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FORMATION OF MUTAGENS FROM TRYPTOPHAN BY THE REACTION WITH NITRITE

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SUMMARY: Tryptophan treated with nitrite under acidic conditions was found to be mutagenic to Salmonella typhimurium tester strains TA 100 and TA 98 both in the presence and in the absence of S-9 mix. Tryptamine, glycyltryptophan and indole were also mutagenic when treated with nitrite, suggesting that the appearance of mutagenic activity from tryptophan was attributable to the reaction of nitrite with the indole ring. Nitrite-treated arginine and proline were not mutagenic in the presence of S-9 mix.

INTRODUCTION

Secondary amines easily react with nitrite at gastric pH to give N-nitroso compounds, many of which are mutagens and/or carcinogens (1). In this respect, amino acids containing imino groups are particularly important, because these are daily ingested as natural constituents of food proteins. Endo et al observed that arginine and arginine-containing peptides showed mutagenic activity when treated with nitrite (2).

This paper describes the appearance of mutagenic activity from tryptophan by the reaction with nitrite under acidic conditions. The mutation tests for nitrite-treated arginine and proline, which had been previously done without S-9 mix (2), were also carried out under the same conditions with S-9 mix: In microbial mutation test, S-9 mix enables detection of various carcinogenic N-nitroso compounds as mutagens (3-5).

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MATERIALS AND METHODS

CHEMICALS

Amino acids were purchased from Kanto Kagaku Co. (Tokyo). Indole was from Nakarai Chemicals Co. (Kyoto). Glyclyltryptophan and tryptamine hydrochloride were from Tokyo Kasei Co. (Tokyo).

REACTION OF AMINO ACIDS WITH NITRITE

(a) To 50 ml of 0.1 N HCl containing 2.45 mmole of amino acid was added 7.35 mmole of sodium nitrite, and the mixture was stirred at room temperature. After a defined period, 2 ml of 25% ammonium sulfamate was added to the reaction mixture, which was then extracted with ethyl acetate (2 X 30 ml). When necessary, the aqueous layer was adjusted to pH 9-10 and extracted again with ethyl acetate as above. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness in vacuo at 40°C. After allowed to stand overnight in a vacuum desiccator, the residue was dissolved in a definite volume of dimethyl sulfoxide (DMSO), and a 0.1-ml portion of the solution was subjected to the mutation test.

(b) Tryptophan (2.45 mmole) was dissolved in 50 ml of 0.1 M sodium citrate, and the solution was adjusted to the desired pH with HCl. Then, 7.35 mmole of sodium nitrite was added to the solution, and the subsequent experiments were done as mentioned above.

MUTATION TEST

The mutagenicity was tested by the method of Ames et al (6) with some modification, including preincubation of test sample with S-9 mix and *Salmonella typhimurium* TA 100 and TA 98 for 20 min at 37°C (7). Liver microsomal fraction (S-9) was prepared from rats that had been injected with PCB as described by Ames et al (6).

RESULTS AND DISCUSSION

Table 1 shows the results of mutation tests for the ethyl acetate extracts of three amino acids treated with nitrite. The aqueous reaction mixture of arginine with nitrite was also subjected to the mutation test, because the extract from that mixture was very small in amount, Without S-9 mix, the extract from tryptophan and the aqueous mixture from arginine were mutagenic. Endo et al reported that arginine showed mutagenicity when arginine and proline were allowed to react with nitrite in strongly acidic solution (2). In the present study, these amino acids were treated with nitrite under milder conditions, but the results of mutation

Table 1. Mutagenicity of amino acids treated with nitrite

amino acid	ethy acetate extract (mg)	dose (mg/plate)	revertant per plate TA 100 -S-9	TA 100 +S-9	TA 98 -S-9	TA 98 +S-9
tryptophan	323.2	0.5	325	360	-[a)]	98
proline	116.2	2.6	102	119	28	38
arginine						
acid fraction	0.8	0.05	92	130	28	27
basic fraction	2.3	0.15	92	128	21	31
aqueous fraction	-	1.7[b)]	180	102	48	29
control			87	115	22	33

Amino acids were treated with nitrite in 0.1 N HCl for 30 min.
a) sterilizing action; b) the dose calculated on the basis of the starting materials.

tests were consistent with those previously reported. In the presence of S-9 mix, the aqueous mixture from arginine lost its mutagenicity and only the extract from tryptophan was mutagenic. The aqueous mixtures from tryptophan and proline after the extraction with ethyl acetate were not mutagenic (data not shown).

Figure 1 shows the time course of appearance of mutagenic activity from tryptophan during the reaction with nitrite. The

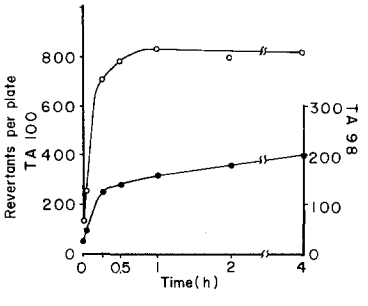
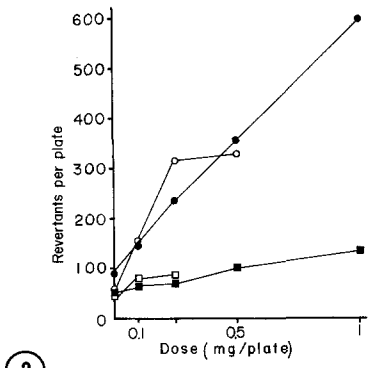
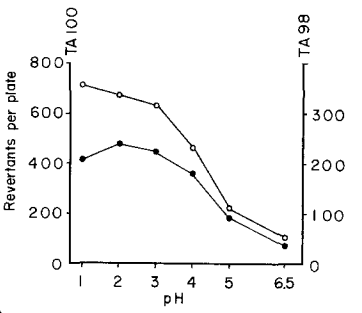


Fig. 1. Time course of formation of mutagens during the reaction of tryptophan with nitrite
Tryptophan was allowed to react with nitrite in 0.1 N HCl, and each extract was dissolved in 25 ml of DMSO. -○- : TA 100 + S-9 mix; -●- : TA 98 + S-9 mix.



(2)



(3)

Fig. 2. Dose dependency of mutagenicity of the ethyl acetate extract from tryptophan treated with nitrite
The extract was prepared as in Table 1. ●— : TA 100 + S-9 mix; —○— : TA 100 without S-9 mix; ■— : TA 98 + S-9 mix: —□— : TA 98 without S-9 mix.

Fig. 3. PH dependency of formation of mutagens from tryptophan by the reaction with nitrite
Tryptophan was allowed to react with nitrite for 30 min in citrate buffer, and each extract was dissolved in 25 ml of DMSO. Symbols used are the same as those in Fig. 1.

reaction proceeded relatively rapidly, and the mutagenic activity of the extract reached a maximum within 1 hour. The relationship between the dose of the extract from tryptophan and the number of revertants induced is shown in Fig. 2. The number of revertants increased almost linearly with the dose level of the extract added when the tests were done in the presence of S-9 mix. The extract was more active toward TA 100 than TA 98. The absence of S-9 mix tended to increase the mutagenic activity, but sterilizing action was observed at the dose over 500 μg/plate and 250 μg/plate for TA 100 and TA 98, respectively.

Figure 3 shows the effect of pH on the formation of mutagens from tryptophan by the reaction with nitrite. The appearance of mutagenic activity from tryptophan depressed with an increase in pH value. The gastric pH range, ca. 1-4, was effective for the formation of mutagens.

Several compounds structurally related to tryptophan were also treated with nitrite under the similar conditions. The extracts from tryptamine, glycyltryptophan and indole were all mutagenic to both tester strains, but the extract from alanine, which corresponded to the side chain of tryptophan, was not mutagenic (data not shown). The appearance of mutagenic activity from tryptophan, therefore, is probably attributable to the reaction of nitrite with the indole ring of tryptophan. That is to say, it is probable that tryptophan reacts with nitrite to give mutagenic compounds not only in free state but also in combined state in peptide chain.

Deaminative hydroxylation of α-C (Van-Slyke reaction) and N-nitrosation of indole ring are generally expected to occur in the reaction of tryptophan with nitrite in acidic solution. However, thin-layer chromatography (plates: silica gel 60F-254 (E. Merk); solvent: chloroform/methanol/28% ammonia (7:4:1, v/v)) of the extract from tryptophan showed that at least 8 compounds were formed by the reaction (detected under UV light). Preliminary tests on mutagenic activity of these components showed that multiple mutagens were present in the extract. These results may indicate that besides N-nitrosation, tryptophan undergoes C-nitrosation: certain indole compounds are known to react with nitrite to give 3-nitroso derivatives (8). Characterization of the above components is now under investigation. Since tryptophan is an essential amino acid and is daily ingested as a food constituent, closer investigations are needed about the mutagens from tryptophan.

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