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Enhancement by Zinc Acetate of 1–Nitropyrene Binding to DNA in the Hypoxanthine–Xanthine Oxidase System

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Mutagenicity and carcinogenicity of nitroaromatic hydrocarbons including 1-nitropyrene(1-NP) in the environment, have been reported (Xu et al. 1982 ; El-Bayoumy et al. 1988) and the nitroreduction(Meissier et al. 1981;Howard et al. 1983; Djuric et al. 1988) of these compounds have been described in relation to an activation pathway in mammals. We have studied the effects of metal compounds on the mutagenicity(Sakai et al. 1985 ; Sakai and Uchida 1992) and carcinogenicity(Yamane et al. 1984;Sakai et al. 1990) of chemical compounds. It has been considered that the nitroreduction pathway is important for the binding of 1-NP to DNA which leads to the mutagenicity or carcinogenicity, not only in bacteria but also in mammals. Howard et al.(1982) have reported that a mammalian nitroreductase, xanthine oxidase [E.C. 1.2.3.2] catalyzes the binding of 1-NP to DNA. This paper reports the enhancing effect of zinc acetate on the *in vitro* binding of 1-NP to DNA isolated from *Salmonella typhimurium* TA100 in the hypoxanthine-xanthine oxidase system.

MATERIALS AND METHODS

N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) was purchased from Dojin Co., xanthine oxidase (from cow's milk), calf thymus DNA (type I) and hypoxanthine were from Sigma Chemical Co. and 1-aminopyrene (1-AP) was from Aldrich Chemical Co. All other reagents used were of the highest grade available. The isolation of DNA from *S. typhimurium* TA100 cells was carried out according to the method of Marmur (1961). Xanthine oxidase was passed through a Sephadex G-25 column equilibrated with water before use. 1-NP was purchased from Tokyo Kasei Kogyo and purified by chromatography on neutral alumina (Merck) following elution with benzene; a single peak was detected by HPLC using a colomn of Zorbax-ODS(Tokiwa et al. 1983). [^{3}H]1-NP was obtained from Amersham International Ltd and further purified as described above. The determination of xanthine oxidase-catalyzed binding of 1-NP to DNA was performed accord-

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ing to the method of Howard et al. modified slightly as follows: Reaction mixtures (2 ml) containing 1 mg DNA of S. typhimurium TA100 or calf thymus, 3.7 mM hypoxanthine, an appropriate concentration of zinc ion, and 25 μM tritiated substrate (9.73 MBq/μmol in 0.1 ml DMSO) in 0.2 M BES-NaOH buffer, pH 7.4 were purged with argon for 5 min. The preincubation of DNA with zinc acetate in the buffer was performed for 10 min before purging with argon. Xanthine oxidase(0.1 units/ml) was added to the reaction mixtures and the solutions (final volume 3 ml) began to be incubated at 37°C. The reactions were terminated by the addition of an equal volume of water-saturated phenol:isoamyl alcohol:chloroform (25:1:24), followed vigorous mixing and centrifugation. This extraction sequence was repeated two additional times and the concentration of sodium chloride in the combined supernatants after centrifugation was adjusted to 5 M by adding sodium chloride. The DNA was precipitated from the aqueous phase by the addition of three volumes ice-cold ethanol. The DNA was by dissolved in 0.2 M BES-NaOH buffer, pH 7.4 containing 0.1 mM EDTA and again precipitated by adding ice-cold the ethanol. After the DNA was redissolved in the BES-NaOH buffer, its concentration was determined spectrophotometrically at 260 nm. The binding of [^{3}H]1-NP to DNA was measured with a Beckman LS 5800 scintillation counter using Aquasol-2 scintillator (New England Nuclear Co.). The determination of uric acid produced by the hypoxanthine-xanthine oxidase system was performed as follows: the reaction mixtures containing 3.7 mM hypoxanthine and an appropriate concentration of zinc ion in 0.2 M BES-NaOH buffer, pH 7.4 were purged with argon for 5 min. Xanthine oxidase(0.1 units/ml) was added to the reaction mixtures (final volume 1.0 ml) which were then incubated for 1 hr at 37°C. The reactions were terminated by the addition of 10% trichloroacetic acid (1 ml) and the proteins precipitated by centrifugation. The pH of the supernatant was adjusted to 8-10 by adding 10% NaOH and the absorption of supernatant measured at 300 nm to determine the concentration of uric acid (Bergmann and Dikstein 1956). The determination of the 1-AP produced by the addition of 1-NP to the hypoxanthine-xanthine oxidase system was performed as follows: the reaction mixtures containing 3.7 mM hypoxanthine and an appropriate concentration of zinc ion in 0.2 M BES-NaOH buffer, pH 7.4 were purged with argon for 5 min. Xanthine oxidase (0.1 units/ml) and 1-NP (25 μM) were added to the reaction mixtures (final volume 2 ml) which were then incubated for 1 hr at 37°C. The reactions were terminated by the addition of 80% acetonitrile and the proteins precipitated by centrifugation. The supernatant was subjected to HPLC (LC-4A, Shimazu Co.) on a reverse-phase column of Nucleosil $5C_{18}$ (4.6 mm X 250 mm) with a mobile phase of 80% CH_3CN-H_2O at a flow rate of 1.5 ml/min. The 1-AP

(retention time, 3.6 min) and 1-NP (retention time, 6.5 min) separated by the HPLC were detected using a fluorescence detector(RF-530, excitation, 360 nm; emission, 425 nm).

RESULTS AND DISCUSSION

The extent of 1-NP binding to DNA by the xanthine oxidase system increased with the increase of incubation time when the reaction mixtures were incubated at 37°C, as shown in the left panel of Figure 1. These results obtained under our experimental conditions agreed with those of Howard et al.(1982). The extent of 1-NP binding to DNA was increased by the addition of 0.25 mM zinc acetate almost linearly during incubating 15 to 60 min and appeared to reach a plateau at 90 min. The extent of 1-NP binding to DNA by the xanthine oxidase system also increased with the increase of zinc concentration in the reaction mixtures, as shown in the right panel of Figure 1. There is no significant difference between DNA of S. typhimurium and calf thymus DNA, in the enhancing effect by the addition of zinc acetate. The increase in 1-NP binding to DNA produced by the addition of 0.25 mM zinc acetate was abolished when DETA (1 mM) was added to the preincubated mixture. The increase in binding of 1-NP to DNA was abolished by the addition of EDTA (5 mM) to the reaction mixtures after the preincubation of DNA with zinc ions (0.25-1.0 mM), as shown in Figure 2.

Zinc ions are known to interact with nucleic acids and proteins that are cellular components (Eichhorn 1973 ; Coleman 1979 ; Mildvan and Loeb 1981). There are many zinc-containing enzymes like DNA and RNA polymerases. As an explanation of the finding that the addition of zinc acetate to the reaction mixtures increases the extent of 1-NP binding to DNA, it is suggested that zinc acetate may have an enhancing effect on the enzyme system. The hypoxanthine-xanthine oxidase system is used as a superoxide anion-radical generator and the hydroxylation of aromatic compounds has been detected (Richmond et al. 1981). Superoxide anion-radicals may be produced by the hypoxanthine-xanthine oxidase system under our experimental conditions and react with 1-NP and DNA in the reaction mixtures. Therefore, it is undertaken as another study to detect the oxidative derivatives produced by the reaction. The effect of zinc acetate on the uric acid concentration produced by the hypoxanthine-xanthine oxidase system was examined. Zinc acetate has no significant effect on the production of uric acid, as shown in Table 1. The effect of zinc acetate on the production of 1-AP from 1-NP in the hypoxanthine-xanthine oxidase system, with the addition of DNA, was also examined. However, neither the production of 1-AP nor 1-NP remaining in the reaction mixtures without adding DNA, was signifi-

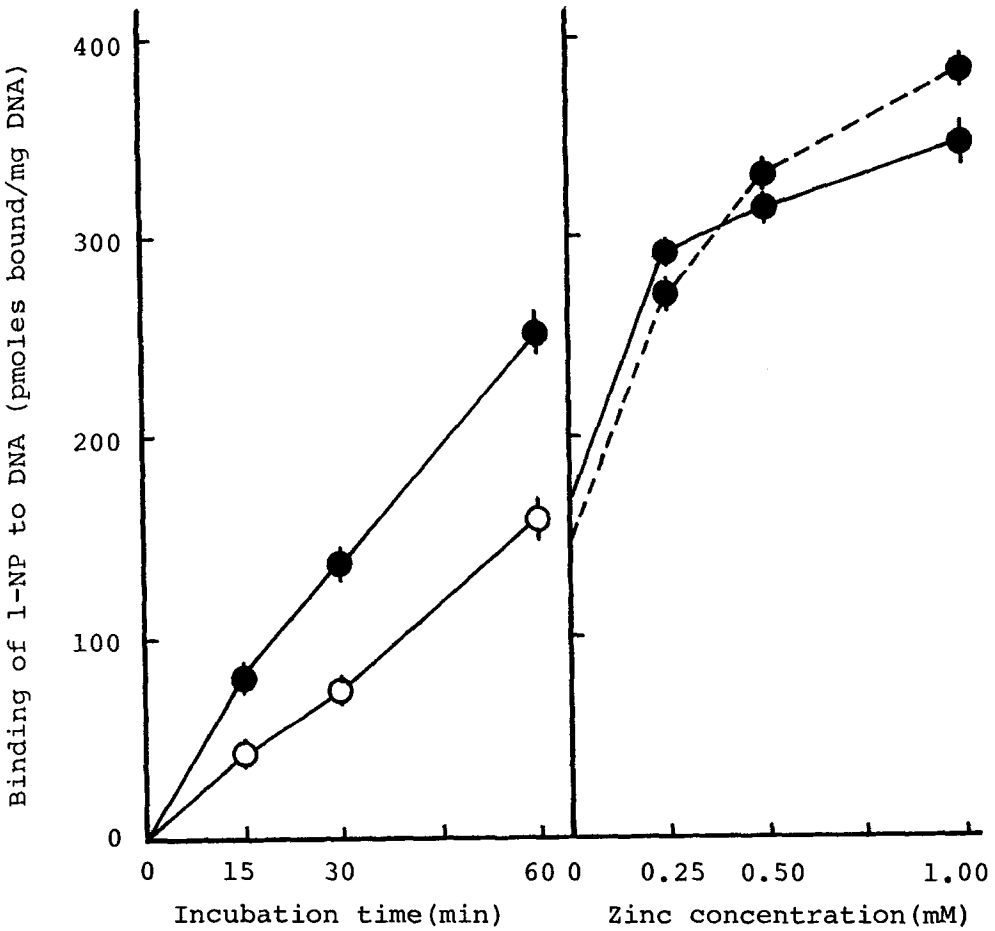

Figure 1. Effect of zinc acetate on the binding of 1-NP to DNA in the hypoxanthine-xanthine oxidase system. The left panel showed the effect of different times of incubation on 1-NP binding to DNA when the reaction mixtures without (—o—) and with (—●—) zinc acetate (0.25 mM) were incubated. The right panel showed the effect of various zinc acetate concentrations on 1-NP binding to DNA when the reaction mixture were incubated for 60 min. DNA (——) from S. typhimurium TA 100 and calf thymus DNA (----) were used. Each point was shown as a mean ± SD. (n=6)

cantly changed by the addition of zinc acetate, as shown in Table 2. Therefore, zinc acetate was considered to have no effect on the activity of xanthine oxidase.

On adding EDTA to the reaction mixtures, the increase in the binding of 1-NP to DNA produced by zinc acetate was abolished, as shown in Figure 2. From these findings it is suggested that the direct interaction of zinc acetate with DNA may have an effect on the binding of 1-NP to DNA.

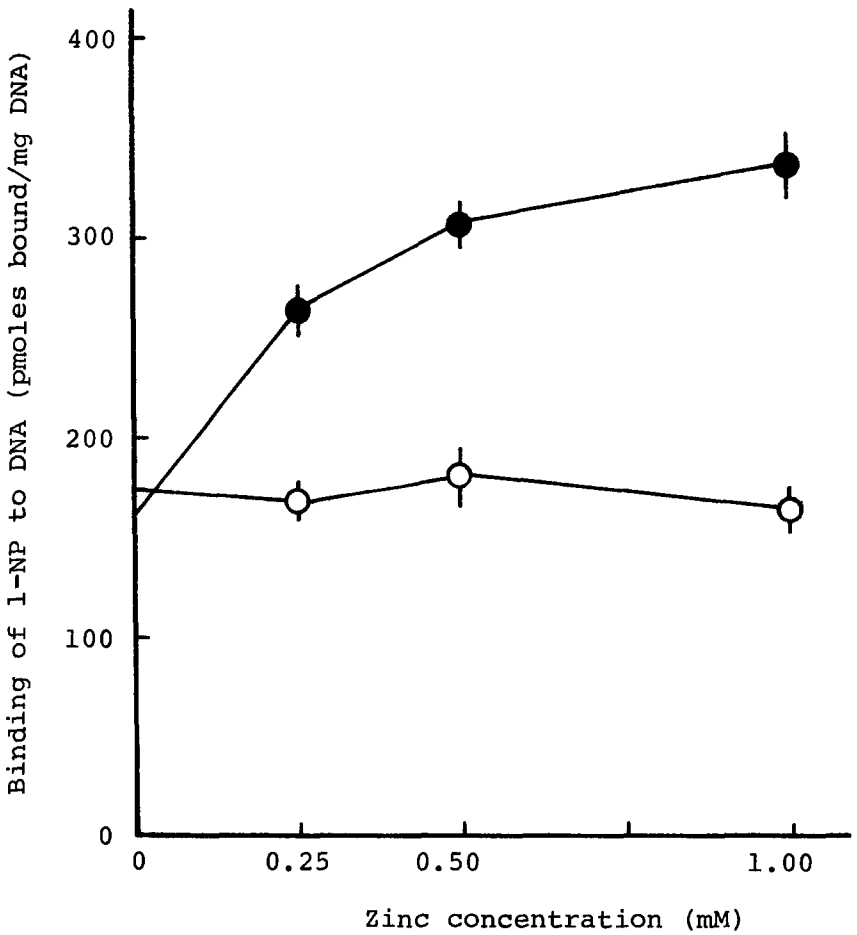

Figure 2. Effects on 1-NP binding to DNA of EDTA treatment after the preincubation of DNA with zinc acetate. The reaction mixtures added 5 mM EDTA were incubated for 60 min at 37°C. Each point was shown as a mean ± SD. (n=4)

-●- without EDTA -o- with EDTA

Zinc ions or complexes have been reported to transform the B-form of poly[dG-dC]·poly[dG-dC] to the Z-form (Fazakerley 1984). Therefore, under the conditions that zinc ions transform the B-form of Z-form, the effect of zinc acetate on the binding of 1-NP to poly[dG-dC]·poly[dG-dC] was examined and an increase of 1-NP binding to poly[dG-dC]·poly[dG-dC] was observed (Data not shown). Under our experimental conditions it is undertaken to investigate that the direct interaction of zinc acetate with DNA may alter the conformation of DNA to be more capable of binding of 1-NP.

Table 1. Effect of zinc acetate on the production of uric acid in the hypoxanthine-xanthine oxidase system

	Zinc concentration (mM)			
	0	0.25	0.50	1.00
Uric acid (produced, μM)	228 ± 4.8	231 ± 6.5	239 ± 9.1	234 ± 5.2

Each value is a mean ± SD (n=6)

Table 2. Effect of zinc acetate on the production of 1-aminopyrene in the hypoxanthine-xanthine oxidase system

	Zinc concentration (mM)			
	0	0.25	0.50	1.00
1-Aminopyrene (produced, μM)	4.1 ± 0.13	4.0 ± 0.16	3.9 ± 0.19	3.8 ± 0.11
1-Nitropyrene (remained, μM)	19.5 ± 0.97	20.6 ± 1.02	20.3 ± 1.18	19.7 ± 0.94

Each value is a mean ± SD (n=8)

The extent of 1-NP binding *in vitro* to DNA in the hypoxantnine-xanthine oxidase system was observed to increase by the addition of zinc acetate to the reaction mixtures. The enhancement by zinc ions of 1-NP binding to DNA was abolished by the addition of EDTA to the mixtures. Zinc acetate had no effect on the hypoxanthine-xanthine oxidase system. It is suggested that the enhancing effect of zinc acetate on the bindin of 1-NP to DNA may result in the alteration of DNA conformation.

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REFERENCES

Bergmann F, Dikstein S (1956) Studies on uric acid and related compounds III. Observations on the specificity of mammalian xanthine oxidases. J Biol Chem 223:765-780

Coleman JE (1979) Zinc in metalloproteins. In: Subcommittee on Zinc Committee on Medical and Biological Effect of Environmental Pollutants (ed), Division of Medical Sciences, Assembly of Life Sciences, National Research Council, University of Park Press, Baltimore,pp.211-223

Djuric Z, Fifer EK, Yamazoe Y, Beland FA (1988) DNA binding by 1-nitropyrene and 1,6-dinitropyrene *in vitro* and *in vivo*: effects of nitroreductase induction. Carcinogenesis 9:357-364

El-Bayoumy K, Rivenson A, Johnson B, DiBello J, Little P, Hecht SS (1988) Comparative tumorigenicity of 1-nitropyrene,1-nitrosopyrene and 1-aminopyrene administered by gavage to Sprague-Dawley rats. Cancer Res 48:4256-4260

Eichhorn GL(1973) Complexes of polynucleotides and nucleic acids. In:Eichhorn GL(ed) Inorganic Biochemistry,Elsevier Scientific Publishing Company, Amsterdam, pp.1210-1243.

Fazakerley GV (1984) Zinc Z-DNA. Nucleic Acids Res 12:3643-3648

Howard PC, Beland FA (1982) Xanthine oxidase catalyzed binding of 1-nitropyrene to DNA. Biochem Biophys Res Commun 104: 727-732

Howard PC, Heflich RH, Evans FE, Beland FA (1983) Formation of DNA adducts *in vitro* and in *Salmonella typhimurium* upon metabolic reduction of the environmental mutagens 1-nitropyrene. Cancer Res 43:2052-2058

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J Mol Biol 3: 208-218

Meissier F, Lu C , Andrews P, McCarry MA, Quilliam MA, McCalla DR (1981) Metabolism of 1-nitropyrene and formation of DNA adducts in *Salmonella typhimurium*. Carcinogenesis 2:1007-1011

Mildvan AS, Loeb LA (1981) The role of metal ions in the mechanism of DNA and RNA polymerases. In: Eichhorn GL, Marzilli LG(eds) Advances in Inorganic Biochemistry, Vol.3, Elsevier North Holland Inc., New York,pp.103-123.

Richmond R, Halliwell B, Chauhan J, Barbre A (1981) superoxide-dependent formation of hydroxyl radical: detection of hydroxyl radicals by the dydroxylation of aromatic compounds. Anal Biochem 118:328-335

Sakai K, Okazaki H, Arai K, Yamane Y (1985) Enhancement of DNA binding and mutagenicity of 1-nitropyrene by zinc acetate in Salmonella typhimurium TA100. Carcinogenesis 6:59-64

Sakai K, Suzuki M, Yamane Y, Takahashi A, Ide G (1990) Promoting effect of basic lead acetate administration on the tumorigenesis of lung in N-nitrosodimethylamine-treated mice. Bull Environ Contam Toxicol 44:707-714

Sakai K, Uchida R (1992) Comparative effects of potassium dichromate on the mutagenicity of some nitrohydrocarbons and methylating agents. Bull Environ Contam Toxicol 48: 541-548

Tokiwa H, Kitamori S, Nakagawa R, Horikawa K, Matamala L (1983) Demonstration of a powerful mutagenic dinitro-pyrene in airborne particulate matter. Mutat Res 121: 107-116

Yamane Y, Sakai K, Umeda T, Murata N, Ishizeki S, Takahashi A, Iwasaki I, Ide G,(1984) Suppressive effect of cupric acetate on DNA alkylation, DNA synthesis and tumorigenesis. Gann 75:1062-1069

Xu XB, Nachtman JP, Jin ZL, Wei ET, Rappaport SM (1982) Isolation and identification of mutagenic nitro-PAH in diesel exhaust particulates. Anal Chim Acta 136:163-174