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THE ENHANCING EFFECT OF CYSTEINE AND ITS DERIVATIVES ON THE MUTAGENIC ACTIVITIES OF THE TRYPTOPHAN-PYROLYSIS PRODUCTS, TRP-P-1 AND TRP-P-2

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Summary Cysteine and its derivatives were found to enhance the mutagenic activity of the tryptophan-pyrolysis products, Trp-P-1 and Trp-P-2, as assayed by the Salmonella-microsome system. Several-fold increase in the number of revertant colonies was caused by addition of cysteine, cysteine ethyl ester and cysteamine at 10 mM concentrations to the reaction mixture containing the bacteria and the mutagen. Studies on the structural requirement for the enhancing effect suggest that both the thiol and the amino groups are necessary in order for the compound to exhibit the effect. The cysteine derivatives did not affect the mutagenic activity of either benzo[a]pyrene or the beef-extract mutagen.

INTRODUCTION

Modification in vitro and in vivo of the activities of mutagens and carcinogens has been extensively studied (1). Search for the modifying factors among components of living organisms may give clues for assessing the activity of the mutagens and carcinogens in vivo. Work in several laboratories has given indications that the reactive electrophilic species of carcinogens can be trapped by nucleophiles (2-6); and cysteine has been shown to diminish the mutagenicity of several carcinogens as tested in vitro by bacterial system (7,8).

Recent demonstration that mutagenic compounds can be formed during cooking of foods prompted us to investigate whether cysteine can modify the activity in vitro. This paper deals with the effect of cysteine and its derivatives on mutagenicities of the tryptophan-pyrolysis products, Trp-P-1 and Trp-P-2 (9,10) and of the beef-extract mutagen (11).

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MATERIALS AND METHODS

Chemicals: Trp-P-1 and Trp-P-2 were kindly supplied by Dr. M. Nagao of the National Cancer Center Research Institute. Beef-extract mutagen was prepared from Bacto beef extract (Difco) by the method of Commoner et al. (11). Mono sodium cysteine-S-sulfonate was a gift from Dr. M. Shikita of the National Institute of Radiological Sciences. Other chemicals used were reagent-grade commercial products. Amino acid derivatives used were of the L form except for homocysteine which was of DL.

Mutation test: The mutation test was carried out by the method of Ames et al. (12) as modified by Yahagi et al. (13), using Salmonella typhimurium TA 98 and measuring the reversion from His^- to His^+. In this process, the mixture containing bacteria, mutagen and S-9 mix was incubated at 37°C for 20 min in the presence and absence of reagents to be tested for the modifying effects. Soft-agar was then added to the mixture, and the whole mixture was poured on agar plate. The plate was maintained at 37°C for two days and the revertant colonies were counted. S-9 fraction was prepared from the livers of rats to which polychlorinated biphenyl had been intraperitoneally administered.

RESULTS

As Figure 1 shows, addition of cysteine and its derivatives at a concentration range of 0.5 - 50 mM to Trp-P-1 during the mutagenesis assay resulted in a remarkable increase in the mutagenic activity. Cysteamine was the most effective among the compounds tested, giving approximately eight-fold increase in the number of revertant colonies by its addition at 10 mM concentration. Several-fold increase in the mutagenicity was noted also for addition of cysteine ethyl ester and cysteine. Little, if any, effect was observed for homocysteine. Compounds that were found not to affect the mutagenic activity were cysteine-S-sulfonate, 2-mercaptoethanol, 3-mercaptopropionic acid, glutathione, methionine, serine, threonine, glycine, lysine and glutamic acid. A mixture of 2-mercaptoethanol and n-propylamine was also without effect, a result suggesting that the intramolecular presence of both a thiol and an amino-group is required for this class of compounds to exhibit the enhancing effect.

Effect of adding cysteamine to several mutagens that are active on Salmonella typhymurium TA 98 in the presence of S-9 mix was investigated. As Table I shows, cysteamine was effective not only for Trp-P-1 but also for Trp-P-2, This was observed at varying doses of both the mutagen and the S-9. Cysteamine showed no effect on the beef-extract mutagen or on benzo[a]pyrene.

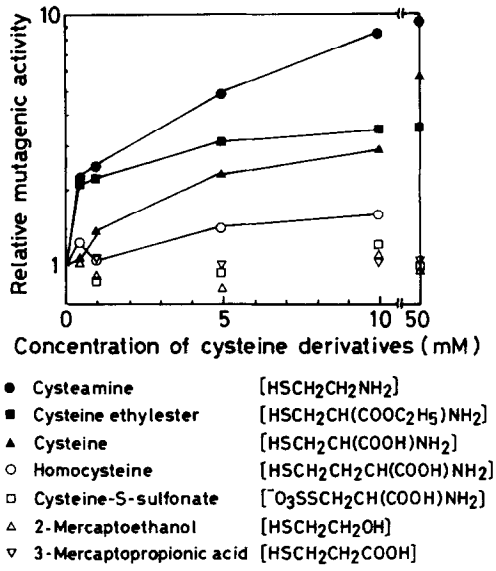

Figure 1. Effect of cysteine derivatives on Trp-P-1 mutagenicity. Salmonella typhimurium TA 98 was incubated with Trp-P-1 (0.2 μg) in the presence of S-9 mix, with or without the cysteine derivative at the concentration indicated in the Figure. The mixture was worked up as described under Methods. The S-9 mix (0.5 ml) contained 10 μl S-9, 0.8 mM NADPH, 0.8 mM NADH, 1 mM D-glucose 6-phosphate and 1 mM ATP. The number of revertant colonies observed for samples without the addition of cysteine derivatives was 939 ± 75. Although not shown in the Figure, no effect to the mutagenic activity was found for glutathione, methionine, serine, threonine, glycine, lysine and glutamic acid.

DISCUSSION

Rosin and Stich (7,8) have shown that cysteine diminishes the mutagenic activity of N-acetoxy-2-acetylaminofluorene, N-methyl-N'-nitro-N-nitrosoguanidine, 4-nitroquinoline-1-oxide and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide when it is added at 10 mM concentration to the Salmonella-microsome test system with which activities of these carcinogenic mutagens are measured. Cysteamine is known as a radioprotective agent in various biological and chemical systems (14), and is also capable of inhibiting the mutagenic and carcinogenic activities of 7,12-dimethyl-benzo[a]anthracene (15). The present finding that cysteine derivatives enhance the activity of the tryptophan-pyrolysis-product mutagens contrasts sharply to these previous reports. Although mammalian tissues contain cysteine up to 0.1 mM (16), it

Table I. Effect of cysteamine on several mutagens

	Mutagen	Amount of mutagen per plate	Amount of S-9 (μl) per plate	His^+ revertants/plate (Relative mutagenic activity[a])			
				Concentration of cysteamine used (mM)			
				0	1	5	10
Exp. 1	Trp-P-1	0.2 μg	50	126 (1)	217 (1.9)	498 (4.7)	647 (6.1)
	Trp-P-2	0.2 μg	10	1380 (1)	1763 (1.3)	5208 (3.6)	6496 (4.8)
	Beef-extract mutagen	20 μl[b]	50	804 (1)	1241 (1.6)	1056 (1.3)	1137 (1.4)
	Benzo[a]pyrene	3 μg	50	795 (1)	565 (0.7)	762 (1.0)	667 (0.8)
	None	-	50	24			
Exp. 2	Trp-P-1	0.15 μg	10	719 (1)	1380 (2.0)	2494 (3.6)	2981 (4.3)
	Trp-P-2	0.02 μg	10	696 (1)	1241 (1.8)	2622 (3.9)	3283 (4.9)
	None	-	10	33			
Exp. 3	None	-	10	23	36	29	30

a) For calculation of the relative mutagenic activity, the background His^+-revertant number (the "None" entry in each experiment) was subtracted from the observed revertant numbers. b) This amount corresponds to 0.08 g of the original Difco Bacto beef extract.

is difficult to assess the biological consequences of this finding.

Studies on the structure-activity relationship (Fig. 1) show that the thiol compounds having an amino group at the β-position are the active enhancers. Under physiological conditions, these amino groups are protonated so that the dissociation of the thiol groups into thiolate anions is greatly facilitated in these compounds: Thus, the pK_{SH} values of the active compounds fall between 7.4 and 8.5 (cysteine, 8.5; cysteamine, 8.35; and cysteine ethyl ester, 7.45), whereas those of the inactive thiol compounds are higher than 9 (17). Consequently, it seems reasonable to assume that the thiolate anion is involved in this mutagen-enhancing effect. Direct chemical reaction between the reagents and the mutagen appear not to be involved in the phenomena observed, since no spectral change of Trp-P-1 was detected on prolonged incubation with cysteine in aqueous solution (data not shown).

The mutagenic activities of chemical agents that require metabolic activation vary greatly by the nature of the S-9 used as well as by the amount of the given S-9 in the incubation mixture (18). This has been shown to be the case also for Trp-P-1 and Trp-P-2 (ref. 19 and the results presented in Table I). The variation in the amount of S-9 did not affect the extent of the enhancement by the thiol compounds as shown in Table I. It should be noted that norharman, a "comutagen", behaves differently in this regard: Norharman is an effective enhancer for Trp-P-1 at high S-9 concentrations, but it is rather a suppressor at low S-9 concentrations (19). When S-9 obtained from rats that had received no "inducer" was used in the present assay, no effect was detectable for cysteamine added to Trp-P-1 (data not shown). This observation suggests that some factors present in the "induced" S-9 is required for the enhancing-effect of the cysteine derivatives.

There could be many ways by which cysteine or its derivatives perturb the metabolisms of Trp-P compounds, resulting in enhancement of the mutagenic activity. One such way is a reaction of Trp-P or its metabolite with

cysteine to produce a compound more potent than Trp-P itself. Hypotheses of this kind are subject to confirmation by future work.

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