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Non-mutagenicity of Fe^{3+}-NTA and NTA in the Ames Salmonella Test

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ABSTRACT. The mutagenic effects of ferric nitrilotriacetate (Fe^{3+}-NTA) and nitrilotriacetate (NTA) were evaluated on 8 Salmonella typhimurium strains (TA97, TA98, TA102, TA1535, TA1537, TA100 1,8-DNP_6 and TA100NR). Neither Fe^{3+}-NTA nor NTA significantly increased the frequency of revertant colonies in any of the different experimental conditions adopted.

Key words : nitrilotriacetate (NTA)—ferric nitrilotriacetate (Fe^{3+}-NTA)—Salmonella typhimurium tests

Nitrilotriacetate (NTA) is a synthetic aminopolycarboxylic acid type chelator used chiefly as a phosphate substitute in household detergent. It has been reported to be a potent carcinogen[1] or a promoter.[2] Questions have arisen, however, regarding its carcinogenic action.[3] Montaldi[4] reported that NTA did not induce chromosomal damage in mammalian cells either *in vivo* or *in vitro*. Fe^{3+}-NTA, which is often used in the laboratory as a specific iron donor to apotransferrin[5] has caused renal cell carcinoma in rats, but no tumors were observed in NTA-treated rats.[6,7] Thus, it is likely that once NTA, which itself has no carcinogenicity to kidney, binds with Fe^{3+} and forms the Fe^{3+}-NTA complex, it participates in renal carcinogenesis. Although the active oxygen generated by Fe^{3+}-NTA may contribute partially to the carcinogenic effects of the Fe^{3+}-NTA complex,[8] the details of this mechanism are still unclear and whether or not the carcinogenic effects of Fe^{3+}-NTA are related to mutagenic events remains to be determined. As far as we know, the mutagenic effects of Fe^{3+}-NTA in the Salmonella typhimurium bioassay have not yet been investigated. Therefore, we attempted to examine mutagenicity studies of Fe^{3+}-NTA and NTA in terms of the reversion frequency in the Salmonella typhimurium bioassay.

MATERIALS AND METHODS

(1) Test substances

Fe^{3+}-NTA solutions were prepared immediately before use according to the method described by Awai.[9] Briefly, ferric nitrate (Wako Pure Chemical

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Ind., Ltd, Osaka) was dissolved in 1N HCl solution, and NTA disodium salt (Tokyo Kasai Kogyo Co., Ltd, Tokyo) was dissolved in distilled water. The molar ratio of Fe to NTA was 1:3 and the pH was adjusted to 7.4 with $NaHCO_3$. The Fe^{3+}-NTA or iron-free NTA solutions were sterilized with a 0.22 μm millipore filter.

(2) Bacterial strains

Eight strains of Salmonella typhimurium (TA97, TA98, TA100, TA102, TA1535, TA1537, TA100 1,8-DNP_6 and TA100NR) were kindly supplied by Professor Hikoya Hayatsu, Department of Pharmaceutical Sciences, Okayama University, Japan.

(3) Assay procedure

The Salmonella test of Ames[10] was carried out using the suspension assay technique of Yahagi.[11] Briefly, each strain of Salmonella typhimurium and desired concentrations of Fe^{3+}-NTA were suspended in Vogel-Bonner minimum medium[12] and incubated in a shaking water bath for 25 minutes at 37°C. Thereafter, the bacteria were seeded onto agar plates and 48 hr later colonies were counted. Each datum on mutagenicity is an average of results found in two plates. 2-Methoxy-6-chloro-9-(3-(ethyl-2-chloroethyl) aminopropylamino)-avidine·2HCl (ICR-170), 4-nitroquinoline-1-oxide (4NQO), 2-nitroflorene (2NF), bleomycin (BLM), and N-methyl-N-nitrosourea (MNU) were used as positive controls.

RESULTS

The results of the mutagenicity test are presented in Table 1. Fe^{3+}-NTA and NTA were examined in the dose ranges of 1-600 μg/plate. Positive responses were judged by two criteria ; 1) the number of revertant colonies was twice the number of spontaneous revertant colonies, and 2) evidence of a sample-dose related increase in revertant colonies. Under these criteria, neither Fe^{3+}-NTA nor NTA exhibited any mutagenic activity in the present experiment, while the positive controls showed the expected increase in the reversion frequency.

DISCUSSION

NTA is generally considered not to be mutagenic, e.g. NTA does not induce a significant increase in the frequency of chromosome aberrations.[4,13] In the review article of Ashby and Tennant[14] (1988), they cited Zeige's works that NTA was not mutagenic in the Salmonella strains, TA98, TA100, TA1535, and TA1537. Montaldi[15] also observed no increase in the sister chromatid exchange (SCE) in Chinese hamster cells (CHO) treated with NTA. However, NTA can chelate heavy metals, and it was shown that the complex of NTA and heavy metals such as Cd, Hg and Ni increased the induction of SCE over that induced by the metal salts alone.[15] Ebina reported that administration of Fe^{3+}-NTA induced renal cell carcinoma in rats, but NTA alone did not induce any tumors.[7] Thus, the mutagenic and carcinogenic potential of NTA is still questionable and perhaps when NTA binds with Fe^{3+}, it exhibits carcinogenic or mutagenic effects.

The Ames Salmonella test has become widely accepted as an initial test

TABLE 1. Mutagenic activity of Fe^{3+}-NTA and NTA in Salmonella typhimurium bioassay

Chemicals	Treatment (μg/100 mm plate)	TA97	TA98	TA100	TA102	TA1535	TA1537	TA100 1,8-DNP_6	TA100 NR
		No. of revertant colonies per plate[1]							
Fe^{3+}-NTA	0	157	23	98	125	9	4	135	159
	1	149	23	116	152	10	10	134	85
	10	166	25	125	135	12	12	160	125
	100	164	19	69	134	6	7	111	78
	600	241	33	124	—	3	7	89	102
NTA	1	187	17	157	120	9	15	181	157
	10	175	22	121	104	19	6	133	180
	100	196	16	127	104	9	10	171	163
	600	198	21	156	—	10	6	151	200
ICR-170	2.0×10^{-4} M	1616	—[2]	—	—	—	2933	—	—
4NQO	2.5×10^{-9}	—	422	—	—	—	—	—	—
2NF	3.0×10^{-8}	—	1408	1473	—	—	—	423	323
BLM	7.0×10^{-7}	—	—	—	251	—	—	—	—
MNU	1.0×10^{-3}	—	—	—	—	2857	—	—	—

1) Each value is the mean of two plates.
2) Not done.

for the identification of chemicals with mutagenic activity and it is reported that there are high correlations between mutagenicity in Salmonella and genetic and carcinogenic effects in mammalian systems.[16–18] However, there are some reports that certain chemical classes such as chlorinated hydrocarbons contain a large number of carcinogens that are not mutagenic in Salmonella.[18] Therefore, the effects of Fe^{3+}-NTA on cells should be investigated in more detail to learn whether or not the chemical is truly mutagenic or carcinogenic at the cellular level.

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