Catalysis of the Covalent Binding of 3-Hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole to DNA by a L-Proline- and Adenosine Triphosphate-dependent Enzyme in Rat Hepatic Cytosol

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ABSTRACT

An enzymatic mechanism involved in the activation of 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole (N-hydroxy-Trp-P-2), a mutagenic intermediate of a tryptophan pyrolysate, was studied in vitro. In hepatic cytosol supplemented with adenosine triphosphate and L-proline, N-hydroxy-Trp-P-2 was converted to a form which reacts readily with DNA. The enzyme responsible for the activation was partially purified and identified as prolyl transfer RNA synthetase as judged by their cofactor requirements, inhibition by pyrophosphate or adenosine monophosphate, and copurification of their activities. The prolyl transfer RNA-dependent covalent binding of N-hydroxy-Trp-P-2 to DNA of hepatic cytosol was highest in rats, followed by mice, hamsters, rabbits, and guinea pigs in that order. The capacity for the binding of N-hydroxy-Trp-P-2 was largely consistent with their prolyl transfer RNA synthetase activity.

With regard to the ultimate form of N-hydroxy-Trp-P-2 for the covalent binding, a possible formation of N,O-prolyl-3-amino-1-methyl-5H-pyrido[4,3-b]indole was proposed.

INTRODUCTION

The esterification of some proximate carcinogens to the corresponding sulfates, acetates, and glucuronides is thought to be necessary to show their mutagenicities and to initiate neoplastic lesions through covalent interaction with nucleic acids (9, 12). Several enzymes involving sulfotransferases and O,O-acyltransferases have been known to exist in hepatic cytosol of mammalian livers. These enzymes produce reactive species and consequently participate in the covalent binding of N-substituted aryl compounds. During studies on the activating mechanism of 4-nitroquinoline 1-oxide, Tada and Tada (20, 21) found that the N-hydroxy intermediate, 4-hydroxyaminoquinoline 1-oxide, was metabolically transformed by serinyl-tRNA synthetase to a form which reacts readily with nucleic acids. The unique activating system had once been considered to be specific for 4-hydroxyaminoquinoline 1-oxide, but thereafter another active intermediate of a tryptophan pyrolysate, N-hydroxy-Trp-P-2, was demonstrated to be converted to a reactive form for the covalent binding by serinyl-tRNA synthetase purified from yeast (24). Hashimoto et al. (3) also suggested that the same enzyme participated in the activation of some N-hydroxy-4-aminoazobenzene dyes. With hepatic cytosol of rats, recent studies in our laboratory indicated that some aminocacyl-tRNA synthetases or related enzymes catalyzed the covalent interaction of N-hydroxy-Trp-P-2 to DNA (23). These results suggest the possible role of this system in mammals for the activation of N-hydroxy arylamines. Thus, in the present study, the enzymatic properties of the amino acid-dependent activation system have been studied using partially purified enzyme obtained from rat hepatic cytosol.

MATERIALS AND METHODS

Chemicals. L-Amino acids, piccocolic acid, and thioproline were obtained from Wako Pure Chemicals, Osaka, Japan, and d-proline, 3,4-dehydro-L-proline, L-prolinamide, and methyl-, butyl-, and tert-butyl esters of L-proline were from Sigma Chemical Co., St. Louis, MO. N-Hydroxy-Trp-P-2, N-hydroxy-2-aminofluorene, 4-hydroxyaminobiphenyl, and 4-hydroxyaminoacetophenone were synthesized by the method using zinc-NH₄Cl or hydrazine:palladium-charcoal for the reduction of the respective nitro compounds (7, 19, 25). [³H]-N-hydroxy-Trp-P-2 was synthesized metabolically from [³H]-Trp-P-2 (specific activity, 344 mCi/mmol) using liver microsomes from polychlorinated biphenyl-treated rats (22, 23). tRNA was purified from rat livers by the method of Russev (17) with minor modifications. L-[³¹C]Proline (specific activity, 283.0 mCi/mmol) was purchased from New England Nuclear, Boston, MA.

Preparation of Cytosol and Purification of Prolyl-tRNA Synthetase.

Adult male Sprague-Dawley rats (6 to 8 weeks old), Syrian golden hamsters, Hartley guinea pigs, and adults of both sexes of BALB/cAnN x DBA/2N (hereafter called CD2F₁) mice, and New Zealand White rabbits were used. Hepatic cytosol was prepared as described (22), except that 2 parts of 50 mm Tris acetate (pH 7.4) containing 0.25 m sucrose and 1 mm DTT were added to 1 part of liver weight. Partial purification of prolyl-tRNA synthetase was performed essentially by the method of Fraser and Klass (2). Briefly, cold 1 m acetic acid was added to liver cytosol to adjust the pH to 5.0. Precipitates formed were separated by centrifugation at 10,000 × g for 5 min and then dissolved in a minimum amount of 10 m NH₄-bis(2-hydroxyethyl)glycine:potassium hydroxide containing 1 m magnesium acetate, 20% glycerol, and 1 m DTT (Buffer A). The precipitates (pH 5 enzyme) were stored at −80°C after adjustment of the pH to 7.8 with 1 m potassium hydroxide. To the pH 5 enzyme, ATP was added to a final concentration of 1 mm. The mixture was treated for 5 min at 55°C and centrifuged at 10,000 × g for 5 min to obtain the supernatant (heat-treated supernatant). Saturated ammonium sulfate solution (pH 7.4) was added to an equal volume of the heat-treated supernatant, and the mixture stood for 10 min at 4°C. The pellet, obtained after centrifugation at 10,000 × g for 5 min, was dissolved with Buffer A. The supernatant was mixed with the saturated ammonium sulfate to give the 80% saturated solution. The precipitates [50 to 80% (NH₄)₂SO₄ fraction] were dissolved in Buffer A after centrifugal separation. The resultant supernatant was dialyzed against Buffer A. Protein concentration was determined by the method of Lowry et al. (10) with bovine serum albumin as the standard.
Incubation Mixture. A typical incubation mixture for the measurement of the covalent binding of Trp-P-2 moiety to DNA consisted of 50 mM potassium-N′-bis(hydroxyethyl)glycine buffer (pH 8.0), 1 mM ATP, 1 mg of calf thymus DNA, 3 mM magnesium acetate, 1 mM L-proline, 1 mM DTT, 3 to 6 μM [3H]N-hydroxy-Trp-P-2, and 0.25 or 1.0 mg of protein of hepatic cytosol, the pH 5 enzyme, or further purified preparation in a final volume of 1 ml. The reaction was started by the addition of [3H]-N-hydroxy-Trp-P-2 dissolved in 50 μl of 20 mM sodium acetate (pH 4.5) and was performed for 30 min at 37°C. To terminate the reaction, 2 ml of ethyl acetate and 1 ml of 1 M sodium acetate were added. After centrifugation, the organic layer was aspirated off, and 2 ml of phenol saturated with 0.1 M Tris acetate (pH 7.4) were added to the aqueous layer. A 1.5-ml portion of the aqueous layer was reextracted with an equal volume of the phenol reagent. To a 1.2-ml portion of the resultant aqueous layer, a 2-fold volume of cold ethanol was added, and the precipitated DNA was washed with 2 ml of ethanol. To a tube containing DNA, a solution (0.4 ml) was added containing 0.15 M sodium chloride; 0.015 M sodium citrate (pH 7.5), and 0.1 mM L-[14C]proline (0.26 μCi). The RNase A solution had been treated previously in a bath at 80°C for 10 min. The mixture was incubated for 1 h at 37°C. The reaction was terminated by addition of 1 ml of chloroform-isooamyl alcohol mixture (24:1, v/v) and 0.5 ml of a solution containing 0.15 M sodium chloride, 0.015 M sodium citrate, 0.1 M EDTA, and 2% sodium dodecyl sulfate. After centrifugation, a 2-fold volume of cold ethanol was added to an 0.8-ml portion of the resultant aqueous layer. The precipitates formed were washed with ethanol and dissolved in 1 ml of 0.5 M sodium acetate. The solution was extracted with an equal volume of ethyl acetate. The radioactivity and DNA content were measured with a liquid scintillation counter (22) and by the method of Burton and Petersen (1), respectively.

Prolyl-tRNA Synthetase Activity. The assay mixture consisted of 2 mM ATP (pH 7.0), 10 mM potassium chloride, 5 mM magnesium acetate, 0.1 M Tris hydrochloride (pH 7.5), 0.1 mM L-[3H]proline (0.26 μCi), 10 absorbance units at 260 nm of tRNA obtained from rat livers, and 0.025 to 0.1 mg of protein of cytosol or purified preparations in a final volume of 0.1 ml. The reaction was started by addition of the enzyme preparation and incubated for 10 min at 37°C. An 80-μl portion of the mixture was impregnated to a filter paper (25 mm in diameter; Whatman MM). After the filter was washed with 5% trichloroacetic acid twice and then once again with diethyl ether-ethanol (1:1), it was immersed in diethyl ether for 5 min. Radioactivity in the dried filter was counted with a Beckman Model LS 3800 liquid scintillation counter.

RESULTS

Effects of Protein Concentration and pH of the Incubation Mixture on the Covalent Binding of [3H]-N-Hydroxy-Trp-P-2. Hepatic cytosol catalyzed the formation of reactive intermediate from N-hydroxy-Trp-P-2 in the presence of ATP and L-proline. Covalent binding to calf thymus DNA was increased curvilinearly with the increase in the amount of cytosolic protein (Chart 1). ATP was an essential cofactor for the reaction. The rate of the binding was increased by the addition of ATP up to 1 mM (Chart 2). ATP caused the binding at a low but significant rate. Another cofactor, L-proline, was also necessary for the maximal binding (Chart 3). The effect of pH on the rate of the covalent binding was also examined. The binding was optimum at pH 7.75 to 8.0 (Chart 4). The rate was decreased with the decrease in the pH of the reaction mixture. The activity when the pH was higher than 8.25 was not examined because of the lability of N-hydroxy-Trp-P-2 in the alkaline ranges. The rate of binding to DNA was also dependent on the concentration of N-hydroxy-Trp-P-2. The binding was linearly increased from 0 to 6 μM (not shown).

Partial Purification of the Proline-dependent Activating Enzyme and Prolyl-tRNA Synthetase. Results with hepatic cytosol suggest that prolyl-tRNA synthetase mediates the activation of N-hydroxy-Trp-P-2 to an ultimate form for the covalent binding. Prolyl-tRNA synthetase has not been purified to homogeneity from mammalian tissues to our knowledge, although the partial purification of this enzyme from rat livers has been reported (2). Thus, we initiated the purification of the proline-dependent activating enzyme using the method described in Table 1.

The activity transferring L-proline to tRNA was found mainly in the precipitates formed at pH 5 (pH 5 enzyme). The specific
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activity was further increased by the treatments described in Table 1. In the 50 to 80% (NH₄)₂SO₄ fraction, 14.5% of the activity of prolyl-tRNA synthetase was recovered with 10.3-fold purification. In accordance with the increase in the activity of prolyl-tRNA synthetase, the specific activity to catalyze the covalent binding of N-hydroxy-Trp-P-2 to DNA was also increased as shown in Chart 5.

With the use of the pH 5 enzyme and the 50 to 80% (NH₄)₂SO₄ fractions, the properties of the enzyme were examined in detail. As seen with the cytosol system, ATP was an essential requirement for the covalent binding of Trp-P-2 moiety to DNA (Table 2). Magnesium ion was required for the maximal activity in a system containing the 50 to 80% (NH₄)₂SO₄ fraction. In addition, the binding was diminished to a 5% level of the complete system in the absence of proline. Apparent K_m values for L-proline and ATP in the binding reaction were calculated to be 93 and 48 μM, respectively, in Lineweaver-Burk plots. The K_m for ATP was largely consistent with that for prolyl-tRNA synthetase activity (30 μM). Comparing the specificity of nucleotides as the cofactor,

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**Table 1**

Partial purification of prolyl-tRNA synthetase from rat livers

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Relative purification (-fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>105,000 x g supernatant</td>
<td>293.4</td>
<td>25,086</td>
<td>85.5</td>
<td>1</td>
</tr>
<tr>
<td>pH 5 precipitate fraction</td>
<td>65.4</td>
<td>17,897</td>
<td>270.6</td>
<td>3.16</td>
</tr>
<tr>
<td>(pH 5 enzyme)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat-treated supernatant</td>
<td>59.2</td>
<td>16,421.3</td>
<td>277.2</td>
<td>3.24</td>
</tr>
<tr>
<td>50–80% (NH₄)₂SO₄ fraction</td>
<td>4.2</td>
<td>3,646.7</td>
<td>876.6</td>
<td>10.25</td>
</tr>
</tbody>
</table>

* Activity unit was defined as pmol proline bound to tRNA/10 min. Prolyl-tRNA synthetase activity was measured as described in "Materials and Methods."

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**Table 2**

Requirements for the covalent binding of N-hydroxy-Trp-P-2 to DNA

<table>
<thead>
<tr>
<th>System</th>
<th>pmol/mg DNA/30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>214.8 (100)</td>
</tr>
<tr>
<td>- ATP</td>
<td>2.6 (1.2)</td>
</tr>
<tr>
<td>- Magnesium ion</td>
<td>ND</td>
</tr>
<tr>
<td>- L-Proline</td>
<td>10.7 (5.0)</td>
</tr>
<tr>
<td>- Enzyme</td>
<td>2.4 (1.1)</td>
</tr>
<tr>
<td>50–80% (NH₄)₂SO₄ fraction</td>
<td>657.4 (100)</td>
</tr>
<tr>
<td>- ATP</td>
<td>19.8 (3.0)</td>
</tr>
<tr>
<td>- Magnesium ion</td>
<td>102.8 (15.6)</td>
</tr>
<tr>
<td>- L-Proline</td>
<td>ND</td>
</tr>
<tr>
<td>- Enzyme</td>
<td>5.6 (0.9)</td>
</tr>
</tbody>
</table>

* Complete system consisted of 0.25 mg of protein of the pH 5 fraction or the 50 to 80% (NH₄)₂SO₄ fraction, 1 mM ATP, 1 mM L-proline, 4.0 μM N-hydroxy-Trp-P-2, 1 mg of calf thymus DNA, 3 mM magnesium acetate, 50 mM potassium-N,N'-bis(2-hydroxyethyl)glycine buffer (pH 8.0) in a final volume of 1 ml.

Numbers in parentheses, percentage of the respective control. ND, not determined.
the covalent binding was the highest with ATP; only <10% of the level was obtained with GTP, CTP, or UTP (Table 3). ADP, which was one-half as active as ATP for prolyl-tRNA synthetase activity (data not shown), also supported the covalent binding at a corresponding rate. AMP was not the cofactor but rather the inhibitor in a dose-dependent manner.

Among 21 amino acids examined, L-proline was the almost exclusive requirement for the covalent binding, although only trace amounts of the binding were detected with L-hydroxyproline or L-asparagine (Chart 6).

**Structural Requirement of L-Proline for the Covalent Binding.** Effects of several proline analogues were examined to ascertain the structural requirement of proline for the binding of N-hydroxy-Trp-P-2 to DNA (Table 4). In a system using *Escherichia coli* prolyl-tRNA synthetase, 3,4-dehydro-DL-proline was reported to inhibit competitively the transfer of L-proline to tRNA and also to act as a cofactor of the ATP-pyrophosphate exchange reaction (15).

In a system containing the pH 5 enzyme, 3,4-dehydro-DL-proline supported the covalent binding at a higher rate than did L-proline. However, p-proline and other proline-related compounds examined were inactive for the binding. These results were largely consistent with the data for the ATP-pyrophosphate exchange reaction reported for proline-tRNA synthetase purified from *E. coli* (16).

**Effects of Various Chemicals on the Covalent Binding of [3H]-N-Hydroxy-Trp-P-2 to DNA.** The covalent binding of Trp-P-2 molecule to DNA was inhibited by 50 or 250 μM pyrophosphate but not by phosphate (Table 5). Sulfate ion also decreased the binding rate. Ferrous ion, which reacts with ATP or hydroxamic acids to form complexes, effectively reduced the amount of the binding.

**Species Difference in the Rate of Binding to DNA.** Species difference in the capacity to form a reactive intermediate for DNA binding was compared using hepatic cytosols from mice, rats, hamsters, rabbits, and guinea pigs. As shown in Table 6, rats showed the highest activity, followed by mice, hamsters, rabbits, and guinea pigs in that order. No significant sex-related difference was observed in rabbits and mice.

To determine whether a low level of activity in hepatic cytosols of guinea pigs is due to the presence of an inhibitor, the guinea pig cytosol was added to the incubation mixture containing the rat liver cytosol. The activity in the rat cytosol was not decreased; a higher binding was observed with both cytosols than with rat cytosol alone or rat cytosol plus heat-denatured guinea pig cytosol.
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Tables

Effects of various chemicals on the pH 5 enzyme-dependent covalent binding of N-hydroxy-Trp-P-2 to DNA

The incubation mixture consisted of 1 mg of cytosol, 1 mM ATP, 1 mg of calf thymus DNA, 1 mM L-proline, 5.79 µM N-hydroxy-Trp-P-2, and other necessary components described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Concentration (µM)</th>
<th>Covalent binding (pmol/mg DNA/30 min)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium pyrophosphate</td>
<td>50</td>
<td>576.5</td>
<td>71.9</td>
</tr>
<tr>
<td>Sodium phosphate</td>
<td>100</td>
<td>796.5</td>
<td>96.7</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>100</td>
<td>879.5</td>
<td>77.3</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>100</td>
<td>325.1</td>
<td>31.8</td>
</tr>
</tbody>
</table>

Experiment 2

None

N-Hydroxy-4-aminobiphenyl (50) 774.2 86.7
N-Hydroxy-2-aminofluorene (50) 538.7 60.3
N-Hydroxy-4-aminoacetophenone (50) 872.2 97.7
4-Hydroxyaminoquinolone 1-oxide (50) 824.1 92.3
Trp-P-2 (50) 702.2 81.1
2-Aminofluorene (50) 929.5 104.1

Chemicals

Concentration (µM) Covalent binding (pmol/mg DNA/30 min) %

Experiment 1

None

Sodium pyrophosphate (50) 1020.8 100
Sodium phosphate (100) 586.7 57.5
Potassium chloride (100) 897.4 96.7
Sodium sulfate (100) 789.5 77.3
Ferrous sulfate (100) 325.1 31.8

Chemicals

Concentration (µM) Covalent binding (pmol/mg DNA/30 min) %

Experiment 2

None

N-Hydroxy-4-aminobiphenyl (50) 774.2 86.7
N-Hydroxy-2-aminofluorene (50) 538.7 60.3
N-Hydroxy-4-aminoacetophenone (50) 872.2 97.7
4-Hydroxyaminoquinolone 1-oxide (50) 824.1 92.3
Trp-P-2 (50) 702.2 81.1
2-Aminofluorene (50) 929.5 104.1

Methanol (10 µl) was used as a vehicle.

cytosol (data not shown). Thus, it was confirmed that species differences in activity were not caused by an inhibitory factor. Supporting this observation, the capacity for the binding of N-hydroxy-Trp-P-2 of these 4 species was largely consistent with their prolyl-tRNA synthetase activities.

DISCUSSION

In the system containing the pH 5 enzyme or the 50 to 80% (NH₄)₂SO₄ fraction, L-proline was almost the sole amino acid among 21 amino acids to support the covalent binding of Trp-P-2 molecule to DNA (Chart 6). ATP and magnesium ion were also necessary, while triphosphate nucleotides other than ATP were inactive for covalent binding (Table 3). In addition, covalent binding was inhibited by the addition of AMP or pyrophosphate. These results, together with the results of copurification of prolyl-tRNA synthetase and the activating ability for N-hydroxy-Trp-P-2, strongly support the idea that prolyl-tRNA synthetase catalyzes the formation of a reactive intermediate(s) from N-hydroxy-Trp-P-2 which reacts readily with DNA.

L-Prolyl-tRNA synthetase, which catalyzes a transfer of L-proline to proline-specific tRNA, is an essential enzyme for the protein synthesis. The enzyme requires ATP and L-proline as cofactors (Chart 7). The reaction is thought to proceed through the formation of an intermediate L-prolyl-AMP:enzyme complex, liberation of pyrophosphate, and then a transfer of L-prolyl group to tRNA (8).

Although the reaction mechanism involved in the covalent binding of N-hydroxy-Trp-P-2 to DNA is not clear as yet, the rate of the covalent binding was decreased by the addition of tRNA obtained from rat livers but not from E. coli. (data not shown). Thus, this reaction is thought to go through the formation of a L-prolyl-AMP:enzyme complex and then of the reactive intermediate from N-hydroxy-Trp-P-2. Amino acid hydroxamates are known to be formed by addition of hydroxylamine to the reaction mixture containing aminoacyl-tRNA synthetase (4) and suggested that acylation of hydroxylamine occurs initially in the oxygen atom of hydroxylamine to yield O-acylhydroxylamine; this is then rearranged to form stable O-acylhydroxylamine (5, 6). These results might implicate the formation of N,O-prolyl-Trp-P-2 as a possible reactive intermediate of N-hydroxy-Trp-P-2.

In a preliminary experiment on the reactivity of the reactive

Table 6

Covalent binding of N-hydroxy-Trp-P-2 to DNA by hepatic cytosols from various animal species in the presence of ATP and L-proline

Incubation systems for the assay of covalent binding of the Trp-P-2 molecule to DNA and for the assay of prolyl-tRNA synthetase activity were the same as described in "Materials and Methods," except that the amounts of N-hydroxy-Trp-P-2 were 5.70 and 3.72 µM in Experiments 1 and 2, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Sex</th>
<th>No. of animals</th>
<th>Covalent binding to DNA (pmol/mg DNA/30 min)</th>
<th>Cytosolic prolyl-tRNA synthetase activity (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Rat</td>
<td>Sprague-Dawley</td>
<td>M</td>
<td>5</td>
<td>207.8 ± 15.0 (100)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>CD2F₁</td>
<td>M</td>
<td>6</td>
<td>157.0 ± 14.3 (75.6)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>CD2F₂</td>
<td>F</td>
<td>6</td>
<td>151.2 ± 23.4 (72.8)</td>
</tr>
<tr>
<td></td>
<td>Hamster</td>
<td>Syrian golden</td>
<td>M</td>
<td>4</td>
<td>117.4 ± 17.0 (56.5)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Rat</td>
<td>Sprague-Dawley</td>
<td>M</td>
<td>5</td>
<td>185.2 ± 14.5 (100)</td>
</tr>
<tr>
<td></td>
<td>Guinea pig</td>
<td>Hartley</td>
<td>M</td>
<td>5</td>
<td>34.0 ± 1.4 (18.4)</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>New Zealand White</td>
<td>M</td>
<td>2</td>
<td>50.7 (27.4)</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>New Zealand White</td>
<td>F</td>
<td>3</td>
<td>41.8 ± 3.2 (22.6)</td>
</tr>
</tbody>
</table>

Mean ± SD.
Numbers in parentheses, percentage of the respective control.
ND, not determined.
intermediate toward nucleotide bases, we observed higher rates of the covalent binding to polyguanylic acid (453 pmol/mg polyguanylic acid/mg protein/30 min) than to polyadenylic acid (24 pmol/mg polyadenylic acid/mg protein/30 min) with the p5 enzyme system, suggesting that the binding occurred with the guanine moiety of polynucleotides.

In rats, the transformation by hepatic cytosol of N-hydroxy-Trp-P-2 to the ultimate metabolite for the covalent binding was not enhanced by pretreatment with phenobarbital or polychlorinated biphenyls. As described in Table 6, the activities of hepatic cytosols differ considerably among rat, mouse, hamster, guinea pig, and rabbit. Although a higher incidence of tumors has been noted in female than in male CD2F1 mice (11), no significant difference was observed between both adult sexes for the specific activity of prolyl-tRNA synthetase for covalent binding of Trp-P-2 molecule to DNA.

N-Hydroxy-Trp-P-2 was reported to bind to DNA nonenzymatically (13, 14), but sulfhydryl compounds, such as DTT and glutathione, and/or cytosol markedly decreased the nonenzymatic binding (data not shown). These results indicate the importance of enzymatic process in the activation of N-hydroxy-Trp-P-2 in vivo. On the other hand, we observed that the binding of N-hydroxy-Trp-P-2 was enhanced by acetyl-CoA-dependent enzymatic acetylation (18, 23) and by nonenzymatic mechanisms in the presence of ATP and acetyl-CoA. Since the covalent binding of N-hydroxy-Trp-P-2 is affected by factors mentioned above, further studies are necessary for evaluation of the significance of these pathways.

REFERENCES


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