Acetyl Coenzyme A Dependent Activation of N-Hydroxy Derivatives of Carcinogenic Arylamines: Mechanism of Activation, Species Difference, Tissue Distribution, and Acetyl Donor Specificity

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ABSTRACT

Acetyl coenzyme A dependent activation of 2-hydroxyaminino-6-methylidipyrido[1,2-a:3',2'-d]imidazole (N-OH-Glu-P-1) and 3-hydroxyaminino-1-methyl-5H-pyrido[4,3-d]imidazole (N-OH-Trp-P-2) was investigated using cytosols from hepatic and extrahepatic tissues of various animal species in comparison with that of N-hydroxy-2-aminofluorene. N-OH-Glu-P-1 and N-OH-Trp-P-2 were metabolized to the reactive species capable of binding to transfer RNA through a putative O-acetylation process by liver cytosols. Kidney, small intestinal mucosa, lung, and bladder from hamsters and rats also mediated the reaction, although their activities were lower than that in the liver. Marked species differences in the enzymatic activities of livers were observed. Hamsters showed the highest ability in the activation for N-OH-Glu-P-1 and N-OH-Trp-P-2, followed by rats. Rabbits with a slow acetylating phenotype, which showed a high activity in the N-acetylation of arylamines, activated N-OH-Glu-P-1 but scarcely N-OH-Trp-P-2. A rabbit with a slow acetylator phenotype, mice, guinea pigs, and a dog showed marginal or nondetectable activities with N-OH-Glu-P-1 and N-OH-Trp-P-2. A typical nonheterocyclic N-hydroxyarylamine, N-hydroxy-2-aminofluorene was also activated by the acetyl coenzyme A dependent system to an intermediate which bound to transfer RNA. However, the acetyl-CoA dependent binding of N-hydroxy-2-aminofluorene was markedly different from those observed with N-OH-Glu-P-1 and N-OH-Trp-P-2 concerning the order of activities among animal species used. In addition to short chain acyl coenzyme A, N-hydroxy-2-aminofluorene also served as an acetyl donor for the activation of N-OH-Glu-P-1 and N-OH-Trp-P-2 in liver cytosol systems. The formation of N-acetyl-N-OH-Glu-P-1, however, was not detected in the cytosolic system of N-OH-Glu-P-1 with acetyl-CoA, suggesting the direct O-acetylation at the N-hydroxy group as a major pathway for the activation of N-hydroxyarylamines.

INTRODUCTION

Carcinogenic-mutagenic heterocyclic arylamines isolated from the pyrolysates of amino acids and proteins, such as Glu-P-1 and Trp-P-2, have been shown to be metabolically activated to N-hydroxy derivatives by mammalian cytochrome P-450 (1-4). The results suggest that a sequential metabolic pathway, the N-acetylation of the arylamines, the N-hydroxylation of aminofluorenes, and the O-acetyl transfer of arylhydroxamic acids, as postulated for 2-AF would be less likely to occur for Glu-P-1 and Trp-P-2. Therefore, the O-acetylation of N-hydroxyarylamines may be an important pathway for the metabolic activation of these heterocyclic arylamines.

On the acetylation of arylamines, marked differences have been noted in both N-acetyltransferase and N-O-acetyltransferase activities among mammalian species and tissues (20, 21). In addition, genetic polymorphism in these activities has been recognized in rabbits, mice, hamsters, and humans (22, 28). The analysis of results with species and tissues in the activation of heterocyclic hydroxylamines, such as N-OH-Glu-P-1 and N-OH-Trp-P-2, by O-acetyltransferase can be important in order to identify the enzyme(s) responsible for the reaction and to clarify the activation mechanisms of these naturally occurring carcinogens. In this report, acetyl-CoA dependent activation of N-OH-Glu-P-1 and N-OH-Trp-P-2 was examined in comparison with that of N-OH-AF using various animal species, tissues, and acyl donors to clarify the characteristics of the metabolic activation pathway of these N-hydroxyarylamines.

MATERIALS AND METHODS

Chemicals. [ring-3H]N-OH-Glu-P-1 or [ring-3H]N-OH-Trp-P-2 (1.46 or 0.74 Ci/mmol) was freshly prepared metabolically from [ring-H]Glu-P-1 or [ring-H]Trp-P-2 using liver microsomes from polychlorinated biphenyl treated rats in the presence of NADPH (1, 4). The N-hydroxylated products were isolated by HPLC and were used after dilution with the corresponding nonradioactive N-hydroxyarylamines synthesized as described previously (29) to the specific activity of 30-50 mCi/mmol. [ring-3H]N-OH-AF (112 mCi/mmol) and [ring-3H]N-OH-AAF (20.4 Ci/mmol) were generous gifts from Dr. Fred F. Kadlubar, National Center for Toxicological Research, Jefferson, AR, and Dr. Snorre S. Thorgergson, National Cancer Institute, Bethesda, MD, respectively. [acetyl-3H]Acetyl-CoA (722.0 mCi/mmol) was obtained from New England Nuclear, Boston, MA; acetyl-CoA derivatives, tRNA (type X), and acetohydroxamic acid were from Sigma Chemical Co., St. Louis, MO; and glass microfiber filter (GF-C, 25 mm) was from Whatman, Kent, England. Other chemicals were of the highest grade commercially available.

Synthesis of N-Acetyl-N-hydroxy-Glu-P-1. N-OH-Glu-P-1 (10 mg), obtained from the reaction of the corresponding nitro derivative as described (29), was dissolved in 10 ml of dry tetrahydrofuran at 0°C. Acetyl chloride in tetrahydrofuran solution was added to the N-OH-Glu-P-1 solution. The reaction was monitored by silica gel thin layer...
and the solution was agitated for 30 min. The mixture was extracted twice with ethyl acetate (10 ml) after the addition of 5 N KOH (1 ml), and then 12 N sulfuric acid was added to change the pH of the solution to neutral. N-acyl-N-OH-Glu-P-1 in the aqueous phase was extracted with ethyl acetate and subjected to a preparative thin layer chromatography as described above. The product, N-acyl-N-OH-Glu-P-1 (R 0.39), was well separated in the thin layer chromatogram from Glu-P-1 (R 0.50) and N-acyl-Glu-P-1 (R 0.58). These compounds were also checked by the HPLC system described below. The product was reduced to N-acyl-Glu-P-1 by the treatment with titanium chloride and showed the absorption maxima at 275 and 361 nm (in methanol), respectively. In addition, the quasi-molecular and fragment ions were observed at m/z 257 [M + 1]+, 241 [M + 1-16]+, and 199 [M + 1-16-42]+ in the mass spectrum which was obtained in a direct inlet chemical ionization mode (Jeol D-300 mass spectrometer). Isobutane was used as a reagent gas.

Preparation of Cytosol. Male Sprague-Dawley rats, male Syrian golden hamsters, male Hartley guinea pigs, male and female BALB/CanN × DBA/2N F1, mice (6–8 weeks old), and female New Zealand White rabbits (1.5 years old), and a male Mongolian dog (4 months old) were used. The animals were decapitated and livers were perfused in situ with 50 mM Tris-HCl buffer (pH 7.4) and 1 mM DTT. Tissues were removed and homogenized with 3 volumes of the above buffer solution. The cytosol (105,000 × g supernatant fraction) was prepared as described previously (30). To determine the acetylator phenotype of the rabbits used, N-acetyltransferase activities of liver cytosols were measured using a polymeric macrast, 2-AF, and a monomorphous arylamine, p-aminobenzoic acid (22), as described previously (14). Among five rabbits examined, four animals showed high activities for 2-AF [12.1 ± 4.1 (SD) nmol/min/mg protein] and one individual showed nondetectable activity (<0.05 nmol/min/mg). Since all five rabbits showed similar activities for p-aminobenzoic acid (3.9 ± 0.2 nmol/min/mg), one individual having nondetectable activity for 2-AF was regarded as a slow acetylator.

Activation of N-Hydroxyarylamines: Nucleic Acid Binding Assay. Nucleic acid binding of N-hydroxyarylamines was assayed by the method of King (31) with modifications. The standard incubation mixture contained 50 mM Tris-HCl buffer (pH 7.0), 1 mM DTT, tRNA (1 mg/ml), 1 mM acetyl-CoA, 20 μM N-[3H]hydroxyarylamine, and cytosolic protein (0.1 mg/ml) in a final volume of 0.5 ml. Reactions were started by addition of N-hydroxyarylamine and incubated for 10 min at 37°C. The reaction was terminated by addition of 1 ml of phenol saturated with 50 mM Tris-HCl (pH 7.0). The mixture was shaken for 5 min and centrifuged at 2500 rpm for 10 min. An aliquot (100 μl) of aqueous layer was pipetted into 5 ml of ethanol containing 2% potassium acetate over a glass filter. The tRNA precipitate was collected on the filter and then washed with each 5 ml of 70% ethanol (3 times), ethanol (3 times), acetone, and diethyl ether. The filter was moistened with 150 μl of water in a counting vial. After addition of 1 ml of tissue solubilizer (Protosol; New England Nuclear) and 9 ml of xylene-based scintillator, the radioactivity was measured by a liquid scintillation counter (Beckman; LS-3800). The recovery of tRNA throughout the precipitation procedure was 95%. The assay was carried out in duplicate. The binding activity is calculated from the radioactivity associated to tRNA recovered from the incubation mixture and is expressed in terms of pmol of arylamine moiety bound to tRNA recovered per min per mg of protein (20).

Assay of the Reaction Products. For reaction with N-OH-Glu-P-1, the incubation mixture contained 50 mM Tris-HCl buffer (pH 7.0), 1 mM DTT, 1 mM [3H]acetyl-CoA, 20 μM N-OH-Glu-P-1, and cytosolic protein (0.2 mg/ml) in a final volume of 0.1 ml. The reaction was initiated by the addition of N-OH-Glu-P-1 and performed at 37°C for definite times (2, 5, 10, and 20 min). After the reaction was terminated by the addition of 0.1 ml of acetonitrile, cold N-acyl-N-OH-Glu-P-1 was added to the mixture as a carrier. The reaction products were extracted with ethyl acetate (2 ml), evaporated with nitrogen gas, and dissolved in 0.1 ml of acetonitrile. An aliquot (60 μl) was subjected to a HPLC (Waters Associates; ALC/GPC 204) equipped with a Nucleosil 5C18 reverse phase column (4 x 300 mm; Macherey-Nagel). A mobile phase of 40% acetonitrile containing 0.07% acetic acid and 0.07% acetoxyhydroxamic acid was used at a rate of 1.2 ml/min. The retention times of N-OH-Glu-P-1, N-acytethyl-N-OH-Glu-P-1, Glu-P-1, and N-acytethyl-Glu-P-1 were 9.6, 6.7, 11.5, and 9.4 min, respectively. The recovery of N-acytethyl-N-OH-Glu-P-1 from the HPLC peak was above 90%. Each fraction of eluent was collected and the radioactivity was determined by a liquid scintillation counter.

RESULTS

Acetyl-CoA Dependent Binding of N-Hydroxyarylamines by Hamster Liver Cytosol. The tRNA binding of N-OH-Glu-P-1 and N-OH-Trp-P-2 was dependent on both acetyl-CoA and liver cytosol of hamsters (Table 1). Previously, Mita et al. (32) reported on the nonenzymatic binding of N-OH-Trp-P-2 to DNA. Actually, both N-OH-Glu-P-1 and N-OH-Trp-P-2 bound to tRNA in the absence of DTT in the incubation mixture. Under the present experimental conditions containing 1 mM DTT, however, the amounts of tRNA binding of these N-hydroxyarylamines were very low without both cytosol and acetyl-CoA (Table 1). When acetyl-CoA was added in the incubations, 30% of complete binding was observed for N-OH-Trp-P-2 without cytosol, indicating that the acetyl-CoA dependent nonenzymatic binding occurred substantially. Non-enzymatic binding of N-OH-AF was much higher than that of N-OH-Trp-P-2. The amounts of binding were increased markedly by the addition of liver cytosol and acetyl-CoA with these N-hydroxyarylamines (complete system in Table 1). When a heat-denatured cytosol (treated at 100°C for 10 min) was added instead of fresh cytosol, these N-hydroxyarylamines were only sparsely bound to tRNA. The reaction was inhibited by iodoacetamide, a sulfhydryl blocking agent, but not by paraoxon, a microsomal deacetylase inhibitor (33, 34). The amount of Glu-P-1 moiety bound to tRNA measured as the radioactivity increased almost linearly with increases in the amounts of cytosolic protein from hamsters, up to 0.2 mg/ml (Fig. 14). Thus, all experiments described below were carried out at a protein concentration of 0.1 mg/ml. The amounts of binding also depended on the concentration of N-OH-Glu-P-1 added; the activity reached a plateau level at about 10 μM (Fig. 18). Similar profiles were also observed using rat liver cytosol for the activation of N-OH-Glu-P-1 or N-OH-Trp-P-2.

Acetyl-CoA dependent binding of N-OH-Glu-P-1 and N-OH-Trp-P-2 was examined using cytosols of several tissues of hamsters and rats (Table 2). Although the activity was the highest in a system with the liver, other tissues also catalyzed the reaction. The cytosols of kidneys in both hamsters and rats showed relatively high activities for N-OH-Glu-P-1. For the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Acetyl-CoA dependent binding of N-hydroxyarylamines by liver cytosol from hamsters</th>
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<tbody>
<tr>
<td>System</td>
<td>Amounts of metabolite bound to tRNA (pmol/min)*</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>N-OH-Glu-P-1</td>
<td>N-OH-Trp-P-2</td>
</tr>
<tr>
<td>Complete</td>
<td>32.7 ± 1.0</td>
</tr>
<tr>
<td>- Acetyl-CoA</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>- Cytosol</td>
<td>0.55 ± 0.13</td>
</tr>
<tr>
<td>- Cytosol + acetyl-CoA</td>
<td>0.22 ± 0.07</td>
</tr>
</tbody>
</table>

*p pmol arylamine moiety bound to tRNA recovered from incubation mixture per min.
Comparison of Acyl Donors. To ascertain cofactor specificity for the activation of N-hydroxyarylamines, the ability of several acyl donors was compared. As shown in Table 3, acetyl-CoA was the most effective followed by propionyl-CoA. Butyryl-CoA and malonyl-CoA showed low activities. N-OH-AAF is a known acetyl donor for mammalian N,O-acetyltransferase, which also served as an acetyl donor.

The effects of the concentration of acetyl-CoA or N-OH-AAF on tRNA binding by hamster liver cytosol were examined (Fig. 2). The amounts of Glu-P-1 moiety bound to tRNA increased with an increase in the concentration of acetyl-CoA up to 1.0 mM. The amount bound also increased with the increase in the concentration of N-OH-AAF up to 0.1 mM but tended to decrease in the range higher than 0.1 mM. A similar profile was observed with rat liver cytosol (data not shown). At 1 mM, the amounts of binding were higher with acetyl-CoA as an acetyl donor than with N-OH-AAF (Table 3). At acetyl donor concentrations lower than 0.1 mM, the amounts bound were higher with N-OH-AAF than that observed with acetyl-CoA in both hamsters and rats.

AAF was reported to be an inhibitor of N,O-acetyltransferase (31). In addition, N,O-acetyltransferase-dependent methylmercapto adduct formation of N-OH-AAF was observed to be enhanced by the addition of N-OH-FAF (35). In the present study, N-OH-AAF dependent binding of N-OH-Glu-P-1 was decreased at higher concentrations of N-OH-AAF (>0.1 mM). The reason is obscure, but it might be partially attributed to the competition between N-OH-Glu-P-1 and liberated N-OH-FAF and/or AAF from N-OH-AAF.

Species Differences in the Activation of N-Hydroxyarylamines by Liver Cytosol. The activities of liver cytosols from several animal species were compared. As shown in Table 4, marked species differences in the enzyme activity were observed. When N-OH-Glu-P-1 was used as a substrate, the highest activity was seen with hamsters, followed in order by rats and rabbits with a rapid acetylator phenotype, which showed high activity in the N-acetylation of 2-AF. Mice and guinea pigs showed very low activities. The slow acetylator rabbit. A large difference between rapid and slow acetylator rabbits was observed, and the dog did not show detectable activities. In the case of N-OH-Trp-P-2, the amounts of binding were smaller than those found with N-OH-Glu-P-1 but the order of activities among the species was similar, except that rabbits and mice showed nondetectable activities.

The activation of N-OH-FAF was measured under the same experimental conditions to ascertain the ability of the enzyme to activate nonheterocyclic N-hydroxyarylamine. The order of the activities among species was not correlated with that seen with N-OH-Glu-P-1 and N-OH-Trp-P-2 as described in Table 4. The highest activity was seen with rapid acetylator rabbits followed in order by hamsters, guinea pigs, mice, rats, and the slow acetylator rabbit. A large difference between rapid and slow acetylator rabbits was observed, and the dog did not show activity.

Species and tissue differences in N,O-acetyltransferase activity have been reported by King and Olive (20). The N,O-acetyltransferase activities of liver cytosols used in the present experiments were also measured (Table 4). The assay was carried out under the same experimental conditions as those

\[\text{Amount bound to RNA (pmol/min/mg protein)}\]

\[\begin{array}{c|c|c|c}
\text{Tissue} & \text{N-OH-Glu-P-1} & \text{N-OH-Trp-P-2} \\
\hline
\text{Hamster} & \\
\text{Liver} & 67.5 (100) & 120.3 (100) \\
\text{Lung} & 24.0 (3.5) & 3.6 (1.0) \\
\text{Kidney} & 94.6 (13.9) & 0.3 (0.2) \\
\text{Small intestinal mucosa} & 85.7 (12.6) & 13.4 (11.1) \\
\text{Bladder} & 12.8 (1.9) & 1.0 (0.8) \\
\hline
\text{Rat} & \\
\text{Liver} & 177.5 (100) & 85.6 (100) \\
\text{Lung} & 34.7 (19.5) & 8.7 (10.2) \\
\text{Kidney} & 57.7 (32.5) & 15.7 (18.3) \\
\text{Small intestinal mucosa} & 22.3 (12.6) & 32.4 (37.9) \\
\text{Bladder} & 25.5 (14.4) & 0.1 (0.1) \\
\end{array}\]

\[\text{Amount bound to RNA (pmol/min/mg protein)}\]

\[\begin{array}{c|c|c}
\text{Acyl donor} & \text{Hamster} & \text{Rat} \\
\hline
\text{Acetyl-CoA} & 566.8 (100) & 125.5 (100) \\
\text{β-Propionyl-CoA} & 219.9 (38.8) & 45.7 (36.4) \\
\text{α-Butyryl-CoA} & 41.7 (7.4) & 11.2 (8.9) \\
\text{Malonyl-CoA} & 32.6 (5.8) & 9.0 (7.2) \\
\text{N-OH-AAF} & 69.2 (12.2) & 28.1 (22.4) \\
\end{array}\]
ACETYL-CoA DEPENDENT ACTIVATION OF N-HYDROXYARYLAMINES

Table 4  Species differences in acetyl-CoA dependent binding by liver cytosol

<table>
<thead>
<tr>
<th>Species</th>
<th>Amounts of metabolite bound to tRNA (pmol/min/mg protein)</th>
<th>N.O-Acetyltransferase activity (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N-OH-Glu-P-1</td>
<td>N-OH-Trp-P-2</td>
</tr>
<tr>
<td>Hamster</td>
<td>656.4 ± 11.2</td>
<td>85.9 ± 31.0</td>
</tr>
<tr>
<td>Rat</td>
<td>1775.3 ± 35.8</td>
<td>62.7 ± 23.4</td>
</tr>
<tr>
<td>Rabbit*</td>
<td>Rapid</td>
<td>Slow</td>
</tr>
<tr>
<td></td>
<td>75.3 ± 24.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>Slow</td>
<td>Slow</td>
</tr>
<tr>
<td>Mouse</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>6.3 ± 0.7</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>1.0 ± 1.0</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>Dog</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

* The rabbits which showed polymorphism to the N-acetylation activity of 2-AF ("Materials and Methods").

for the binding assay to tRNA of N-hydroxyarylamines except
that 20 μM [3H]-N-OH-AAF was added instead of the N-
hydroxyarylamine and acetyl-CoA. The highest activity was
observed in hamsters, followed in order by rapid acetylator
rabbits and rats. The slow acetylator rabbit, mice, and the dog
did not show detectable activities.

Analysis of the Products Formed by the Reaction of N-Hydroxyarylamines with Acetyl-CoA and Liver Cytosols. To clarify
the pathway of the acetyl-CoA dependent activation of N-
hydroxyarylamines, metabolites were analyzed by HPLC. Typ-
cal chromatographic separation of the metabolites formed
upon the incubation of N-OH-Glu-P-1 with [3H]acetyl-CoA and
cytosols from hamsters is shown in Fig. 3. Radioactivity
was not coeluted with synthetic N-acetyl-N-OH-Glu-P-1
(peak A) added as a carrier, indicating that the formation of
N-acetyl-N-OH-Glu-P-1, if any, was too low to be detected. Glu-
P-1, a reduced product of putative N-acetoxy-Glu-P-1
in the presence of DTT (36), was formed (peak C). The amounts
of Glu-P-1 increased almost linearly with the increase of incuba-
tion time up to 10 min (data not shown). Small amounts of
radioactive product were eluted at peak B, at which the authen-
tic standards of N-OH-Glu-P-1 and N-acetyl-Glu-P-1 were
eluted, indicating that N-acetyl-Glu-P-1 was produced during
incubation. The formation of N-acetyl-Glu-P-1 was not linear
with incubation time but was observed after the appearance of
Glu-P-1, suggesting that N-acetyl-Glu-P-1 is formed mainly
through N-acetylation of Glu-P-1. The formation of Glu-P-1
and N-acetyl-Glu-P-1 was observed only in the presence of both
acetyl-CoA and cytosol. With liver cytosols from rats and
rabbits with rapid acetylator phenotype, Glu-P-1 formation was
observed but N-acetyl-N-OH-Glu-P-1 was not detected. The
amounts of Glu-P-1 formed were the largest with hamster
cytosols, followed by rats and rabbits (pmol/min/mg protein:
1180 with hamsters, 325 with rats, and 125 with rapid acety-
lator rabbits). N-Acetyl-Glu-P-1 was not detected with rat cy-
tosols during the period of incubation, since rat hepatic cytosols
were reported to show a low activity of N-acetylation of heter-
ocyclic arylamine (14).

Synthetic N-acetyl-N-OH-Glu-P-1 was incubated with ham-
ster cytosol and analyzed by HPLC. After incubation at 37°C
for 10 min, formation of Glu-P-1, N-OH-Glu-P-1, or N-acetyl-
Glu-P-1 and loss of N-acetyl-N-OH-Glu-P-1 by enzymatic
process were not observed. These results suggest that N-acetyl-
N-OH-Glu-P-1 is not a good substrate for cytosolic N-O-acetyl-
transferase in hamster liver.

DISCUSSION

The present studies clearly showed that N-hydroxyarylamines
are activated to the reactive species capable of binding to tRNA
by a cytosolic enzyme(s) in several animal tissues in the presence
of acetyl-CoA. Recently, acetyl-CoA dependent activation of
several N-hydroxyarylamines was reported by us (8, 13) and
Flammand et al. (37).

In the literature, highly electrophilic N-acetoxyarylamines
are reported to be produced by chemical reaction of N-hydroxy-
arylamines with acetic anhydride or ketene and are subse-
quently bound to nucleophiles (35, 38). N-Acetoxyarylamines
are produced enzymatically by N-O-acetyl transfer of arylhy-
droxyaromatic acids such as N-OH-AAF (15, 17, 31, 35, 38, 39).

The ultimate metabolites derived from N-hydroxyarylamines in
the present experiments are also assumed to be N-acetoxy-
arylamines formed by O-acetylation

Two pathways would be possible for the acetyl-CoA depend-
ent formation of N-acetoxyarylamines from N-hydroxyarylamines. One is direct O-acetylation of the hydroxy group and another is sequential N-acetylation and N,O-acetyl transfer. When N-OH-Glu-P-1 was incubated with acetyl-CoA and cytosols from hamsters, rats, or rabbits with rapid acylator phenotype, N-acetyl-N-OH-Glu-P-1 was not detected by HPLC (Fig. 3). To ascertain the absence of N-acetyl-N-OH-Glu-P-1 in the acetyl-CoA supported system, a portion of the incubation mixture was treated with TICl (40, 41). The amounts of Glu-P-1 were increased by the reduction of N-OH-Glu-P-1 but no increase in the amounts of N-acetyl-Glu-P-1 was observed. This result also supports the lack of N-acetyl-N-OH-Glu-P-1 formation (data not shown). In addition, synthetic N-acetyl-N-OH-Glu-P-1 was not a substrate for cytosolic N,O-acetyltransferase as was a certain arylhydroamic acid (37). Thus, the reaction through the formation of arylhydroamic acid is unlikely to be the case for acetyl-CoA dependent activation of N-OH-Glu-P-1, and the direct O-acetylation at the N-hydroxy group is conceivable for the mechanism.

Acetyl-CoA dependent binding of N-OH-AF, but not N,O-acetyl transfer activity of N-OH-AAF, was observed with slow acylator rabbit, mouse, and guinea pig cytosol (Table 4). Although the N,O-acetyl transfer mechanism could not be excluded, these results suggest the role of a direct O-acetylation pathway for metabolic activation of N-OH-AAF.

Among the animal species used, the highest activity for the activation of N-OH-Glu-P-1 and N-OH-Trp-P-2 was observed in liver cytosol of hamsters, which showed high activities of either N,O-acetyl transfer (Table 4) or N-acetylation of Trp-P-2 and 2-AF (14). Although the rate of N-acetylation of Trp-P-2 by hamster cytosol was about 100-fold slower than that of 2-AF (14), no such marked species difference was observed in the activation of the corresponding N-hydroxyarylamines, N-OH-Trp-P-2 and N-OH-AF. The rat cytosol, which showed relatively high activities in the activation of N-hydroxyarylamines, also exhibited high N,O-acetyl transfer activity (Table 4) but very low activity in N-acetylation of aryamines (14). These results indicate that the relative activities of several types of acetyl transfer reactions are different among species, by substrate used and by type of reaction.

In rabbits, genetic polymorphism in both N,O-acetyltransferase and N-acetyltransferase has been reported (17, 22, 26). We have noted that similar polymorphism is seen in the N-acetylation of heterocyclic amines such as Trp-P-2 (13, 14). In the present experiments, the rapid acylator rabbits showed much higher activities in the activation of N-OH-Glu-P-1 and N-OH-AAF than did the slow one (Table 4), indicating that this activation reaction has a similar polymorphism. N-OH-Trp-P-2, however, was not activated by cytosols from all rabbits. The dog, which did not possess detectable activities in either N,O-acetyl transfer or N-acetylation reactions, was also deficient in any activity for the activation of N-hydroxyarylamines.

The properties of an enzyme(s) in hamsters and rats catalyzing the activation of N-hydroxyarylamines are consistent with those of N,O-acetyltransferase (15–17, 19, 20) or N-acetyltransferase (16, 17, 21, 22), in terms of being inhibited by sulfhydryl inhibitors and unaffected by an esterase inhibitor, paraoxon.

N-Acetyltransferase and arylhydroamic acid N,O-acetyltransferase were reported to be the properties of the same enzyme in rabbit liver (17). We have recently purified an acetyl-CoA dependent N-hydroxyarylamine activating enzyme to an electrophoretic homogeneity from hamster liver cytosol, which also catalyzes N-acetylation of aryamines and N,O-acetyl transfer of N-OH-AAF (42). In the N-acetylation reaction, a Ping Pong Bi-Bi mechanism has been postulated, i.e., acetyl residue is first transferred to enzyme to form an acetylated enzyme intermediate and then is transferred from the acetylated enzyme to substrate (43). Intermolecular transfer of acetyl group from arylhydroamic acid to aryamines (44, 45) or N-hydroxyarylamines (35, 44) by rat tissues has also been noted. The presence of the enzyme catalyzing various types of acetyl transfer reactions in hamster liver may be explained by postulating that the enzyme accepts an acetyl group from acetyl-CoA or N-OH-AAF to form acetylated enzyme and transfers the acetyl group to the O-atom of N-hydroxyarylamines or the N-atom of aryamines or N-hydroxyarylamines.

In our recent experiment, we have purified another enzyme from hamster liver which catalyzes N-acetylation of aryamines but not acetyl-CoA dependent activation of N-OH-Glu-P-1. Species and substrate related differences observed in the acetylation reactions may be due to the presence of an enzyme catalyzing various types of acetyl transfer reactions with different affinities for acetyl donors and substrates, and/or due to the presence of multiple acetyltransferases in each animal species.

The present data indicate a possible involvement of the direct O-acetylation pathway in the carcinogenesis of aryamines, especially Glu-P-1 and Trp-P-2. Further detailed studies on the O-acetylation pathway of N-hydroxyarylamines will be needed for the clarification of species and tissue specificity in carcinogenesis by these heterocyclic aryamines (3, 46, 47) in relation to other metabolic activation and detoxication pathways.

REFERENCES


ACETYL-CoA DEPENDENT ACTIVATION OF N-HYDROXYARYLAMINES


Acetyl Coenzyme A Dependent Activation of N-Hydroxy Derivatives of Carcinogenic Arylamines: Mechanism of Activation, Species Difference, Tissue Distribution, and Acetyl Donor Specificity

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