetylase activity has been demonstrated only in the liver of a few of the several species tested. Furthermore, the guinea pig, which is not susceptible to the induction of liver tumors by *N*-hydroxy-FAA [18], has much higher levels of this enzyme than does the rat, which is susceptible to the induction of liver tumors by *N*-hydroxy-FAA [19].

If the alteration of tissue macromolecules plays an important role in the induction of tumors by chemicals, the metabolic activation of aromatic amines and other carcinogens to reactive derivatives may be crucial to the carcinogenic process [20]. Although the conjugation of *N*-hydroxy-FAA with sulfate has been implicated in the development of liver tumors in the rat [21–23], this metabolic pathway is apparently not involved in the production of tumors in other tissues of the rat or other species [21, 24]. Enzymes capable of activating arylhydroxamic acids by N→O acyltransfer are widely distributed in tissues that develop tumors, as well as in tissues that have not been shown to be susceptible to carcinogenic aromatic amines [5, 6, 14, 25]. Still other tissues develop tumors but have not been shown to possess a metabolic activation system [14]. Consequently, it seems that no single metabolic pathway is uniquely associated with the carcinogenicity of these compounds.

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