Enhancing Effect of Zinc Acetate on the Binding of 1-Nitropyrene to Polydeoxyguanylic-polydeoxycytidylic Acid in the Hypoxanthine–Xanthine Oxidase System

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The main aim of this research was to investigate whether conformational alteration of polydeoxyguanylic-polydeoxycytidylic acid (poly[dG-dC]·poly[dG-dC]) by zinc acetate would have an effect on the binding of 1-nitropyrene (1-NP) to poly[dG-dC]·poly[dG-dC]. The binding of 1-NP to poly[dG-dC]·poly[dG-dC] in the hypoxanthine–xanthine oxidase system in vitro was increased by zinc acetate. This increase was abolished when EDTA was added to the preincubated mixture of poly[dG-dC]·poly[dG-dC] and zinc ions. Neither the production of 1-aminopyrene and its expected intermediates, N-hydroxy-1-aminopyrene and 1-nitrosopyrene, nor the 1-NP remaining in the reaction mixture was altered by the addition of zinc acetate without poly[dG-dC]·poly[dG-dC]. From these findings, it seems that the hypoxanthine–xanthine oxidase system is not activated by zinc acetate. On the other hand, under the same reaction conditions but the hypoxanthine–xanthine oxidase system and 1-NP, it has been reported that zinc ions or complexes transform the B-form of poly[dG-dC]·poly[dG-dC] to the Z-form. Therefore, these findings indicate that the formation of the Z-form or intermediates from the B-form by zinc ions may promote the binding of 1-NP to poly[dG-dC]·poly[dG-dC].

Keywords 1-nitropyrene; hypoxanthine–xanthine oxidase system; zinc acetate; polydeoxyguanylic-polydeoxycytidylic acid adduct

The mutagenicity and carcinogenicity of nitroaromatic hydrocarbons, including 1-nitropyrene (1-NP) have been reported.1,2 We have studied the effects of metal compounds on the mutagenicity3,4 and carcinogenicity5,6 of a variety of chemical compounds and recently reported7 that metal compounds enhance the mutagenicity of 1-NP and its binding to DNA in Salmonella typhimurium cells. We believe that the enhanced binding of 1-NP to DNA by zinc ions may alter the DNA conformation.3,5 Howard et al.8 have reported that a mammalian nitroreductase, xanthine oxidase [EC 1.2.3.2] catalyzes the binding of 1-NP to DNA and Fazakerley9 has reported that zinc ions or complexes transform the B-form of polydeoxyguanylic-polydeoxycytidylic acid (poly[dG-dC]·poly[dG-dC]) to the Z-form. However, it is not known if the altered conformation of poly[dG-dC]·poly[dG-dC] by zinc ions may enhance the binding of 1-NP to poly[dG-dC]·poly[dG-dC]. This paper reports the enhancing effect of zinc acetate on the binding of 1-NP to poly[dG-dC]·poly[dG-dC] in the hypoxanthine–xanthine oxidase system under the same experimental conditions used by Fazakerley.

MATERIALS AND METHODS

Xanthine oxidase (EC 1.2.3.2; from cow’s milk), hypoxanthine and poly[dG-dC]·poly[dG-dC] were obtained from Sigma Chemical Co.; 1-aminopyrene (1-AP) was from Aldrich Chemical Co. All other reagents used were of the highest grade available. 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 column of Zorbax ODS.10 [3H]1-NP was obtained from Amersham International Ltd. and further purified as described above.

The determination of the hypoxanthine–xanthine oxidase-catalyzed binding of 1-NP to poly[dG-dC]·poly[dG-dC] was performed according to the method of Howard et al.8 modified slightly as follows: Reaction mixtures (2 ml) containing 0.25 mg poly[dG-dC]·poly[dG-dC], 3.7 mM hypoxanthine, an appropriate concentration of zinc ion, and 25 μM tritiated substrate (9.73 MBq/μmol in 0.1 ml dimethyl sulfoxide (DMSO)) in 20 mM sodium cacodylate buffer, pH 7.1, were purged with argon for 5 min. The preincubation of poly[dG-dC]·poly[dG-dC] 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) incubated for 1 h at 37°C. The reactions were terminated by the addition of an equal volume of water-saturated phenol: isooamyl alcohol:chloroform (25:1:24), followed by vigorous mixing and centrifuging. This extraction sequence was repeated twice and the concentration of sodium chloride in the combined supernatants after centrifugation was adjusted to 5 m by adding sodium chloride. Poly[dG-dC]·poly[dG-dC] was precipitated from the aqueous phase by the addition of three volumes ice-cold ethanol. After poly[dG-dC]·poly[dG-dC] was redissolved in the cacodylate buffer, its concentration was determined spectrophotometrically at 260 nm. The binding of [3H]1-NP to poly[dG-dC]·poly[dG-dC] was measured with a Beckman LS 5800 scintillation counter using Aquasol-2 scintillator (New England Nuclear Co.).

Determination of the 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 20 mM sodium cacodylate buffer (pH 7.1) 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 h 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 absorbance of supernatant measured at 300 nm to determine the concentration of uric acid.13)

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 20 mM sodium cacodylate buffer (pH 7.1) 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 h 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 5C18 (4.6 × 250 mm) with a mobile phase of 80% CH3CN–H2O 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 HPLC were detected using a fluorescence detector (RF-530, excitation, 360 nm; emission, 425 nm).

RESULTS AND DISCUSSION

Zinc ions or complexes have been reported to transform the B-form of poly[dG-dC]·poly[dG-dC] to the Z-form in 2 mM sodium cacodylate buffer (pH 7.1).19) Fazakerley has stated that the addition of zinc ions to mixtures containing poly[dG-dC]·poly[dG-dC] produces a cooperative transition with an isobestic point at 278 nm, leading to the inverted spectrum typical of the Z-form. Circular dichroism spectra of poly[dG-dC]·poly[dG-dC] in the presence of zinc acetate were examined according to the method of Fazakerley. The alterations in the circular dichroism spectra in our experiments also agreed with those of Fazakerley.

The binding of 1-NP to poly[dG-dC]·poly[dG-dC] was catalyzed by the hypoxanthine–xanthine oxidase system using 20 mM sodium cacodylate buffer (pH 7.1) and the degree of this binding increased with time when the reaction mixtures were incubated at 37 °C, as shown in Fig. 1. Our results are very similar to those of Howard et al.8) The addition of zinc acetate (0.25 mM) to the reaction mixtures increased the degree of 1-NP binding to poly[dG-dC]·poly[dG-dC] over the incubation period, as shown in Fig. 1. The extent of 1-NP binding to poly[dG-dC]·poly[dG-dC] in the hypoxanthine–xanthine oxidase system increased with increasing zinc concentration, as shown in Fig. 2. By adding 0.25, 0.5 and 1.0 mM zinc acetate, the degree of 1-NP binding to poly[dG-dC]·poly[dG-dC] increased 1.6-fold, 1.9-fold and 2.3-fold respectively, compared with the control (224 ± 7.1 pmol bound/mg deoxynucleotides, without addition of zinc acetate). On adding EDTA to the reaction mixtures, the increase in 1-NP binding to poly[dG-dC]·poly[dG-dC] produced by zinc acetate was abolished.

As an explanation of the finding, that the addition of zinc acetate to the reaction mixtures increases the binding of 1-NP to poly[dG-dC]·poly[dG-dC], it is suggested that zinc acetate may have an enhancing effect on the enzyme system. Therefore, the effect of zinc acetate on the amount of uric acid produced by the hypoxanthine–xanthine oxidase system was examined when it was found that zinc acetate had no significant effect, as shown in Fig. 3A. The effect of zinc acetate on the production of 1-AP from 1-NP in the xanthine oxidase system, without the
addition of poly[dG-dC]·poly[dG-dC], was also examined. However, neither the production of 1-AP nor the 1-NP remaining in the reaction mixtures, without adding poly[dG-dC]·poly[dG-dC], was significantly altered by the addition of zinc acetate, as shown in Fig. 3B. Furthermore, the addition of zinc acetate to the reaction mixtures without poly[dG-dC]·poly[dG-dC] produced no significant alteration in the peaks (over a 12 min development time) in the HPLC elution profiles under our experimental conditions. These profiles would contain the peaks that were considered to be expected intermediates, N-hydroxy-
1-aminopyrene (retention time, 2.5 min) and 1-nitrosopyrene (retention time, 4.7 min), prepared in situ by the reduction of 1-NP with palladium on carbon and hydrazine at \(-5^\circ C\). Therefore, zinc acetate was considered to have no effect on the activity of xanthine oxidase.

An other explanation for the finding that zinc acetate increases the binding of 1-NP to poly[dG-dC]·poly[dG-dC] is that the direct interaction of zinc ions with poly [dG-dC]·poly[dG-dC] may alter its conformation to give a form more capable of binding 1-NP.

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REFERENCES