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ENHANCEMENT OF BINDING OF N-HYDROXY-TRP-P-2 TO DNA BY SERYL-tRNA SYNTHETASE

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SUMMARY: Covalent binding to DNA of a mutagenic metabolite of Trp-P-2, N-hydroxy-Trp-P-2, was examined in the presence of seryl-tRNA synthetase. Both ATP and L-serine were essential requirements for this binding. In the absence of seryl-tRNA synthetase, the binding was reduced to about 14% of the complete system. These results indicate that seryl-tRNA synthetase which is widely distributed in tissues of experimental animals might act as the activating enzyme of N-hydroxy-Trp-P-2.

3-Amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) is a compound which was isolated from tryptophan-pyrolysis products once was found to be a causative substance of bacterial mutagenicity (1,2). Trp-P-2 is contained in grilled foods (3). Moreover, Matsukura et al. (4) recently demonstrated the tumourigenicity of Trp-P-2 to mice.

In our experiments using microsomal systems, we found that Trp-P-2 was N-hydroxylated to the 3-N-hydroxyl derivative (N-hydroxy-Trp-P-2). This metabolite isolated by HPLC was highly mutagenic to Salmonella typhimurium TA98, without the addition of activating enzymes (5).

Covalent bindings of chemicals to macromolecules, especially to DNA, are considered to be critical events for their carcinogenicities. Most carcinogenic amines require further enzymatic activations for their binding to DNA and

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RNA after their N-hydroxylations. Among the several activation pathways, Tada and Tada (6) reported an unique activation mechanism of an active intermediate of 4-nitroquinoline 1-oxide, 4-hydroxyaminoquinoline 1-oxide (4-HAQO). This compound is acylated by seryl-tRNA synthetase to the seryl derivative which is thought to be an ultimate form to bind to macromolecules (7). In addition, chemical N-O-acetylation of N-hydroxy-Trp-P-2 was reported to stimulate the covalent binding to DNA (8). Thus, we examined the possibility that seryl-tRNA synthetase may be involved in the activation of N-hydroxy-Trp-P-2 to an ultimate form for the binding to macromolecules.

MATERIALS and METHODS

Acetic acid salt of 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) was kindly donated by Dr. T. Sugimura, National Cancer Center Research Institute, Tokyo, Japan. ^{3}H-[ring labeled]-Trp-P-2, which was synthesized by tritiation of Trp-P-2 according to the method reported by Hashimoto et al. (9), was obtained from New England Nuclear, Boston, Mass. The specific activity was 344 mCi/mmole and the purity was over 99% as checked by thin layer chromatography (10). ATP, dithiothreitol (DTT) and calf thymus DNA (type I) were purchased from Sigma Chemicals, St. Louis, Mo. 4-Hydroxyaminoquinoline 1-oxide (4-HAQO) was obtained from Tokyo Kasei Chemical Co., Tokyo, Japan. Seryl-tRNA synthetase was purified from baker's yeast as previously reported (6). The purified preparation obtained was homogeneous in SDS-polyacrylamide gel electrophoresis and catalyzed the serylation of seryl-tRNA at a rate of 0.60 μmol per mg protein per min. N-Hydroxy-Trp-P-2 was biosynthesized from Trp-P-2 using hepatic microsomes from PCB-treated rats (5). N-Hydroxy-Trp-P-2 in the microsomal incubation mixture was separated with a ALC/GPC 204 liquid chromatograph equipped with a μBondapak C_{18} column (0.4 x 30 cm). Acetonitrile-0.02 M sodium acetate (pH 4.5) was used as the mobile phase. After an injection of diethylether extract of the incubation mixture, eluate containing N-hydroxy-Trp-P-2 was collected and then acetonitrile in the eluate was evaporated under a current of nitrogen gas. The resultant solution (50 μl) was used for a binding assay.

A typical incubation system (1 ml) consisted of 3 mM ATP, 10 mM L-serine, 3 mM magnesium acetate, 1 mM DTT, 50 mM potassium bicine (pH 8.0), 1 mg of calf thymus DNA, 10 μg of seryl-tRNA synthetase and 3 to 13 nmols of N-hydroxy-Trp-P-2.

The reaction was initiated by addition of N-hydroxy-Trp-P-2 dissolved in 50 µl of 20 mM sodium acetate (pH 4.5) and incubated for 30 min at 37°C. One ml of 2 M cold sodium acetate solution was added to the reaction mixture and then the substrate was extracted twice with ethylacetate (2 ml). To the aqueous phase, 2 ml of phenol saturated with 0.1 mM Tris-HCl (pH 7.7) was added. After shaking and centrifugation at 3000 rpm for 15 min, a 1.5 ml portion of the aqueous phase was transferred to another tube and re-extracted with the phenol reagent (1.5 ml). DNA was precipitated by addition of 2 volume of cold ethanol to 1.2 ml of the aqueous phase and then washed with ethanol. DNA thus precipitated was dissolved in 1 ml of 1 M sodium acetate and re-extracted with ethylacetate (1 ml). The amount of DNA and radioactivity in the resultant aqueous phase was quantified to determine the binding of N-hydroxyTrp-P-2 to DNA. DNA concentration was assayed by the method of Burton and Petersen (11). Radioactivity was measured with an Aloka liquid scintillation spectrometer after mixing the aqueous phase (100 to 200 µl) with a scintillation cocktail (Aquasol II). The results given are the mean of at least duplicate determinations.

RESULTS

To directly determine whether seryl-tRNA synthetase stimulates the covalent binding of N-hydroxy-Trp-P-2, a metabolite of Trp-P-2, we used a reconstituted system including seryl-tRNA synthetase purified from baker's yeast, L-serine and ATP. In a complete system (Table 1), 20.7 pmol of N-hydroxy-Trp-P-2 bound to 1 mg of calf-thymus DNA during a 30 min incubation period. This value represents the binding of 0.55% of the applied hydroxylamine. ATP was an essential cofactor and in its absence the binding was reduced to 4.6% of the complete system. Omission of L-serine or seryl-tRNA synthetase from the complete system also resulted in the reduced binding to DNA.

In the complete system, the binding of N-hydroxy-Trp-P-2 increased linearly with an increase in the incubation period up to 60 min (Fig. 1). Moreover, as shown in Fig. 2, increasing the amount of seryl-tRNA synthetase resulted in an enhanced binding of N-hydroxy-Trp-P-2 to DNA.

Table 1. Requirements for the covalent binding of N-hydroxy-Trp-P-2 to calf thymus DNA

System	Amount of N-hydroxy-Trp-P-2 bound to DNA (pmol/mg DNA)	%
Complete*	20.72	100
- ATP	0.95	4.6
- L-Serine	2.68	12.9
- Seryl-tRNA synthetase	2.89	14.0

* Complete system consisted of 3 mM ATP, 10 mM L-Serine, 3 mM magnesium acetate, 1 mM DTT, 50 mM potassium bicine (pH 8.0), 1 mg of calf thymus DNA, 10 μg of seryl-tRNA synthetase and 3.77 nmoles of N-hydroxy-Trp-P-2 in a final volume of 1 ml.

Less than 10% amounts of the binding were observed when 4 nmol of Trp-P-2 was added, instead of N-hydroxy-Trp-P-2, to the reaction mixture. Addition of ten-fold amounts of Trp-P-2 did not produce any further increase in the binding rate (data not shown).

The binding of 4-HAQO to DNA is catalyzed by seryl-tRNA synthetase (7). Moreover, aminoacylation by several aminoacy-tRNA synthetase were reported to be inhibited by

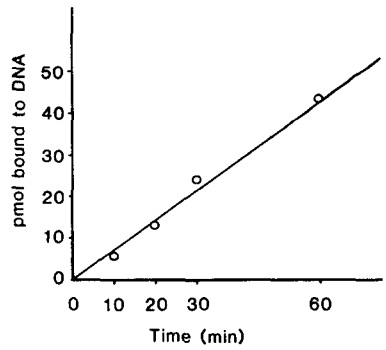

Fig. 1. Time course of the binding of N-hydroxy-Trp-P-2 to DNA. Experimental details are described in Materials and Methods. The reaction mixtures were incubated for the varying periods.

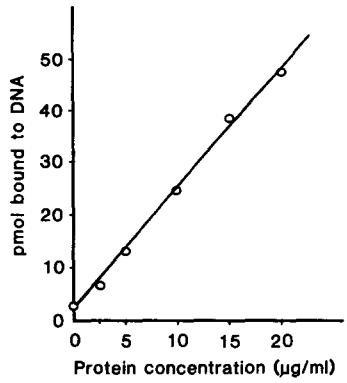

Fig. 2. Dependence of the binding of N-hydroxy-Trp-P-2 to DNA on the concentration of seryl-tRNA synthetase. Experimental conditions are described in Materials and Methods.

addition of pyrophosphate to the reaction mixture (12,13). Thus, we examined the effects of 4-HAQO and pyrophosphate on the binding of N-hydroxy-Trp-P-2 to DNA. As described in Table 2, the binding of N-hydroxy-Trp-P-2 was inhibited by addition of sodium pyrophosphate (50 μM). 4-HAQO also reduced the rate of the binding to DNA.

Table 2. Effects of 4-HAQO and pyrophosphate on the binding of N-hydroxy-Trp-P-2 to DNA

Compound	Concentration (mM)	Amount of N-hydroxy-Trp-P-2 bound to DNA (pmole/mg DNA)	%
None	--	24.10	100
Sodium pyrophosphate	0.05	15.88	65.9
Control*	--	25.78	100
4-HAQO	0.1	15.18	58.9
"	1.0	14.80	57.4

* 30 μl of dimethylsulfoxide was added as the vehicle.

DISCUSSION

Arylamines and arylamides, such as 4-aminobiphenyl and 2-acetylaminofluorene, exert their mutagenicity and carcinogenicity through N-hydroxylations. In the metabolic activation of Trp-P-2, the N-hydroxylation was also an obligatory step for the mutagenic activation (5,10,14,15). With regard to the covalent binding to macromolecules, several pathways including sulfation and acetylation are postulated as mechanisms for the activation of N-hydroxylamines (16,17). Nemoto et al. (18) reported that the binding of Trp-P-2 to DNA was enhanced by adding cytosol fraction and ATP or GTP to the microsomal activation system. 4-HAQO is activated by seryl-tRNA synthetases purified from yeast and liver of rats, and ATP is an essential cofactor for this reaction (6,7). Therefore, we examined the covalent binding of N-hydroxy-Trp-P-2 using purified seryl-tRNA synthetase. As described in Table 1, N-Hydroxy-Trp-P-2 was activated by seryl-tRNA synthetase to a form which could bind readily to DNA. Requirements of ATP and L-serine for the binding suggest that N-hydroxy-Trp-P-2 is activated by the same mechanism which is reported for 4-HAQO (7). This idea was further supported by the observation that pyrophosphate and 4-HAQO inhibited the binding to DNA.

Seryl-tRNA synthetase is an essential enzyme for protein synthesis and in mammals is localized in the cytosol fraction (105,000 x g supernatant). Ubiquitous distribution of seryl-tRNA synthetase in tissues of experimental animals might play a role in the carcinogenesis of Trp-P-2 although the rate of binding was not so rapid as that of 4-HAQO. In Table 1, a trace binding of N-hydroxy-Trp-P-2

to DNA was observed in the absence of seryl-tRNA synthetase. The non-enzymatic phosphorylation of N-hydroxy-Trp-P-2 may be responsible for the binding (19,20).

Recently, Hashimoto et al. found that the binding of 4-hydroxyaminoazobenzene to DNA was increased by addition of a seryl-tRNA synthetase from yeast (submitted for publication).

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