BioMetals 1992, **5**, 157–161

Mutagenic activity of copper(II) chromate and dichromate complexes with polypyridines

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Received 16 April 1992; accepted for publication 29 April 1992

Copper(II) chromate and dichromate complexes with 2,2′-bipyridyl and 1,10-phenathroline were tested for their mutagenic activity in the standard Ames test. All of six tested complexes exhibited markedly lower mutagenic activity than the reference compounds—potassium dichromate and sodium chromate. The blockage of Cr(VI) reduction capability in the presence of the complex Cu^{2+} ion and the competition between copper and chromium ions in the interaction with cellular components are discussed in the light of the results of our previous chemical study.

Keywords: Ames test, chromium, mutagenicity, polypyridines

Introduction

The mutagenic and carcinogenic properties of hexavalent Cr(VI) are well established (Leonard & Lauwerys 1980, Bianchi & Lewis 1987, Fan & Harding-Barlow 1987) although the genotoxic potency of various Cr(VI) compounds differs significantly (Petrilli *et al.* 1986, De Flora *et al.* 1990). The reason for such a divergency is still not clear except for the solubility of chromium compounds (Lewis & Majone 1981, Beyersmann 1989). Data for the mutagenic activity of simple chromates are available; however, Cr(VI) complexes have not yet been studied.

The aim of the present paper is the study of the mutagenic activity of Cu(II) chromate and dichromate complexes with 2,2′-dipyridyl (bpy) and 1,10-phenathroline (phen) to elucidate the influence of complex cations on the mutagenic potency of Cr(VI) ions in the Ames test.

The tested complexes belong to the M(HB)A-type (where M represents a metal ion, HB a heterocyclic base and A an oxidant), which are successfully used as mild and efficient reagents in organic synthesis (Firouzabadi *et al.* 1984, Firouzabadi & Sardarian 1986) ($M = Ag^+$, Cu^{2+}; BH = py, bpy; A = MnO_4^-, $Cr_2O_7^{2-}$).

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Materials and methods

Chemicals

The tested complexes were synthesized by M. Cieślak-Golonka (Cieślak-Golonka *et al.* 1991). Standard mutagens, i.e. 4-nitroquinoline-*N*-oxide (NQNO), methyl methanesulfonate (MMS), 2-aminofluorene (2AF), potassium dichromate and sodium chromate, were purchased from Sigma (USA). NADP and glucose-6-phosphate were also obtained from Sigma. Other chemicals used for buffers and media preparation were obtained from POCh (Poland). Oxoid nutrient (Oxoid, UK), Difco nutrient broth and Difco agar (Difco, USA) were applied for bacterial growth. $CuphenCl_2$, kindly donated by Dr T. Tlaczala, was studied for comparison purposes.

Mutagenicity

The mutagenic activity of potassium dichromate, sodium chromate and copper chromate as well as copper chromate and dichromate complexes with organic ligands were tested according to Ames (Ames *et al.* 1975, Maron & Ames 1983) with *Salmonella typhimurium* TA100 and TA102 strains (kindly supplied by Dr Bruce N. Ames) with and without an activation system (S9 fraction). The efficiency of histidine prototrophy reversion was measured by subtracting the spontaneous reversion background of

the particular strain from the plate counts in each experiment; the averages of these backgrounds were 132 and 376 revertants per plate, respectively, for TA100 and TA102. The tested salts and complexes were dissolved in formamide and assayed in the dose range from 25 to 400 nmol per plate, which depended on their toxicity to bacterial cells. The only exception was copper chromate, insoluble in formamide, which was dissolved in water acidified to pH 2.0. Activating S9 fraction was prepared according to Ames (Ames *et al.* 1975), i.e. from the liver of Aroclor 1254 treated male Wistar rats. S9 fraction was used at a volume of 50 μl (containing 1.5–1.75 mg of protein) per plate.

The Cr(VI) complexes were tested in three independent experiments using three repetitions of each dose. In all experiments control assays of bacterial strains were performed to check the efficiency of strains actually used in mutagenicity searching. The standard mutagens were NQNO (at a dose of 0.5 μg per plate) for TA100, MMS (1 μl per plate) for TA102 and a promutagen 2AF (5 μg per plate) for TA100 with fraction S9.

Results

Mutagenic activities of Cr(VI) complexes in comparison to the reference Cr(VI) compounds (sodium chromate and potassium dichromate) are shown in Figures 1 & 2. Only 1 nmol concentration of each chromium complex was given in Figures 1 & 2. It was the highest concentration of each particular complex which caused a mutagenic effect without any toxic influence on bacterial cells. The complexes inhibited bacterial growth at concentrations higher than those which were given in Figures 1 & 2. In the case of reference compounds, from the broad range of tested doses we have chosen only that part for which the dose–response effect was linear. Regression equations were calculated for these dose ranges.

The results obtained with strain TA100 are shown in Figure 1. It can be seen that at least three groups of Cr(VI) complexes might be distinguished according to their responses in the Ames test. These are (i) the group of highly toxic complexes, where slight or no mutagenic effect was observed (D, F and G), (ii) the group of low toxicity and small mutagenic activity complexes (A, B and C), and (ii) one complex which exhibited relatively high mutagenic activity and a low toxic effect (E).

The same is true in the case of results from experiments with TA102 (Figure 2) with one difference: complexes B and C were more toxic for TA102 than for TA100.

It can be concluded from Figures 1 & 2 that from the tested chromium complexes, only $Cu(bpy)Cr_2O_7$ (E) exhibited marked mutagenic activity. However,

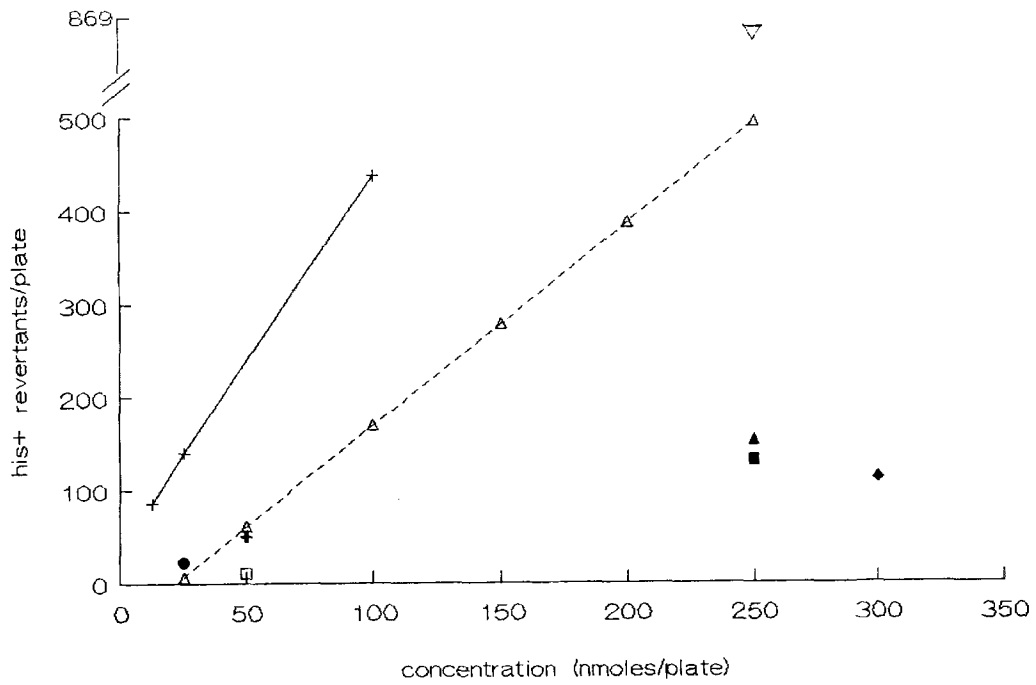

Figure 1. Mutagenic activity of Cr(VI) complexes on *S. typhimurium* TA 100 strain. (+) $K_2Cr_2O_7$ ($y = 31.62 + 4.35x$; $r = 0.916$); (△) Na_2CrO_4 ($y = -48 + 2.18x$; $r = 0.980$); (◆) A ($CuCrO_4$); (■) B ($[Cu(bpy_2)]_2CrO_4 \cdot 2H_2O$); (▲) C ($[Cu(bpy)_2]_2CrO_4Cl_2 \cdot 2H_2O$); (●) D ($[Cu(bpy)_2]_2CrO_4(NO_3)_2 \cdot 4H_2O$); (▽) E ($Cu(bpy)Cr_2O_7$); (+) F ($Cu(phen)_2Cr_2O_7$); (□) G ($Cu(phen)_2CrO_4 \cdot 2H_2O$).

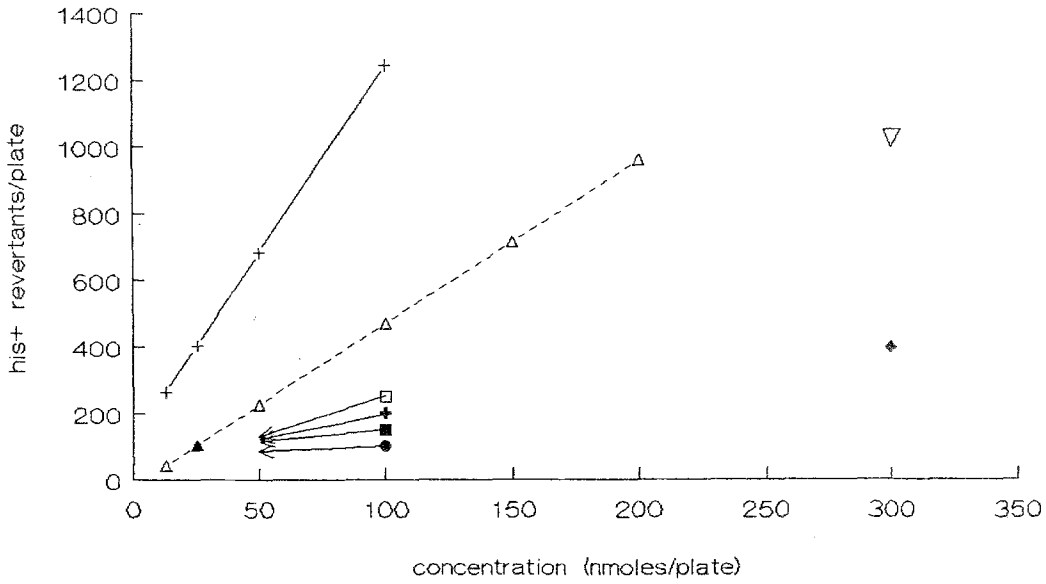

Figure 2. Mutagenic activity of Cr(VI) complexes on *S. typhimurium* TA 102 strain. (+) $K_2Cr_2O_7$ ($y = 122.8 + 11.2x$; $r = 0.987$); (△) Na_2CrO_4 ($y = -19.2 + 4.89x$; $r = 0.994$). The graphic and alphabetical codes of metal complexes are the same as given in Figure 1.

if the mutagenic activities of this complex are compared with the mutagenicity of the reference compound (potassium dichromate) at lower doses (data not presented in Figures 1 & 2) it can be seen that the mutagenic potency of $Cu(bpy)Cr_2O_7$ was markedly lower. For instance, at a concentration of 100 nmol per plate the average number of *his*$^+$ revertants was 100 in the case of $Cu(bpy)Cr_2O_7$ versus 424 in the case of potassium dichromate for TA100, and 258 versus 1208 for TA102.

Table 1 contains the data of the specific mutagenicity of Cr(VI) complexes (expressed as the number of *his*$^+$ revertants per plate induced per nanomole of complex), estimated with TA100 and TA102. Significant differences in the toxicities of various chromium complexes led to the differences in dose numbers taken into account in the calculation of average specific activities ($\bar{X} \pm$ SD); toxic dose results were excluded.

As can be seen from Table 1, strain TA102 was more sensitive to chromium complexes than TA100, especially with regard to the determination of the mutagenicity of chromate complexes. As compared with sodium chromate, the specific mutagenic

Table 1. Specific mutagenic activity of Cr(VI) complexes on *S. typhimurium* strains

	Revertants/nmol/plate[a]			
	TA100		TA102	
Compound	$\bar{X}$	SD	$\bar{X}$	SD
$CuCrO_4$	0.190	0.093	1.082	0.226
$Cu(bpy)_2CrO_4 \cdot 2H_2O$	0.211	0.180	1.840	0.520
$[Cu(bpy)_2]_2CrO_4Cl_2 \cdot 2H_2O$	0.477	0.097	2.920	0.880
$[Cu(bpy)_2]_2CrO_4(NO_3)_2 \cdot 2H_2O$	0.651	0.249	2.171	0.469
$Cu(bpy)Cr_2O_7$	1.762	1.050	3.600	0.990
$Cu(phen)_2Cr_2O_7$	1.047	0.159	3.390	0.968
$Cu(phen)_2CrO_4 \cdot 2H_2O$	0.292	0.092	3.155	0.605
$K_2Cr_2O_7$	4.900	1.367	15.525	3.309
Na_2CrO_4	1.419	0.522	4.950	1.573

[a]Values derived from number of revertants obtained for two to six doses of complexes, depending on their toxicities to bacterial cells.

activities of chromium complexes were from 1.5 and 1.7 times lower for $Cu(phen)_2CrO_4 \cdot 2H_2O$ and $Cu(bpy)_2CrO_4 \cdot 2H_2O$, respectively, to 2.3 and 2.7 times lower for $[Cu(bpy)_2]_2CrO_4(NO_3)_2 \cdot 2H_2O$ and $Cu(bpy)_2CrO_4 \cdot 2H_2O$. Specific mutagenic activities of dichromate complexes were generally more than 4 times lower than the specific activity of the reference compound (potassium dichromate).

In the case of strain TA100 the decrease of specific mutagenic activity of chromate complexes as compared with the reference compound (sodium chromate) ranged from 2.2 times for $[Cu(bpy)_2]_2CrO_4(NO_3)_2 \cdot 2H_2O$ to 7 times for $Cu(bpy)_2CrO_4 \cdot 2H_2O$ and 7.5 times for copper chromate. Dichromate complexes also exhibited lower specific mutagenic activities than the reference compound (potassium dichromate): in the case of $Cu(phen)_2Cr_2O_7$ an almost 4.7 times and for $Cu(bpy)Cr_2O_7$ an almost 3 times decrease of activity was observed.

In all tested Cr(VI) complexes the presence of S9 fraction caused a significant decrease of *his*$^+$ revertant induction, even to the level of background spontaneous revertant numbers (therefore the data are not presented).

The mutagenic potency of the organic ligands, i.e. 1,10-phenanthroline, 2,2′-bipyridyl as well as $Cu(phen)Cl_2$, was assesed under the same conditions as used for Cr(VI) complexes. At doses of 25–50 nmol per plate none of these compounds was active in either TA100 or TA102 (both in the presence and in the absence of S9 fraction). Organic ligand concentrations higher than 50 nmol per plate were toxic to bacterial cells.

Discussion

It has been well documented that Cr(VI) compounds are mutagenic in a number of bacterial systems (Venit & Levy 1974, Petrilli & De Flora 1977, Baker *et al.* 1984, Zakour & Glickman 1987, De Flora *et al.* 1990) while most Cr(III) compounds are not mutagenic in those systems (Langerwerf *et al.* 1985, De Flora *et al.* 1990). However, some of the trivalent chromium complexes showed mutagenic activity in bacterial tests and it was suggested that the mutagenicity of Cr(III) complexes with various organic ligands depends mainly upon the chemical character of a ligand (Warren *et al.* 1981). Cr(III) complexes containing aromatic amine ligands like 2,2′-bipyridyl or 1,10-phenanthroline exhibited DNA-damaging capability in repair assays as well as mutagenic activity in the Ames test (Warren *et al.* 1981).

According to Ames (Ames *et al.* 1975) a compound is mutagenic if it generates a linear dose-response and causes more than double the background number of spontaneous revertants. Among six Cr(VI) complexes tested here, only one, i.e. $Cu(bpy)Cr_2O_7$, fits these rules; however, its mutagenic activity was still significantly lower than that of the reference compound, i.e. potassium dichromate, at comparable doses (Table 1 and Figures 1 & 2). Two of the Cr(VI) complexes, i.e. $Cu(bpy)_2Cr_2O_7$ and $[Cu(bpy)_2]_2CrO_4Cl_2 \cdot 2H_2O$, showed only low mutagenic activity (i.e. they induced about double the background number of spontaneous revertants) and three of the complexes, i.e. $[Cu(bpy)_2]_2CrO_4(NO_3)_2 \cdot 2H_2O$, $Cu(phen)_2CrO_4 \cdot 2H_2O$ and $Cu(phen_2)Cr_2O_7$, exhibited no measurable mutagenicity in the Ames test (Figures 1 & 2).

Our previous electronic and molecular investigations (Cieślak-Golonka *et al.* 1991) indicated that determination of the physicochemical properties and reactivity of these complexes would be helpful in predicting and explaining the mutagenic activity data. It seems to be especially important in explaining the decrease of the observed Cr(VI) mutagenicity in the compounds studied. The mutagenic activity of the six valent chromium can be linked to its strong oxidation tendency towards many organic and inorganic species (Connett & Wetterhahn 1983, Mitewa & Bontchev 1985). This redox process seems to be a condition *sine qua non* for the appearance of mutagenic and toxic activity (Connett & Wetterhahn 1983, Appenroth *et al.* 1991).

In the complexes of Cu(II) and Ag(I) chromates with polypyridines, as well as in other metal ion complexes with various ligands, the bridging $^{-O}_{-O}\!>CrO_2$ type coordination of C_{2v} symmetry for the chromate ion has been proposed on the basis of the IR spectroscopic (Cieślak-Golonka *et al.* 1988, 1991) and crystallographic (Cieślak-Golonka 1991) studies. That means that, at least in the solid state, no ionic tetrahedral chromates exist both in copper chromate and its complexes. A lower tendency for coordination was observed in the dichromate ion (Puglisi 1970). It may be presumed that the coordination of the chromate anion to the metal ions via its oxygen atoms may also block two of four oxygen atoms in solution. If so, it prevents the formation of the $CrO_3(OR)^-$ entity (where R is an organic part), a pre-redox intermediate form found in the Cr(VI) –biomolecule systems (Brauer & Wetterhahn 1991), and thus prevents the redox process taking place.

The role of Cu(II) ions in the diminution of the mutagenicity of Cr(VI) complexes also should not be neglected; in our experiments (Table 1) Cu(II) chromate exhibited the lowest mutagenic activity. It

is well known that the Cu(II) ion exhibits a strong potency for coordination to various organic molecules and could markedly diminish any possible interaction of Cr(VI) ions with cellular components.

All of the above mentioned aspects, i.e. the oxidation tendency of Cr(VI), its coordination ability preventing the redox process and the role of Cu(II) ions, should be taken into account when attempting to explain the results of our biological studies. The results show that copper chromate and dichromate complexes with polypyridines, as well as copper chromate itself, exhibited lower mutagenic effects than the reference chromate (sodium) and dichromate (potassium). Detailed explanation of our biological results needs further investigation.

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