AN ABSTRACT OF THE THESIS OF

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Title: [Cr3O(O2CCH2CH3)6(H2O)3]+ (Cr3) toxicity potential in bacterial and mammalian cells

Abstract approved:

Chromium(III) has generally been considered to be essential for proper carbohydrate and lipid metabolism, and despite recent evidence to the contrary, chromium(III)-containing compounds remain one of the most popular commercial dietary supplements. Cr3, or $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$, is a trivalent chromium compound that is a promising chromium nutritional supplement. Studies with Cr3 have indicated that it is nontoxic in developmental and short- and long-term exposures studies in rodents, but the safety of this compound to chromosomes and cells has not been explored. The current study evaluates the mutagenicity, cytotoxicity, and clastogenicity of Cr3 in bacterial and mammalian cells. Mutagenicity was tested in *Escherichia coli* FX-11 after treatment with Cr3 or chromium picolinate (CrPic) from 0.025 mM to 1.0 mM for 1 h/48 h. *Salmonella typhimurium* (TA 98 and TA 100) were also tested for mutagenicity after treatment with Cr3 or CrPic up to 10,000 µg/plate with and without S9 metabolism. Cytotoxicity was measured as a decrease in plating efficiency relative to controls after treatment with 4.0 µg/cm^2, 20 µg/cm^2, 40 µg/cm^2, and 80 µg/cm^2 of Cr3 and CrPic for 24 h in CHO K1 cells. Clastogenicity was measured by counting the number of metaphases damaged and of the total number

chromosomal aberrations in CHO K1 cells. Mutagenesis assays in *E. coli* and *S. typhimurium* were negative both with and without S9 mixture. All treatments of Cr3 produced ≥ 84% plating efficiency except 80 μg/cm^2, which reduced the plating efficiency to 62%. Cr3 at the above treatments did not produce a significant increase in the number of cells with abnormal metaphases, while treatments ≥ 40 μg/cm^2 of CrPic elevated the number significantly. These data suggest that Cr3 is neither mutagenic in bacteria cells nor clastogenic in CHO K1 cells.

$[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ (Cr3) TOXICITY POTENTIAL IN BACTERIAL AND MAMMALIAN CELLS

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PREFACE

This thesis was prepared following the publication style of *Biological Trace Element Research.*

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INTRODUCTION

The element chromium (Cr) in its trivalent oxidation state has been thought to be essential for proper carbohydrate and lipid metabolism for nearly forty years [1-3]. In 2004, the U.S. National Academy of Sciences recommended the daily intake of chromium(III) be 50 μg-200 µg for adults [4]. Rats fed a low-chromium diet increased areas under the curve for insulin in glucose tolerance tests, suggesting the development of insulin resistance [5]. Despite recent evidence to the contrary [6, 7], the sale of chromium(III)-containing compounds mainly in the form of chromium picolinate (CrPic), has been a multimillion dollar business [8]. Chromium picolinate has been the focus of numerous studies, and the results of some suggest that it may help to control the level of blood glucose in patients with type 2 diabetes [9, 10]. However, the safety of CrPic has been questioned, as it was reported to cause chromosomal aberrations 3-fold to 18-fold above control levels for concentrations from 0.05 mM to 1.0 mM in Chinese hamster ovary (CHO) cells after 24 h of exposure [11]. CrPic also induced mutagenesis in L5187Y mouse lymphoma cells at relatively non-toxic levels [12]. Previous studies have indicated that CrPic may induce vertebral malformation in mouse fetuses exposed to high levels of the supplement [13]. The results of a behavioral study with picolinic acid, the ligand in CrPic, suggest that it may cause behavioral deficits in mouse pups exposed

through gestation and weaning, although the results of that study were not statistically significant [14].

As CrPic may have adverse effects, a new form of chromium for use as a dietary supplement and as part of a potential treatment for diabetes is needed. A low-molecular-weight chromium - binding substance (LMWCr), an oligopeptide, has been proposed to be the active form of chromium, and it enhances the tyrosine kinase activity of insulin receptors up to 8-fold in the presence of insulin *in vitro* [15]. However, LMWCr is susceptible to hydrolysis under acidic conditions, and it might be degraded when taken orally [16]. Davis and co-workers synthesized a propionate complex of chromium, $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$, or Cr3, to mimic the functions of LMWCr, and Cr3 has been shown to be stable in aqueous and acidic solutions [17]. Cr3 lowers the levels of fasting plasma triglycerides, total cholesterol, low density lipoprotein (LDL) cholesterol, and plasma insulin in healthy and type 2 diabetic rats [18]. Cr3 has not been shown to increase the incidence of vertebral abnormalities [19], and long-term administration of the compound to adult male Wistar rats did not reveal any adverse effects [6].

Not only do multiple animal studies indicate that Cr3 causes no adverse effects, the compound also provides more bioavailable chromium than any other supplement currently on the market. When ingested orally, Cr3 can be absorbed with up to 60% efficiency, which is greater than chromium chloride or CrPic (0.5-2%) [20]. As a promising dietary supplement, regulatory agencies such as the US Food and Drug

Administration require assessments of cytotoxicity and mutagenicity before a product is made available for human consumption. To date, no such studies of Cr3 have been published. As a dietary supplement, this compound would generously be outside the jurisdiction of most regulatory agencies, but nevertheless, information about the cytotoxicity and mutagenicity of Cr3 is helpful. Thus, the current study investigated the mutagenicity of Cr3 in *Escherichia coli* FX-11 and *Salmonella typhimurium* cells in the presence and absence of S9 metabolism. Additionally, the cytotoxicity and clastogenicity of both Cr3 and CrPic were examined in Chinese hamster ovary (CHO) K1 cells.

MATERIALS AND METHODS

Bacterial strains and test chemicals

The *Escherichia. coli* FX-11 bacteria were kindly provided by Dr. Yixin Yang from Emporia State University (Emporia, KS). Two strains (TA 98 and TA 100) of *Salmonella typhimurium* and 10% S9 rat liver mixtures were purchased from Moltox, Inc (Boone, NC). The 10% S9 rat liver mixture was prepared by mixing Aroclor 1254-induced Sprague Dawley male rat liver S9 with NADPH-regenerating system cofactors and phosphate buffer by the company. Chinese hamster ovary (CHO) K1 cells were originally purchased from American Type Culture Collection (Manassas, VA, #CCL-61) and transferred from Dr. Tim Burnett's laboratory from Emporia State University (Emporia, KS).

Cr3 and CrPic were synthesized and generously provided by the John B. Vincent laboratory at The University of Alabama (Tuscaloosa, AL). Cr3 was synthesized according to the methods of Earnshaw et al. [21]. CrPic was synthesized according to the methods of Press et al. [22]. The authenticity of both was established by high resolution electron impact mass spectrometry [23, 24]. Deionized (DI) water was used as the solvent for Cr3 and CrPic in all assays. *N*-ethyl-*N*-nitrosourea (ENU) was also generously provided by Dr. Yixin Yang.

E. coli mutagenicity assay

The assay was carried out as described by Yang and Fix [25]. Due to a UAA defect in the mRNA encoding amino acid number 161 of the *tyrA* gene product, *E. coli* FX-11 are auxotrophic for tyrosine [26].

In this mutagenicity assay, *E. coli* cultures were grown overnight in A-O minimal media [27] at 37^{o}C to the density of OD_{450} = 0.5. Cells were divided into two groups based on treatment time. In both assays, *E. coli* cells were treated with 0.025 mM, 0.05 mM, 0.1 mM, 0.5 mM and 1.0 mM of Cr3 or CrPic.

One hour assay

In this assay, mutagenesis and viability were assayed to determine mutation frequency. After one hour of treatment, the cells were centrifuged and resuspended in A-O buffer. ENU was applied as the positive control at the final concentration of 0.5 mM. To test viability, cells were diluted 100,000 times, and were plated on A-O semi-enriched minimal agar plates (0.02% nutrient broth) containing leucine (20 µg/ml) but lacking tyrosine. The plates were then incubated at 37^{o}C for 24 h, and the colonies that grew out were counted as viable cells. Mutagenesis was determined by plating cells directly onto A-O semi-enriched minimal agar, and the number of revertant colonies was counted after 48 h incubation at 37^{o}C. Mutation frequency was calculated by using the number of revertants divided by the number of viable cells.

Forty-eight hour assay

In this experiment, test chemicals were not removed prior to plating. A mixture of the appropriate test chemicals (at the concentrations used in the one hour assay) and cells was applied directly onto the surface agar plates to test mutagenesis. The plates were the same as those previously described. After 48 h incubation at 37ºC, visible colonies were counted as revertants.

Ames assay

This assay was performed as described by Mortelmans and Zeiger with or without S9 mixture [28]. *Salmonella* strains TA 98 and TA 100 were both histidine auxotrophs. They were grown overnight in Oxoid nutrient broth No. 2 (Sigma) at 37ºC to the density of 1-2×10^9 cells/ml. To the sterile glass tubes, 0.5 ml AO buffer or S9 mixture, 0.05 ml of DI water or test chemical, and 0.1 ml bacteria culture were added. After a 20 min incubation, 2.0 ml of molten top agar was added to each tube, and the mixture was poured onto the surface of glucose minimum agar plates that contained no histidine. Five concentrations from 100 μg/cm^2 to 10,000 μg/cm^2 of Cr3 and CrPic were tested in duplicate for each strain. After 48 h incubation at 37ºC, colonies were formed as a result of cells being reverted to histidine independence. The number of colonies was counted after incubation. The criteria for a tested compound to be considered positive were: it must increase the mean number of revertants (over the solvent control) per plate 2-3 fold and the increase in number must be dose-dependent [29].

Colony forming assay

Chinese hamster ovary (CHO) K1 cells were grown in McCoy's 5A culture medium supplemented with 10% fetal bovine serum, and 10 units penicillin/ml, and 10 ug/ml streptomycin (Sigma). In each T25 flask, $5x10^5$ cells were seeded and incubated at 37ºC in a 5% CO_2 atmosphere for 20 h. Cells were treated with 4.0 µg/cm^2, 20 µg/cm^2, 40 µg/cm^2, or 80 µg/cm^2 of Cr3 or CrPic for 24 h. Deionized (DI) water was used as the solvent for Cr3 and CrPic. After 24 h, cells were rinsed with Hank's balanced salt solution (HBSS, Sigma), and harvested with 0.25% trypsin/EDTA (Sigma). Each treatment group was reseeded in quadruplicate, with 200 cells replated in a 6-well plate with 3 ml culture medium. Cells were incubated at 37ºC in 5% CO_2 grown for 7 days, and the medium was changed every 3 days. After 7 days, cells were fixed with freshly prepared Carnoy's fixative (3:1 methanol/acetic acid) and stained with crystal violet. Colonies were counted and averaged for quadruplicate doses. Plating efficiency was calculated as percent colonies in treated groups relative to controls.

Chromosomal aberration assay

The chromosomal aberration assay was conducted using a standard assay as described by Klein et al. [30]. Cells were seeded and treated in the same way as in the cytotoxicity assay at 37ºC in a 5% CO_2 incubator for 24 h. Two hours before harvesting cells, demecolcine solution (Sigma) was added to cell cultures at a concentration of 0.1 µg/ml. Following the 2-h demecolcine treatment phase, cells were trypsinized and

transferred to 15 ml tubes and centrifuged. Cell pellets of each treatment group were resuspended in 4 ml of 0.075 M KCl. After a 10-min period, 5 ml of a Carnoy's fixative solution was added to cell suspensions, refrigerated at 4ºC for 30 min, and centrifuged 10 min at 300xg at 4ºC. The 40 min KCl/Carnoy's fixation process was repeated three times. After centrifuge at the third time, cells were resuspended in 1 ml of Carnoy's fixative for slide preparation.

Glass slides were chilled overnight at 4ºC. To each slide, 3-4 drops of cells suspension were applied. Fixed cells were stained with 10% Giemsa solution for 1 min. The Giemsa solution consisted of 9 ml Sorenson's phosphate buffer and 1 ml Giemsa stain. For each dose group, 100 cells in metaphase were examined under oil immersion for chromosomal aberrations. Cell samples were coded by a person not involved in the project and scored by the author without any knowledge of treatment condition. The percentage of metaphases with chromosomal damage and total aberrations per 100 metaphases population were analyzed for each group before the code was revealed. Experiments were carried out in triplicate, with 100 metaphase cells examined for each dose level. A total of 300 metaphase cells were analyzed for each treatment group.

Statistical methods

Data were analyzed by one-way ANOVA using SPSS (SPSS, Inc., Chicago, IL) followed by a two-way Dunnett's t test to determine specific significant differences ($p \leq 0.05$).

RESULTS

Mutagenicity assays

In the Escherichia coli mutagenicity assay, mutagenesis was measured as an increase in the number of revertants relative to the solvent control after 1 h or 48 h exposure varying concentrations of Cr3, CrPic, or ENU. After 1 h of treatment, no significant differences ($p \leq 0.05$) were present among the groups with regard to mutation frequency except for the ENU 0.5 M group (positive control) (Fig. 1). After 48 hours of exposure, there was no major difference in the number of revertants between Cr3 and CrPic groups, and neither of them caused a significant increase in the number of revertants compared to untreated controls (Fig. 2). In the Ames assay, Cr3 and CrPic were tested in the S. typhimurium Ames assay with tester strains TA 98 and TA 100 at dose levels up to and including 10,000 µg/plate with and without S9 metabolic activation. Both Cr3 and CrPic were clearly negative in the Salmonella strains tested (Table 1).

The results of the E. coli mutagenicity assay show that treatment with Cr3 and CrPic up to 1.0 mM did not produce mutagenesis. All of the data reported in this assay were gathered and analyzed without the addition of the S9 mixture, which induced a large increase of revertants in all treatment groups regardless of dosage. As E. coli FX-11 does not have the ability to synthesize tyrosine, it is possible that trace amounts of tyrosine were released after S9 mixture was added, causing wild growth in all groups. Consequently, the data from this assay with the addition of S9 mixture was not reported. Traditionally, an

S9 metabolism is only used in the Ames assay, so excluding these data does not compromise the integrity of the results of this project.

Colony forming assays

CHO K1 cells were exposed to solvent (DI water) alone and to four concentrations of Cr3 and CrPic ranging from 4.0 µg/cm2 to 80 µg/cm2. CHO K1 cells were treated for 24 h with various concentrations of Cr3 and CrPic. Toxicity was measured as a reduction in plating efficiency relative to the solvent control. According to the criteria established by the World Health Organization (WHO), a concentration giving rise to > 50% plating efficiency can be considered non-toxic [36]. Recently, a new limit of 40% cytotoxicity (60% plating efficiency) was suggested to prevent the observation of false positive results [37]. In the current study, we took 60% plating efficiency as the cut-off between cytotoxic and noncytotoxic. As shown in Table 2, treatments of 4.0 µg/cm2, 20 µg/cm2, and 40 µg/cm2 Cr3 produced colonies > 84% of control. Cr3 at 80 µg/cm2 significantly decreased the plating effciency to 62%, however, it is still considered nontoxic based on the criteria set forth by the WHO. Chromium picolinate at concentrations 4.0 µg/cm2 and 20 µg/cm2 produced >60% plating efficiency, while concentrations over 40 µg/cm2 produced a significant decrease in the number of plating efficiency.

Clastogenicity assays

Chromosomal aberrations were measured for CHO K1 cells treated with the same concentrations of Cr3 and CrPic as in colony forming assay described above for 24 h. The number of chromosomal aberrations produced by Cr3 ranged from 2 ± 1 to 5 ± 2 per 100 cells in metaphase (Table 3), and there was no significant difference among any of the Cr3 doses and the solvent control cells. The 4.0 µg/cm2 group of CrPic yielded 5 ± 1 chromosomal aberrations, the clastogenicity of which was almost the same as that of the highest dose of Cr3. Though 4.0 µg/cm2 and 20 µg/cm2 doses of CrPic did not produce significant clastogenicity compared to untreated controls, the number of chromosomal aberrations was still higher than equivalent doses of Cr3. For the 80 µg/cm2 CrPic, the number of chromosomal aberrations was 15 ± 2, which was 3 times higher than the number produced at the quivalent dose of Cr3 (Table 3). None of the Cr3 doses produced a significant increase in the percentages of damaged mataphases, while the percentages for CrPic at 40 µg/cm2 and 80 µg/cm2 were significantly elevated compared to the cells exposed to solvent alone (Table 3). Therefore, CrPic appeared to be more cytotoxic and clastogenic than Cr3 at all dose levels in CHO K1 cells, and both of the effects were dose-dependent.

DISCUSSION

The mutagenicity of CrPic in S.typhimurium TA 98 and TA 100 with and without S9 metabolism was tested and reported negative by National Toxicology Program (NTP) [31], and the results of our study on CrPic are consistent with the results obtained by the NTP and of that reported by Whittaker et al. [12]. The solvent used in NTP was not reported, and the solvent used by Whittaker et al. was dimethyl sulfoxide (DMSO). The extreme water solubility of Cr3 meant that DI water, rather than a traditional solvent such as DMSO, was able to be used to solubilize the compound for testing. Chromium supplements have been shown to generate reactive oxygen species in cells, such as superoxide anion, hydroxyl radicals, and hydrogen peroxide[32-34]. Considerable evidence indicated that reactive oxygen species play an important role in the process of cellular injury which can lead to cancer. During all those Cr supplements, chromium picolinate was found to enhance the production of hydroxyl groups [33]. DMSO is known to be a radical scavenger with high affinity for hydroxyl radicals [35], so it should decrease the reactive oxygen species produced through Fenton-type chemistry [32], thus decreasing the DNA damage that leads to mutations. The exclusion of DMSO in this study ensures that the negative results reported in this study are because Cr3 is not mutagenic and not because of solvent interference. It should be noted, however, the reported solubility of CrPic in water is 0.6 mM at room temperature, so the 1.0 mM of CrPic was partially suspended in DI water, but at all other concentrations, CrPic was dissolved. The

mutagenicity of Cr3 has not been previously reported, and our results indicate that indeed it is not mutagenic to S. typhimurium in the strains tested.

The data in the present study strongly suggest that CrPic reduces plating efficiency at far lower levels than Cr3. A 4.0 µg/cm2 dose of CrPic produced a 79% plating efficiency, which was a statistically significant decrease compared to the 98% in Cr3 4.0 µg/cm2 group (Table 2). Similar results are reported for other doses of CrPic, and only the 4.0 µg/cm2 and 20 µg/cm2 concentrations were considered acceptably noncytotoxic according to the criteria (> 60% plating efficiency). Cytotoxicity for concentrations of 40 µg/cm2 and 80 µg/cm2 of CrPic produced 57% and 29% plating efficiency, respectively. The plating efficiency of 80 ug/cm2 dose is consistent with the results about CrPic reported by Stearns et al., who produced 24 ± 11% plating efficiency in CHO AA8 cells though the CrPic was also suspended in acetone in their study [38]. CrPic treatment at all levels was more cytotoxic than the corresponding concentrations of Cr3. This difference in plating efficiency between Cr3 and CrPic suggests that Cr3 can be considered safer in mammalian cells. We can assume that if CrPic was completely dissolved, the cytotoxicity it produced possibly would be even higher.

The clastogenicity of CrPic has been studied previously in CHO K1 cells, and no chromosome damage was found with doses up to 770 µg/mL, which is equivalent to 123 µg/cm2 [39]. However, the hydroxyl radical scavenger DMSO was used as the solvent for CrPic, increasing the possibility the free radicals released by CrPic were trapped by

DMSO. In the current study, clastogenicity was observed after treatments with 40 µg/cm2 and 80 µg/cm2 of CrPic, and the response was concentration-dependent from 4.0 µg/cm2 to 80 µg/cm2 (Table 3). Consistent with our results, the clastogenicity of CrPic at 40 µg/cm2 was also observed in CHO cells after a 24 h exposure, producing chromosomal aberrations 16-fold above control levels [11]. A 24 h exposure to any of the tested concentrations of Cr3 did not produce any significant increase in the number of cells with abnormal metaphase. According to previous studies, people who took Cr in the form of CrPic were found to have a serum Cr level of 16 nM after 2 months [40], and the tissue Cr levels may as much as 100 times higher than serum Cr levels [41]. The highest concentration for Cr3 in our study is 80 µg/cm2, which is equivalent to 0.7 mM, exceeding the Cr amount that is likely to exist in human tissues over by 400 times. However, the highest dose that did not increase the number of chromosomal aberrations significantly for CrPic was 20 µg/cm2 (Table 3).

The results of this study are consistent with several other well-known published studies [12, 31, 38] on CrPic and support the results of in vivo studies that show no evidence that Cr3 causes adverse effects. Cr3 appears noncytotoxic to bacterial and mammalian cells at the concentrations tested, but further studies would be needed to establish its safety in humans.

TABLE 1. Mutagenicity of Cr3 and CrPic in *Salmonella typhimurium*[a]

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Dose (µg/plate)	TA 98		TA 100	
	(-) S9	(+) S9	(-) S9	(+) S9
DI water	38 ± 3	36 ± 10	23 ± 2	33 ± 8
Cr3 100	41 ± 3	42 ± 4	28 ± 8	33 ± 3
Cr3 333	45 ± 4	48 ± 9	26 ± 6	33 ± 7
Cr3 1000	49 ± 9	45 ± 7	28 ± 5	34 ± 8
Cr3 3333	47 ± 7	46 ± 7	26 ± 5	35 ± 7
Cr3 10000	51 ± 9	62 ± 8	29 ± 6	38 ± 11
CrPic 100	51 ± 9	41 ± 4	23 ± 8	34 ± 9
CrPic 333	49 ± 5	40 ± 6	37 ± 23	41 ± 13
CrPic 1000	47 ± 7	43 ± 8	30 ± 11	32 ± 5
CrPic 3333	52 ± 13	44 ± 6	33 ± 6	39 ± 9
CrPic 10,000	55 ± 8	53 ± 7	39 ± 8	37 ± 10
Positive control	402 ± 19	385 ± 14	457 ± 30	487 ± 9

[a]Average revertants/plate ± standard deviation.

[b]The positive controls without S9 were 2-nitrofluorene (1.0 ug/plate) for TA98, sodium azide (1.0 ug/plate) for TA100. With S9 mixture, 1-aminoanthracene (1.0 ug/plate) was used for both tested strains.

TABLE 2. Cytotoxicity of Cr3 and CrPic in CHO K1 cells after 24 h treatment.

Treatment	Dose	Plating efficiency (%)	*P* value
DI water		100	
Cr3	4.0 μg/cm^2	98	n.s.[a]
	20 μg/cm^2	96	n.s.
	40 μg/cm^2	84	n.s.
	80 μg/cm^2	62	< 0.0001
CrPic	4.0 μg/cm^2	79	< 0.0001
	20 μg/cm^2	74	< 0.0001
	40 μg/cm^2	57	< 0.0001
	80 μg/cm^2	29	< 0.0001

Each data represents the average of three independent experiments.

[a]Not significant.

TABLE 3. Chromosomal aberrations in CHO K1 cells after 24 h exposure to Cr3 and CrPic.

Treatment	Dose	Metaphases with damage (%)	Total aberrations (per 100 metaphases)
DI water		1 ± 1	1 ± 1
Cr3	4.0 µg/cm^2	2 ± 1	2 ± 1
	20 µg/cm^2	4 ± 1	4 ± 1
	40 µg/cm^2	3 ± 1	3 ± 1
	80 µg/cm^2	5 ± 1	5 ± 2
CrPic	4.0 µg/cm^2	5 ± 1	5 ± 1
	20 µg/cm^2	5 ± 1	6 ± 1
	*40 µg/cm^2	9 ± 1	10 ± 1
	*80 µg/cm^2	13 ± 2	15 ± 2
Mitomycin C	*0.016 µg/ cm^2	58 ± 4	83 ± 3

Data is presented as mean ± standard deviation. 300 metaphases (100 per experiment) were analyzed for each treatment group. * indicates significant difference of metaphases with damage and of chromosomal aberrations ($p < 0.05$).

FIG. 1. Effect of different concentrations of Cr3 and CrPic on mutation frequency in *E.coli* FX-11after 1 h treatment. Mutation frequency ($x10^{-7}$) = number of revertants/number of viable cells. Data are presented as mean±standard deviation.

* $p < 0.05$.

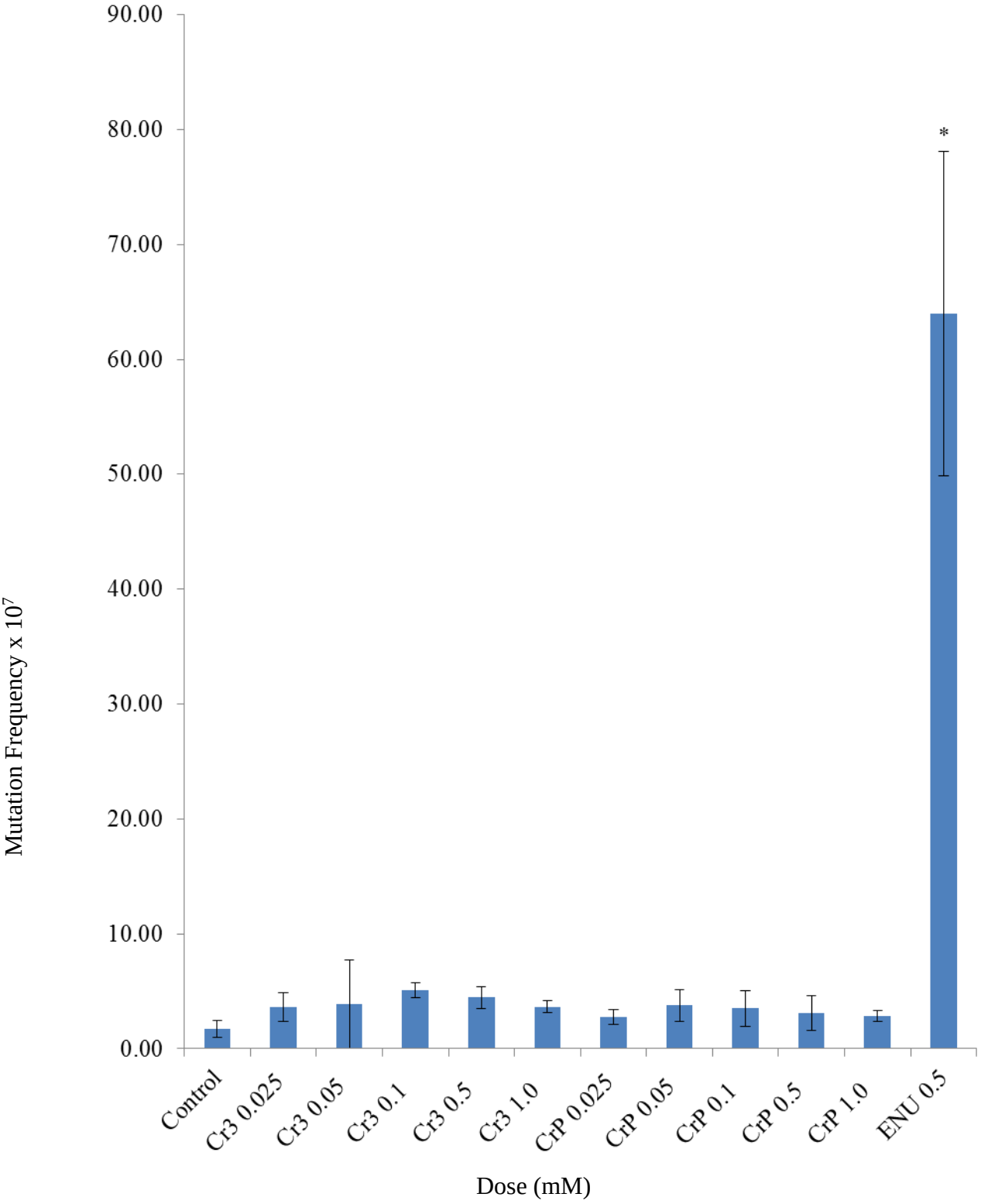

90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Mutation Frequency x 10^7
*
Control
Cr3 0.025
Cr3 0.05
Cr3 0.1
Cr3 0.5
Cr3 1.0
CrP 0.025
CrP 0.05
CrP 0.1
CrP 0.5
CrP 1.0
ENU 0.5
Dose (mM)

FIG. 2. Effect of different concentrations of Cr3 and CrPic on numer of revertants in *E.coli* FX-11after 48 h treatment. Data are presented as mean±standard deviation.

* $p < 0.05$.

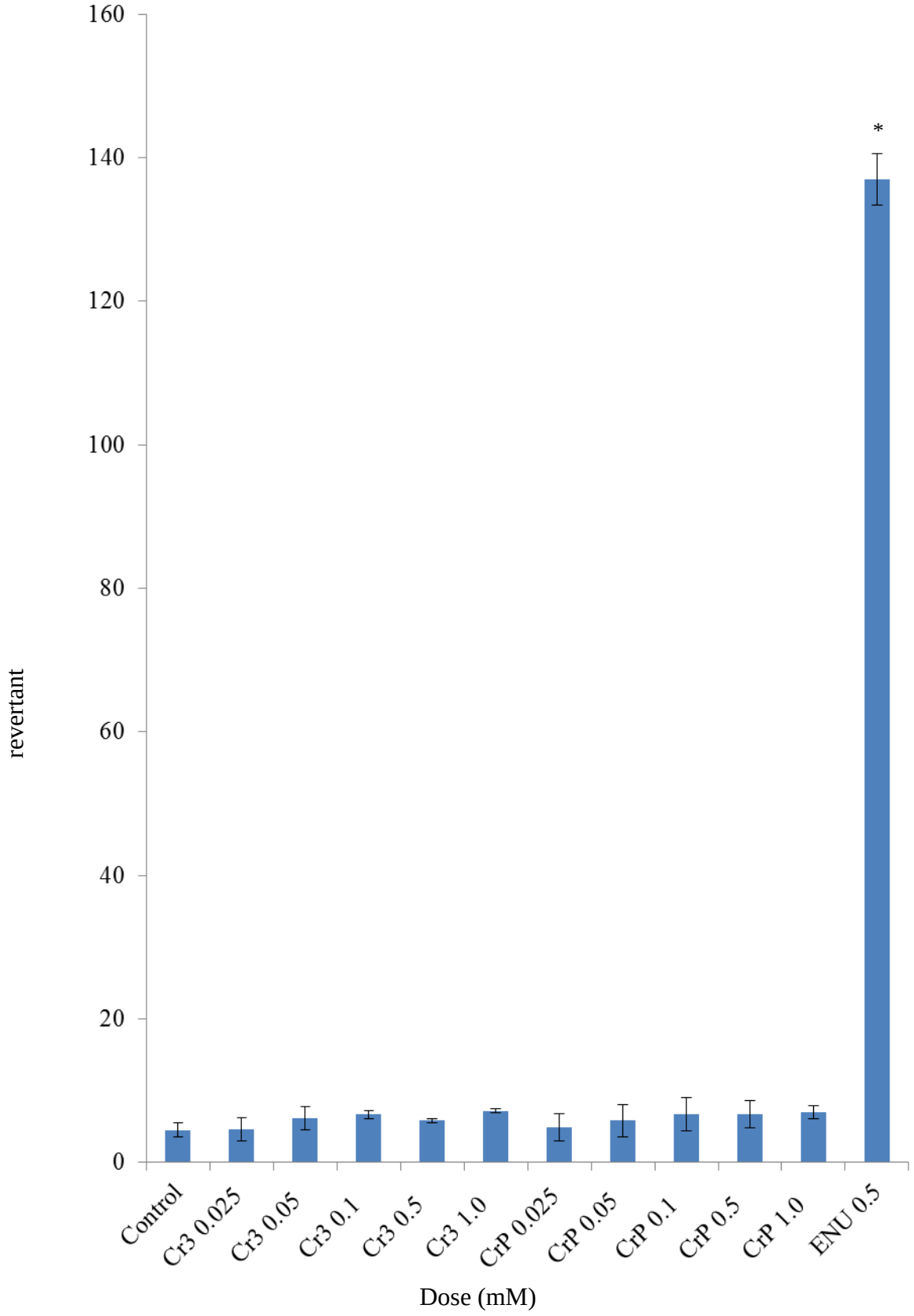
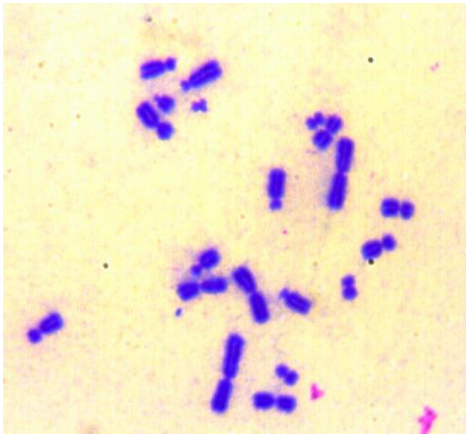
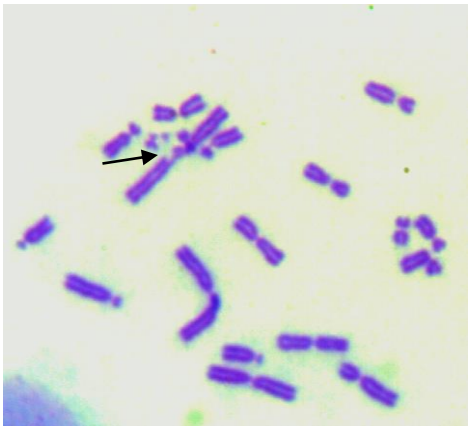

160
140
120
100
80
60
40
20
0
revertant
*
Control
Cr3 0.025
Cr3 0.05
Cr3 0.1
Cr3 0.5
Cr3 1.0
CrP 0.025
CrP 0.05
CrP 0.1
CrP 0.5
CrP 1.0
ENU 0.5
Dose (mM)

FIG. 3. Effect of different concentrations of Cr3 and CrPic on CHO K1 chromosomes under 1000× magnification. (a) shows chromosomes without aberrations in DI water treatment group; (b) shows chromosomes with aberrations in mitomycin C 0.016 μg/cm^2; (c) shows chromosomes after treated with 80 μg/cm^2 Cr3; (d) shows chromosomes after treated with 80 μg/cm^2 CrPic. Arrows indicate aberrations.

(a)



(c)



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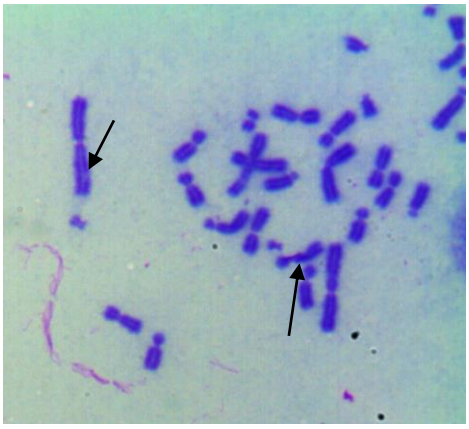

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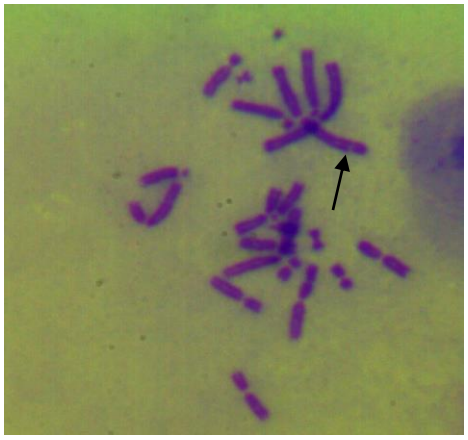

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