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Comparative Effects of Potassium Dichromate on the Mutagenicity of Some Nitrohydrocarbons and Methylating Agents

Kazuo Sakai and Riichiro Uchida

Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Chiba 260, Japan

Chromium compounds are used in the metallurgical industry, particularly in relation to the production and use of ferrochromium alloys and stainless steel, in the pigment, paint and dying industries and as an important component of refractory materials such as bricks, glass and ceramics. Chromiun (VI) compounds such as potassium dichromate(Cr(VI)) have been shown to be potent mutagens and carcinogens. In various bacterial assay systems containing Ames test, mutagenicity of chromium(VI) has been reported(Lofroth and Ames 1978;Leonard and Lauwerys 1980; Bianchi et al. 1983). It has been considered as leading to mutagenesis and carcinogenesis that DNA single strand break and cross-linking of DNA nuclear proteins are caused by the reaction of DNA with chromium (III) inside the cell. Methylating agents such as methylmethanesulfonate(MMS) and methylnitrosourea(MNU) are well-known mutagens and carcinogens. They have been reported to modify the residues of bases such as O-6-position of guanine by reaction with DNA, leading to mutagenesis and carcinogenesis (Gerchman and Ludlum 1973;Singer 1975). 1-Nitropyrene (1-NPy) and 2-nitropropane(2-NPro) are also mutagens and carcinogens (Chiu et al.1978; Speck et al.1982; Hirose et al.1984;Fiala et al.1987) and the modification of 8-position of guanine in DNA by them has been reported to lead to mutagenesis and carcinogenesis (Howard et al. 1983 ; Kuchino et al. 1987). In this report the comparison of effects of Cr(VI) on the mutagenicity of nitrohydrocarbons and methylating agents was examined.

MATERIALS AND METHODS

Cr(VI) and 2-NPro was obtained from Wako Pure Chemical Industries (Osaka), 1-NPy was from Tokyo Kasei Kogyo, Ltd (Tokyo), MNU was from Sigma Chemical Co. (St Louis, MO), MMS was from Nakalai Tesque, Inc.(Kyoto). All other reagents used in this study were of special grade. 1-NPy was purified by chromatography on neutral alumina (Merck, Darmstadt) with benzene as the eluant. Bacterial strain used in the experiment is Salmonella typhimuium TA100

Send reprint requests to Kazuo Sakai at the above address.

and the authors are grateful to Dr.B.N. Ames, University of California at Berkeley, California , for a generous gift of the strain. The mutagenicity test was performed as follows. The liquid preincubation method (Sakai et al. 1985), a modified method of the test described by Ames et al. (1975) was used. Mixtures of overnight culture (0.1 ml; 5 x 10^7 cells) and Cr(VI) in 0.2 M BES-NaOH buffer (0.5 ml; pH 7.4) were preincubated for 30 min at 37 ° C. After the pretreatmet , other direct-acting mutagens in dimethylsulfoxide (0.1 ml) was added to the mixtures and incubation was continued for an additional 30 min at 37° C. After addition of 2 ml of soft agar(0.8% Difco agar supplemented with 0.1 µmol of L-histidine and 0.1 µmol of D-biotin in 0.6% sodium chloride), the mixtures were poured onto minimal glucose agar plate with Vogel-BonnerE medium and incubated for 48 hr in the dark at 37° C. The colonies were counted as revertant colonies.

RESULTS AND DISCUSSION

The effect of pretreatment with Cr(VI) on the mutagenicity of MNU or MMS was examined. As shown in Figure 1-A, the number of revertant colonies induced by MNU (0.5 mM) was decreased by the pretreatment of the cells with Cr(VI) in the preincubation mixtures, under the experimental conditions used. The number of revertant colonies induced by MNU (0.5 mM) alone were much more than that induced by Cr(VI) alone. This may show that the pretreatment with Cr(VI), the weaker mutagen supresses the mutagenicity of MNU. It is known that the methylated bases containing O^6-methylguanine in DNA produced by MNU may lead to mutagenesis. The complex formation of chromium ion with DNA may have an effect on the methylation site of DNA which leads to mutagenesis. Therefore the complex formation of chromium ion,the weaker mutagen with DNA by the pretreatment of Cr(VI) may lead to the suppression of mutagenicity of MNU which is stronger than that of Cr(VI). As shown in Figure 1-B, the number of revertant colonies induced by MMS (2 mM) did not appear to be altered by the pretreatment of the cells with Cr(VI) in the preincubation mixtures. The number of revertant colonies induced by MMS (2 mM) is approximately same as that induced by Cr(VI)(0.10-0.15 mM) alone. This may show that the decreasing extent of mutagenicity of MMS by the pretreatment with Cr(VI) is equivalent to the increasing extent of mutagenicity of Cr(VI) itself.

The effect of pretreatment with MNU or MMS on the mutagenicity of Cr(VI) was examined. The number of revertant colonies induced by MNU (0.5 mM) or MMS (2 mM) was not altered by the addition of Cr(VI) after the pretreatment of the cells with MNU or MMS in the preincubation mixtures, as shown in Figures 2-A and 2-B. This may show that the methylation of bases containing O-6-position of

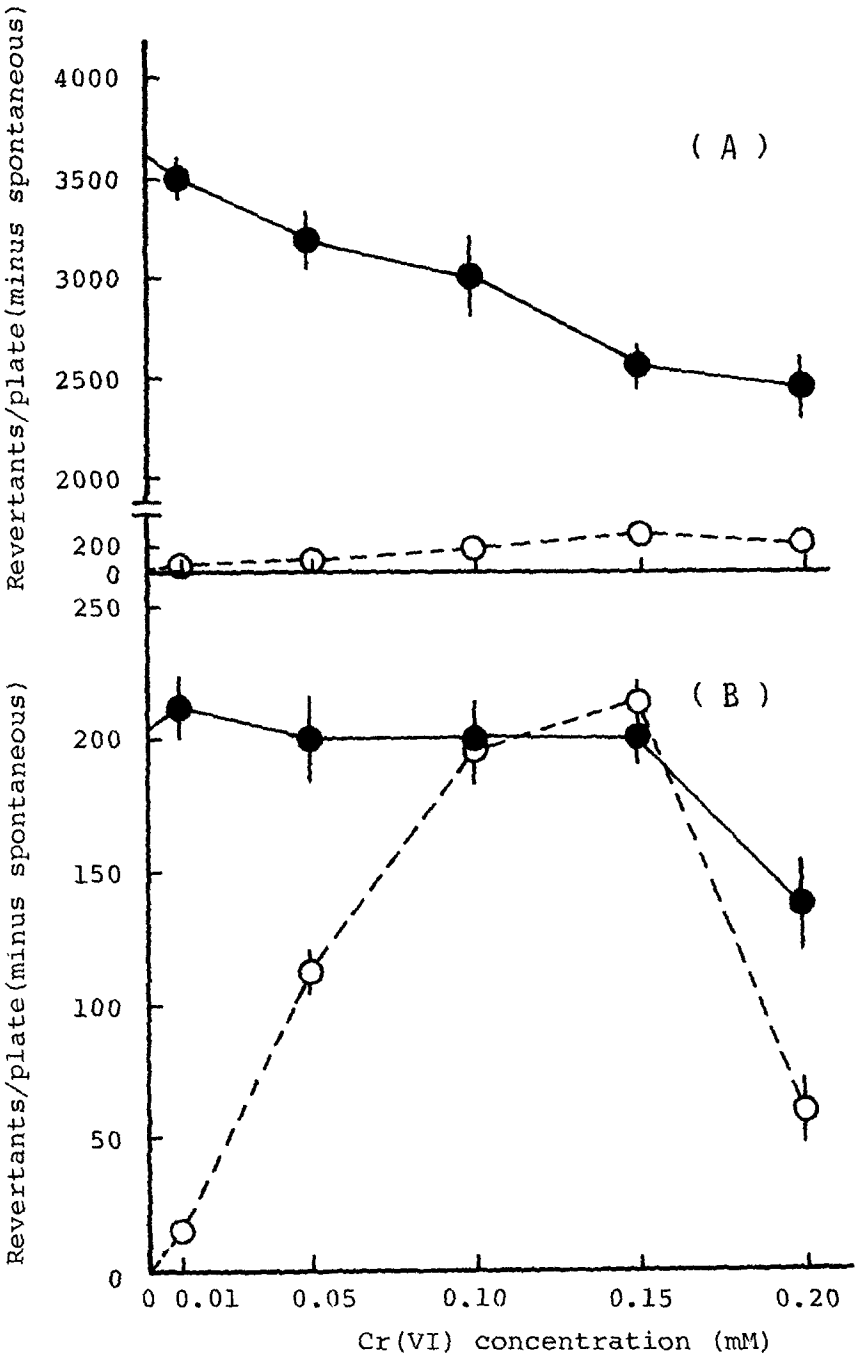

Figure 1. Effect of pretreatment with potassium dichromate on the mutagenicity of methylnitrosourea (0.5 mM, MNU) and methylmethanesulfonate (2 mM, MMS) in Salmonella typhimurium TA100. Each point is shown as mean ± SD of 5 or 6 plates.
(A) -O-, Cr; -●-, Cr + MNU (B) -O-, Cr; -●-, Cr + MMS

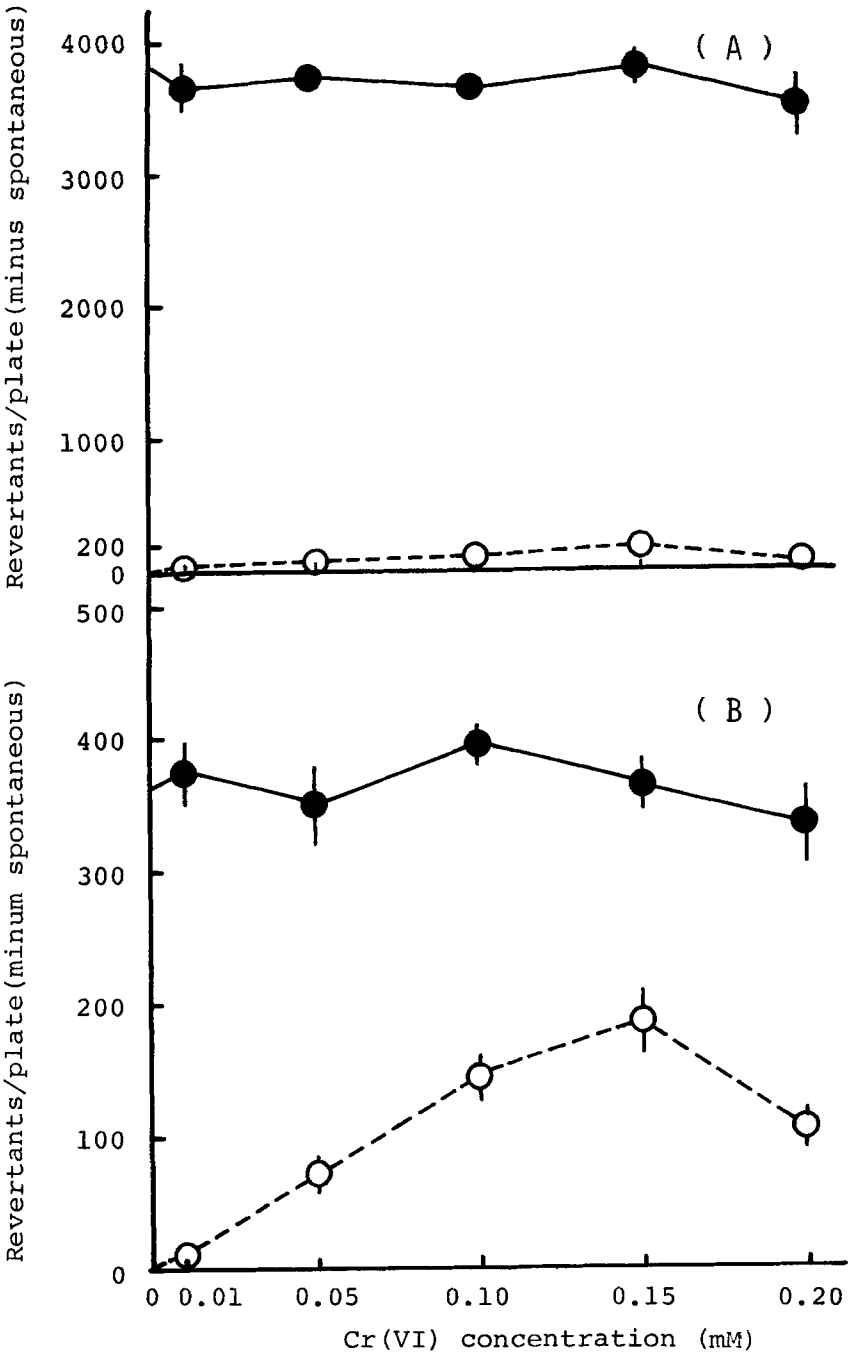

Figure 2. Effect of pretreatment with methylnitrosourea (0.5 mM, MNU) or methylmethanesulfonate (2 mM, MMS) on the mutagenicity of potassium dichromate in Salmonella typhimurium TA100. Each point is shown as mean ± SD of 5 or 6 plates.
(A) -O-, Cr; -●-, MNU + Cr (B) -O-, Cr; -●-, MMS + Cr

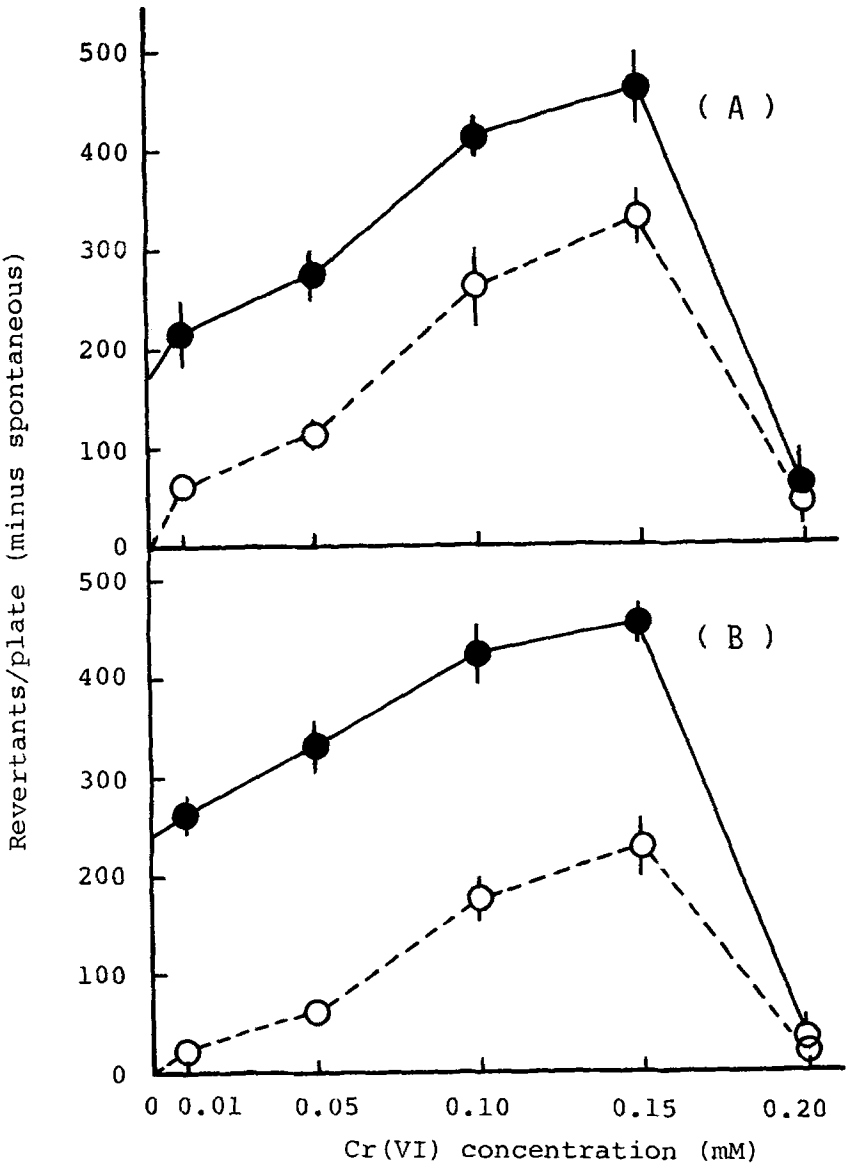

Figure 3. Effect of pretreatment with potassium dichromate on the mutagenicity of 1-nitropyrene (1 μM, 1-NPy) and 2-nitropropane (50 mM, 2-NPro) in Salmonella typhimurium TA100. Each point is shown as mean ± SD of 5 or 6 plates.
(A) -O-, Cr; -●-, Cr + 1-NPy
(B) -O-, Cr; -●-, Cr + 2-NPro

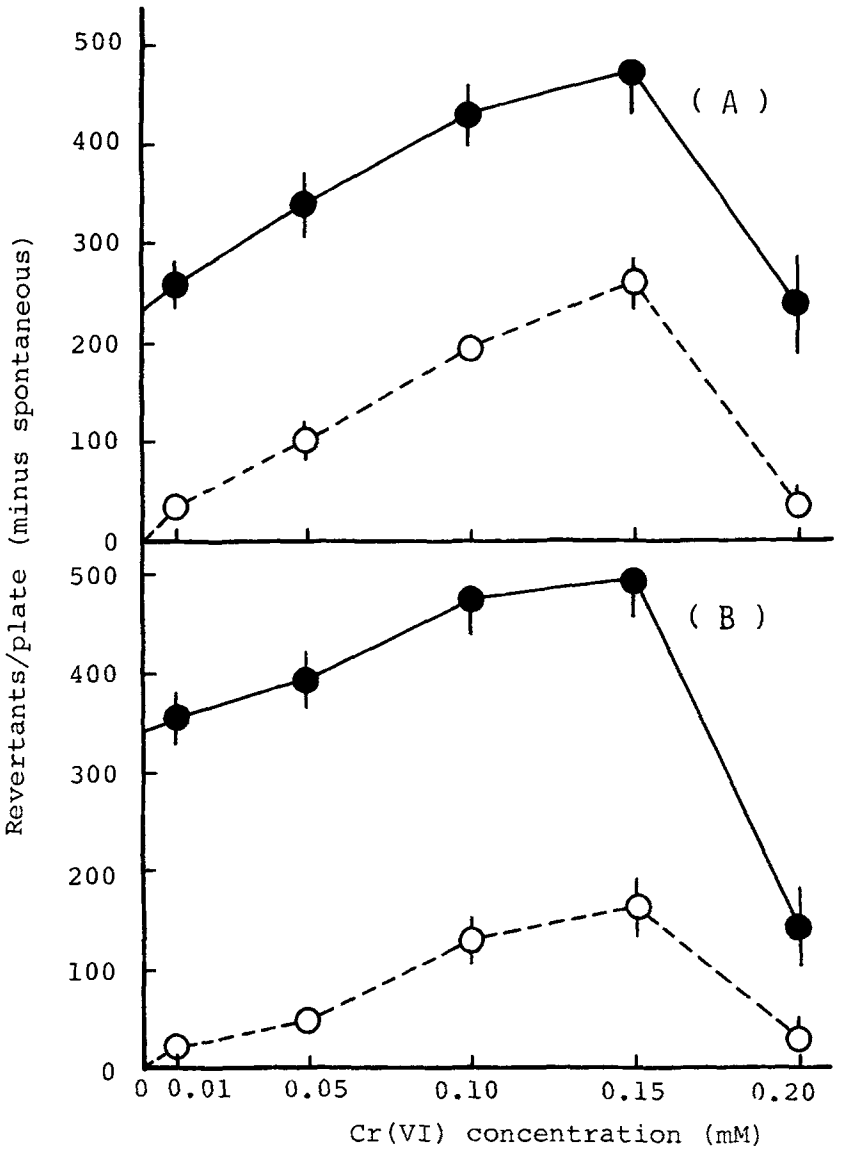

Figure 4. Effect of pretreatment with 1-nitropyrene (1 μM, 1-NPy) or 2-nitropropane (50 mM, 2-NPro) on the mutagenicity of potassium dichromate in Salmonella typhimurium TA100. Each point is shown as mean ± SD of 6 plates.
(A) -O-, Cr; -●-, 1-NPy + Cr
(B) -O-, Cr; -●-, 2-NPro + Cr

guanine in DNA by MNU or MMS have a great suppressive effect on the complex formation of chromium ion with DNA and the mutagenicity of Cr(VI).

It has been reported that the major DNA adduct in the DNA-binding species formed by the reaction of 1-NPy with DNA in the cell is N-(deoxyguanosine-8-yl)aminopyrene (Howard et al. 1983). Recently 2-NPro has been reported to produce 8-hydroxydeoxyguanosine in DNA in vivo (Fiala et al.1989), which leads to mutagenesis and carcinogenesis (Kuchino et al. 1987). Under our experimental conditions, the 8-position of guanine in DNA in the cell may be modified by 1-NPy or 2-NPro and is different from the position of bases in DNA modified by MNU and MMS. Therefore the effect of pretreatment with Cr(VI) on the mutagenicity of 1-NPy or 2-NPro was examined. As shown in Figure 3-A, the number of revertant colonies induced by both Cr(VI) and 1-NPy appears to be same as the sum of those induced by Cr(VI) alone and 1-NPy alone. This may show that the complex formation of chromium ion with DNA by the pretreatment of Cr(VI) has little or no effect on the modification of 8-position of guanine and the mutagenicity by 1-NPy. The effect of pretreatment with 1-NPy on the mutagenicity of Cr(VI) was also examined. As shown in Figure 4-A, the number of revertant colonies induced by both 1-NPy and Cr(VI) appears to be same as the sum of those induced by 1-NPy alone and Cr(VI) alone.

It has been reported that 2-NPro is mutagenic but the mechanism of action for mutagenicity of 2-NPro is not clear. The recent report that 2-NPro produces 8-hydroxydeoxyguanosine in DNA in vivo (Fiala et al. 1989) suggests that in our experimental system 2-NPro may produce 8-hydroxydeoxyguanosine in DNA which leads to mutagenesis. It is of interest to know whether the formation of 8-hydroxydeoxyguanosine by 2-NPro contributes mainly to mutagenesis. Therefore the effect of pretreatment with Cr(VI) on the mutagenicity of 2-NPro was examined. As shown in Figure 3-B, the number of revertant colonies induced by both Cr(VI) and 2-NPro appears to be same as the sum of those induced by Cr(VI) alone and 2-NPro alone. This may show that the pretreatment with Cr(VI) has little or no effect on the mutagenicity of 2-NPro. The effect of pretreatment with 2-NPro on the mutagenicity of Cr(VI) was also examined and the number of revertant colonies induced by both 2-NPro and Cr(VI) appears to be same as the sum of those induced by 2-NPro alone and Cr(VI) alone, as shown in Figure 4-B. These results may show that the formation of 8-hydroxydeoxyguanosine in DNA by 2-NPro contributes mainly to mutagenesis.

Under the conditions tested, the pretreatment with Cr(VI) had a suppressive effect on the mutagenicity of MNU and MMS, while the pretreatment with Cr(VI) had little or no

effect on the mutagenicity of 1-NPy and 2-NPro.

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