Vol. 114, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 29, 1983 Pages 626-631

ULTIMATE FORMS OF MUTAGENIC AND CARCINOGENIC HETEROCYCLIC AMINES PRODUCED BY PYROLYSIS

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Received June 13, 1983

SUMMARY: A mutant strain of *Salmonella typhimurium* TA98/1,8-DNP$_6$ isolated by McCoy et al. (1) was reported to be defective in esterifying activity. We have found that 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-1), 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-2), 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) were not mutagenic to TA98/1,8-DNP$_6$ with S9 mix, while these compounds were strongly mutagenic to the original TA98 with S9 mix. The mutagenicities of some of these heterocyclic amines to TA98 were inhibited by pentachlorophenol, an aryl sulfotransferase inhibitor. These results indicate that the ultimate forms of these heterocyclic amines are probably sulfate esters of heterocyclic amine N-hydroxides. Contrary to this, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) were definitely mutagenic to TA98/1,8-DNP$_6$, although less than to TA98.

McCoy et al. (2) isolated a mutant strain, TA98/1,8-DNP$_6$, from *Salmonella typhimurium* TA98, as a nitroreductase deficient mutant. Another lesion in this strain was later suggested to be involved with the conversion of arylhydroxylamine to its ultimate from (1), based on the evidence that TA98/1,8-DNP$_6$ responded only slightly to N-hydroxy-2-acetylaminofluorene, although it did respond to N-acetoxy-2-acetylaminofluorene as strongly as to TA98.

Using this strain of TA98/1,8-DNP$_6$, we tested the mutagenicities of heterocyclic amines obtained from pyrolyzates of amino acids and protein foods, some of which have been shown to be carcinogenic to mice or rats (3,4,5). We report here that the ultimate forms in Salmonella of Glu-P-1,

Abbreviations used: Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole; Glu-P-2, 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole; IQ, 2-amino-3-methylimidazo-[4,5-*f*]quinoline; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quionline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; AαC, 2-amino-9*H*-pyrido[2,3-*b*]indole; MeAαC, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]-indole; PCP, pentachlorophenol

0006-291X/83 $1.50

Glu-P-2, IQ, MeIQ and MeIQx are probably sulfate esters of the N-hydroxy derivatives of these amines.

Materials and Methods

Materials: Trp-P-1·CH_3COOH, Trp-P-2·CH_3COOH, AαC·CH_3COOH, MeAαC·CH_3COOH, IQ, MeIQ and MeIQx were obtained from Nard Institute, Ltd., Osaka. Glu-P-1·HC and Glu-P-2·HCl were from Katsura Chemical Co. Ltd., Tokyo. Pentachlorophenol (PCP), analytical grade, was obtained from Wako Pure Chemical Industry, Ltd., Osaka.

Salmonella typhimurium TA98/1,8-DNP_6 was kindly provided by Dr. H. S. Rosenkranz, Case Western Reserve University, Cleveland, Ohio.

Methods: Mutagenicity tests were performed by the preincubation method as described previously (6). Heterocyclic amines, and PCP were dissolved in dimethyl sulfoxide in a total of 0.1 ml. The S9 was prepared from livers of SD rats which had received polychlorinated biphenyls (Kanechlor 500) by the procedure described by Ames et al. (7). The S9 mix consisted of 10 µl S9, 2 µmol NADPH and 2.5 µmol G6P, 4 µmol $MgCl_2$, 16.5 µmol KCl and 50 µmol sodium phosphate buffer (pH 7.4), in a total volume of 0.5 ml.

Results

Mutagenicity of Heterocyclic Amines to TA98/1,8-DNP_6. Fig. 1 (a-e) shows the dose-response curves of Glu-P-1, Glu-P-2, IQ, MeIQ and MeIQx for mutagenicities to *Salmonella typhimurium* TA98/1,8-DNP_6 and TA98. All five heterocyclic amines induced very small numbers of revertants of TA98/1,8-DNP_6 at concentrations which were sufficient to induce revertants of TA98 in a dose-dependent manner. AF-2, a nitrofuran derivative, had the same mutageni-

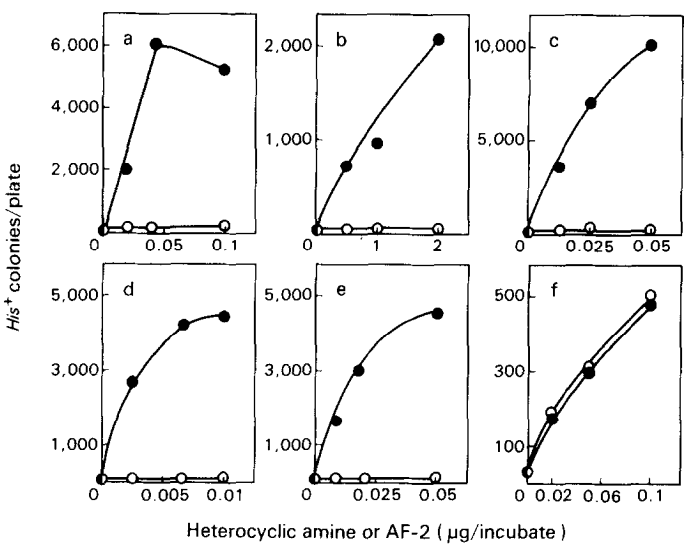
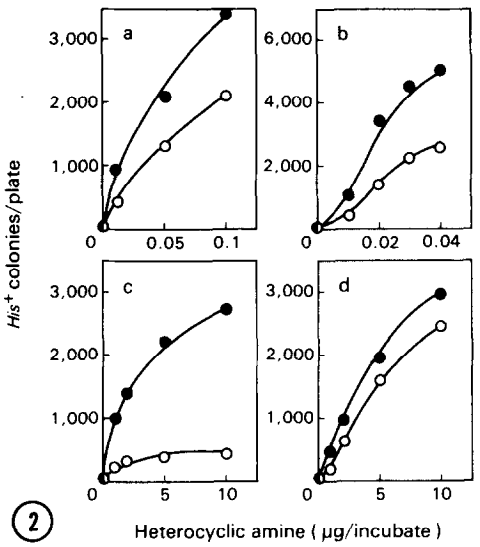


Fig. 1. Dose-response curves of the mutagenicities of heterocyclic amines and AF-2 to TA98/1,8-DNP_6 (○) and TA98 (●). a, Glu-P-1; b, Glu-P-2; c, IQ; d, MeIQ; e, MeIQx and f, AF-2. Heterocyclic amines were tested in the presence of S9 mix.



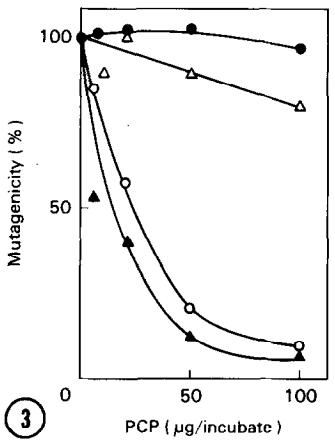


Fig. 2. Dose-response curves of the mutagenicities of heterocyclic amines to TA98/1,8-DNP_6 (o) and TA98 (●). a, Trp-P-1; b, Trp-P-2; c, MeAαC and d, AαC. All compounds were tested in the presence of S9 mix.

Fig. 3. Effect of PCP on the mutagenicities of heterocyclic amines and 2-acetylaminofluorene with TA98 and S9 mix. 0.02 μg of Glu-P-1 (o), 0.01 μg of IQ (▲), 0.02 μg of Trp-P-2 (●) and 10 μg of 2-acetylaminofluorene (△) were preincubated with S9 mix, bacteria and various amounts of PCP. The S9 mix used with 2-acetylaminofluorene contained 30 μl instead of 10 μl of S9 in 0.5 ml of S9 mix.

city to TA98/1,8-DNP_6 and TA98, as shown in Fig. 1f. The sensitivities of these two bacterial strains to AF-2 were expected to be the same as to niridazole, another derivative of nitrofuran (1).

On the other hand, Trp-P-1, Trp-P-2, MeAαC and AαC induced revertants of TA98/1,8-DNP_6 with sensitivities of 62%, 50%, 12% and 80%, respectively of those of TA98 to these compounds, as shown in Fig. 2 (a-d).

Effect of PCP on the Mutagenicities of Heterocyclic Amines. We tested the effect of PCP, an aryl sulfotransferase inhibitor, on the mutagenicities of Glu-P-1, IQ and Trp-P-2 with TA98. Various concentrations of PCP were added to 0.02 μg of Glu-P-1 or 0.01 μg of IQ, and then to the preincubation mixture which contained S9 mix and bacteria. Between concentrations of 10 and 100 μg/incubate, PCP inhibited the mutagenicities of both Glu-P-1 and IQ (Fig. 3). However, at these concentrations, it did not affect the mutagenicity of 0.02 μg of Trp-P-2 (Fig. 3). PCP itself was not mutagenic,

with or without S9 mix. The PCP at 50 μg/incubate also had no effect on the mutagenicity of Trp-P-2 to TA98/1,8-DNP_6 (data not shown).

The present results suggest that the lack of responsiveness of TA98/1,8-DNP_6 to Glu-P-1, Glu-P-2, IQ, MeIQ and MeIQx is due to its inability to form sulfate esters. This defect might be caused by decreased sulfotransferase activity or an inadequate supply of an active sulfate, adenosine 3'-phosphate 5'-phosphosulfate. This study also suggested that the ultimate forms of Glu-P-1, Glu-P-2, IQ, MeIQ and MeIQx in Salmonella are the sulfate esters of their N-hydroxy derivatives.

Discussion

The N-hydroxy derivatives of the carcinogenic heterocyclic amines Glu-P-1 (8), Trp-P-2 (9), AαC (10) and IQ (11) were reported to be their proximate forms, and these derivatives of Glu-P-1 (12), Trp-P-2 (9) and AαC (10) were reported to show direct-acting mutagenicity to TA98. N-OH-Trp-P-2 itself was shown to bind to DNA *in vitro*, although the acetyl ester of this compound was found to bind to DNA more efficiently than the free N-hydroxy derivative (13). Serine and seryl-tRNA synthetase in yeast (14), and proline and prolyl-tRNA synthetase in rat liver (15) enhanced the binding of N-OH-Trp-P-2 to DNA, in a similar way to 4-hydroxyaminoquinoline 1-oxide (16). 4-Nitroquinoline 1-oxide showed almost the same mutagenicity in the two strains TA98 and TA98/1,8-DNP_6 (2). Since amino acyl-tRNA synthetase is essential for bacterial growth, the defect in the mutant strain TA98/1,8-DNP_6 can not be involved with any amino acyl-tRNA synthetase reaction. Therefore, our finding of a marked mutagenic response of TA98/1,8-DNP_6 to Trp-P-2 is consistent with previous results (13,14,15).

The ultimate form of 2-acetylaminofluorene in mammals is known to be the sulfate ester of N-hydroxy-2-acetylaminofluorene (17,18). N-Hydroxy-2-acetylaminofluorene was reported to show no mutagenicity to TA98/1,8-DNP_6 without S9 mix (1), and we found that 2-acetylaminofluorene was not mutagenic to TA98/1,8-DNP_6 with S9 mix. However, the mutagenicity of 2-acetylaminofluorene to TA98 was not inhibited by PCP as shown in Fig. 3. More than 10 μg/plate

of 2-acetylaminofluorene was necessary to obtain sufficient mutants without PCP. Since PCP is a competitive inhibitor of sulfotransferase with respect to the sulfate acceptor (19), a high concentration of PCP must be required for its inhibition of the mutagenicity of 2-acetylaminofluorene. But at a higher concentration of PCP, this inhibition can not be demonstrated because of the killing action of PCP.

The inhibitory effect of PCP on the mutagenicities of Glu-P-1 and IQ with TA98 and S9 mix strongly suggested that the ability to form sulfate esters is deleted in TA98/1,8-DNP$_6$. Our results strongly suggest that the ultimate forms of Glu-P-1, Glu-P-2, IQ, MeIQ and MeIQx are the sulfate esters of their N-hydroxy derivatives.

Although TA98/1,8-DNP$_6$ seems to have another defect resulting in ability to metabolize some kinds of nitro compounds, this strain is useful for obtaining information on the ultimate forms of mutagens.

Acknowledgements

This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare of Japan.

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