

The Effects of Vitamin C on Oxidative DNA Damage and Mutagenesis**

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Received: November 4, 2005

Accepted: February 17, 2006

Summary

DNA damage induced by reactive oxygen species is involved in mutagenesis and generation of mutation-related diseases, including cancer. Therefore, study of antigenotoxic potential of antioxidants is of great importance for protection of human health. Vitamin C is well known as a potent antioxidant, but its prooxidative effects have also been reported. In this work, antigenotoxic properties of vitamin C in *E. coli* K12, *E. coli* WP2 and *S. cerevisiae* D7 reversion assays, as well as in *S. cerevisiae* comet assay were examined. *t*-Butyl hydroperoxide (*t*-BOOH) was used to induce oxidative mutagenesis, and hydrogen peroxide (H_2O_2) was used to induce DNA strand breaks in the comet assay. Vitamin C reduced *t*-BOOH-induced and spontaneous mutagenesis in repair proficient and mismatch repair (MMR) deficient strains of *E. coli* K12 respectively, as well as *t*-BOOH-induced mutagenesis in *S. cerevisiae* D7 strain. However, in *E. coli* K12 strains that carry plasmid with microsatellite sequences, treatment with vitamin C slightly stimulated microsatellite instability. Vitamin C also showed mutagenic effects in WP2 *oxyR* strain, probably due to its prooxidative potential, amplified in the strain deficient in antioxidative defense. In the yeast comet assay, contradictory results were obtained: while low concentration (0.05 μ M) of vitamin C inhibited oxidative damage, higher concentrations (0.1–10 μ M) stimulated it. The obtained results indicate that vitamin C can exhibit antigenotoxic or genotoxic effects, depending on the dose, genetic background and other experimental conditions.

Key words: antigenotoxic potential, vitamin C, *E. coli*, *S. cerevisiae*, reversion assay, yeast comet assay

Introduction

It has been established that DNA damage induced by reactive oxygen species (ROS) is involved in aging and different human diseases such as atherosclerosis, cancer, neurodegenerative diseases and AIDS (1,2). The oxidative DNA damage arises from the attack of ROS on DNA bases or deoxyribose residues to produce damaged bases or strand breaks. In addition, ROS can attack protein or lipid molecules (process of lipid peroxidation)

and generate intermediates that can react with DNA to form adducts. The most frequent type of oxidized bases is 8-oxo-guanine (8-oxo-G), which mispairs with adenine and causes GC→TA transversion. Strand breaks caused by ROS are predominantly single-strand breaks, but if single-strand breaks are in close proximity, ROS can induce double-strand breaks (3). Oxidative DNA damage can also increase the frequency of strand slippage intermediates occurring during DNA replication or repair synthesis, especially in microsatellite sequences,

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**This paper was presented at the 2nd Congress of Croatian Geneticists in Supetar, Island of Brač, Croatia, September 24–27, 2005

and thus it can contribute to genomic instability. It is supposed that increasing the formation of slipped intermediates occurs through the formation of either base modifications or strand breaks (4–6).

Ascorbic acid (vitamin C) is an essential micronutrient for growth and development of most living organisms; its deficiency in humans may result in vascular, skeletal, immunological, neuroendocrinological, and various other disorders (7). It is a potent water-soluble antioxidant with high potential to protect cytosol and membrane components from oxidative damage. Mechanisms of antioxidative action of vitamin C are direct scavenging and blocking of ROS, as well as regeneration of other antioxidative systems (8). It plays a range of biological roles, one of which is to restrict the propagation of multiple reactive electrophilic end-products from ROS, produced either by normal cellular processes or from toxic exogenous precursors (9). Still, the role of vitamin C in protecting against oxidative DNA damage is controversial: although numerous studies demonstrate its antioxidant effects, *in vitro* studies are often confounded with the prooxidative effects, especially in the presence of free transition metals.

It has been reported that vitamin C significantly decreased spontaneous mutagenesis in MMR-deficient human cells (10) as well as in *mutT* strains of *E. coli* (11). There are literature data about its potential to reduce H₂O₂-induced mutagenesis in human kidney 293T and human myeloid HL-60 cells (12), as well as ethyl methanesulphonate (EMS) induced mutagenesis in V79 hamster cell-line and cultured human lymphocytes (13). Protective effects of vitamin C against ionizing radiation damage have also been extensively documented (14–16). Numerous investigations, both *in vitro* and *in vivo*, have shown the efficacy of vitamin C in inhibiting carcinogenesis. Ascorbic acid reduced the incidence of 3-methylcholanthrene-induced cervical cancer, as well as UV-induced skin cancer in mice (17,18). Protective effects against oxidative DNA damage were also confirmed by reduction of the number of micronuclei in human lymphocytes after supplementation of vitamin C to smokers (19).

However, there are also reports indicating that vitamin C may cause cytotoxic and genotoxic effects. Its high concentrations can enhance radiation-induced DNA damage (16). In the presence of transition metals, it can stimulate Fenton reaction and thereby increase the production of ROS, which may cause oxidative DNA damage (20). This property of vitamin C can explain its ambivalent influence on DNA damage in the presence or absence of copper, reported by Cai *et al.* (21). Their experiments confirmed that vitamin C could act as: (i) an antioxidant that protects DNA from ionizing radiation, or (ii) a prooxidant agent in the presence of copper that induces DNA damage.

In this study we tested vitamin C for antimutagenic and antigenotoxic activity against oxidative mutagens in microbial assays. The influence of vitamin C on spontaneous and *t*-butyl hydroperoxide (*t*-BOOH)-induced mutagenesis was tested in *E. coli* K12 and WP2 reversion assays, as well as in *S. cerevisiae* D7 reversion test (22–25). In addition, the alkaline comet assay or single cell gel electrophoresis (SCGE), adapted for *S. cerevisiae* 3A (26),

was also used in order to measure antigenotoxic potential of vitamin C against H₂O₂-induced DNA strand breaks.

Materials and Methods

Chemicals

Stock solutions of vitamin C (CAS No. 50-81-7, Gale-nika a.d.), *t*-butyl hydroperoxide (CAS No. 75-91-2, Aldrich) and hydrogen peroxide (CAS No. 7722-84-1) were freshly prepared in distilled water.

Tester strains

The tester strains used in this study are listed in Table 1.

Table 1. Tester strains

Strain	Relevant marker	Origin	Reference
<i>E. coli</i> K12			
SY252	<i>argE3</i>	Lab. collection	(22)
IB103	as SY252 but <i>mutS::Tn10</i>	Lab. collection	(22)
IB122	as SY252 but pAJ47	Lab. collection	(23)
IB123	as IB122 but <i>mutH::Tn5</i>	Lab. collection	(23)
<i>E. coli</i> WP2			
IC185	<i>trpE65</i>	E. Witkin	(24)
IC202	as IC185 but <i>oxyR/pKM101</i>	M. Blanco	(24)
<i>S. cerevisiae</i>			
D7	<i>ade2-40/119 trp5-12/27 ilo92/92</i>	F.K. Zimmermann	(25)
3A	<i>a/α gal1 leu2 ura 3-52</i>	G. Miloshev	(26)

Media and growth conditions

The bacteria were grown overnight at 37 °C, strains containing plasmid pAJ47 in 2×YT medium (5 g of NaCl, 16 g of bactotryptone, 10 g of yeast extract, 1000 mL of distilled water), and all the remaining bacterial strains in LB medium (5 g of NaCl, 10 g of bactotryptone, 5 g of yeast extract, 1000 mL of distilled water). *S. cerevisiae* D7 and 3A strains were grown in YPD medium (10 g of yeast extract, 20 g of bacto-peptone, 20 g of dextrose, 1000 mL of distilled water) at 30 °C with aeration. Semi-enriched minimal medium (SEM) for *E. coli* K12 reversion assay was minimal agar medium supplemented with 3 % (by volume) NB (22,23,27). In the test of microsatellite instability, 2×YT agar plates (2 × yeast-tryptone agar, Difco) supplemented with appropriate antibiotics were used for the survival (12.5 µg/mL of tetracycline, Sigma) and mutation (12.5 µg/mL of tetracycline and 100 µg/mL of carbenicillin, Fluka) assays. All media used for WP2 reversion test had previously been described by Blanco *et al.* (24). Solid YPD and selective C medium (4 g of yeast nitrogen base without amino acids, 10 mg of tryptophan, 20 g of D-glucose, 15 g of agar, 1000 mL of distilled water) were used in *S. cerevisiae* reversion test.

Detection of antimutagenic potential in repair proficient strain of *E. coli* K12

The overnight culture of *E. coli* SY252 strain was washed by centrifugation and resuspended in 0.01 M MgSO₄. To detect antimutagenic potential against induced mutagenesis, water solution of *t*-BOOH was added in cell suspension (the final concentration was 150 µg/plate) and the samples (0.1 mL) were immediately plated onto 3 % SEM plates with or without different concentrations of vitamin C (plating in duplicates). The same procedure, but without *t*-BOOH, was used to measure the effect on spontaneous mutagenesis. To determine the effect of vitamin C on the cell survival, appropriately diluted untreated and *t*-BOOH-treated cell suspensions were plated onto 3 % SEM plates with or without different concentrations of vitamin C. The incubation was at 37 °C for 48 h.

Detection of antimutagenic potential in the MMR deficient strain of *E. coli* K12

The overnight culture of IB103 (*mutS*) strain was washed by centrifugation and resuspended in 0.01 M MgSO₄. For the mutation assay, a twofold diluted cell suspension was plated on 3 % SEM plates with or without vitamin C. Appropriately diluted cell suspension was used for the determination of cell survival. The number of Arg⁺ revertants and viable cells was determined after incubation at 37 °C for 48 h.

Detection of inhibition of microsatellite instability

The test was performed as described by Jackson and Loeb (4). The exponential cultures (~5·10⁸ cells/mL) of strains containing microsatellite repeats on plasmid pAJ47 were collected by centrifugation at 13 000 rpm, washed with 1×M9 buffer (1 g of NH₄Cl, 15 g of Na₂HPO₄·12H₂O, 3 g of KH₂PO₄, 0.5 g of NaCl, 0.4 g of MgSO₄·7H₂O, 1000 mL of distilled water, pH=7.2) and resuspended in it. *t*-BOOH was used to induce frameshifts in microsatellite sequences in the repair proficient strain (IB122), while spontaneous frameshifts were detected in isogenic *mutH* strain (IB123). For induction of mutagenesis, 150-µL aliquots of cell suspension were mixed with an equal volume of *t*-BOOH dissolved in 1×M9 buffer (the final concentration was 225 µg/mL) in 1.5-mL Eppendorf tubes and incubated at 37 °C, with shaking at 200 rpm for 30 min. The exposure was terminated by centrifugation of the cells followed by resuspension in 300 µL of 2×YT. Appropriate dilutions of the cells were plated on 2×YT plates containing 12.5 µg/mL of tetracycline (the survival assay) and 2×YT plates containing 12.5 µg/mL of tetracycline plus 100 µg/mL of carbenicillin (the mutation assay), with or without vitamin C. The number of carb^Rtet^R and tet^R colonies was determined after incubation at 37 °C for 24 h.

Detection of antimutagenic potential in IC202

WP2 reversion test was performed by mixing 0.1 mL of fresh overnight culture of IC202 strain, water dilution of *t*-BOOH (final concentration 25 µg/plate), appropriate volume of vitamin C solution and 3 mL of molten top agar (45 °C). The mixture was poured onto minimal ET4 plates (minimal medium supplemented with 0.5 µg/mL of tryptophan). The number of Trp⁺ revertants

was determined after incubation at 37 °C for 48 h. Simultaneously, the influence of vitamin C on spontaneous mutagenesis was examined. Plating on E4 plates (minimal medium, no added tryptophan) was used to evaluate the number of preplating mutants originated during overnight growth. The cultures with low number of preexisting revertants (<15 revertants/plate) were used for experiments (24).

Detection of antimutagenic potential in *S. cerevisiae* D7

The reversion assay was performed by mixing 0.1 mL of fresh overnight culture of *S. cerevisiae* D7 strain, water dilution of *t*-BOOH (final concentration 500 µg/plate), appropriate volume of vitamin C solution and 3 mL of molten top agar (45 °C). The mixture was poured onto selective C medium to score Ilv⁺ revertants. The survival assay was performed in the same manner, but the cell suspension was appropriately diluted and the mixture was plated onto YPD. Plates were incubated at 30 °C for 72 h.

Yeast comet assay

S. cerevisiae 3A was grown to middle logarithmic phase (5·10⁷ cells/mL) and then, for pretreatment experiments, treated with vitamin C for 15 min at room temperature. After washing, the cells were treated with H₂O₂ for 10 min at 4 °C. In the case of cotreatment, H₂O₂ and vitamin C were applied simultaneously for 10 min at 4 °C. Appropriately treated cells, as well as untreated control, were washed twice with S buffer (1 M sorbitol, 25 mM NaH₂PO₄, pH=6.5). The spheroplasting with zymolyase (6 µg/mL, concentration that induced less than 5 % of comets) was carried out in the gel. The cells were mixed with low-melting agarose (final concentration 0.7 %), spread on microscope slides precoated with 0.5 % agarose, covered with cover slips and placed at 4 °C for 5 min. The cover slips were gently removed and the slides were placed at 37 °C for 10 min. The slides were submersed into lysing solution (1 M NaCl, 50 mM EDTA, pH=8, 30 mM NaOH, 0.1 % *N*-lauroyl sarcosine) for 1 h and after unwinding the DNA in alkaline conditions at pH=12.6 for 1 h (30 mM NaOH, 10 mM EDTA, pH=8), the slides were subjected to electrophoresis (0.45 V/cm for 15 min) in the same buffer. Following electrophoresis, the microgels were dehydrated in 95 and 75 % ethanol, both for 5 min and allowed to dry at room temperature. The DNA was stained with SYBR Green (CAS No. 163795-75-3, Fluka) and comets were visualized under the fluorescent microscope (Leica DMLS). The comets and spheroplasts were scored visually at fifty randomly selected fields for each slide and the percentage of comets was calculated.

Statistical analysis

The Student's *t*-test was employed for statistical analysis. The significance was tested at the *p*<0.05 level. The results presented in tables are expressed as the means obtained from three (or more) independent experiments, with the standard error of the mean. In all reversion tests, the antimutagenic potential was expressed as percentage of inhibition of mutagenesis ($I/\% = (1 - N_i/N_c) \cdot 100$), where *N_i* is the number of revertants in the sample with

vitamin C, and N_c is the number of revertants in the control sample), as described by Wall *et al.* (28).

Results and Discussion

This study included evaluation of antimutagenic and antigenotoxic potential of vitamin C in microbial tests, performed by using reversion assays and yeast comet assay. In order to induce oxidative damage, potent oxidative mutagens *t*-BOOH and H_2O_2 were used. In our preliminary reversion tests, more reproducible results were obtained with *t*-BOOH than with H_2O_2 as a mutagen. It was also more stable and could not be eliminated by catalase (29). However, in yeast comet assay, H_2O_2 induced more comets and it was chosen to induce DNA damage (data not shown).

E. coli K12 reversion assay

The repair-proficient and MMR-deficient *mutS* strains of *E. coli* K12 (Table 1) were used in order to estimate the capability of vitamin C to reduce spontaneous and *t*-BOOH-induced mutagenesis. The mutations monitored were *argE3*→ Arg^+ reversions. *argE3* mutation is ochre (UAA) and it can be reverted by several molecular events including transition or transversion of AT base pairs (30). Since *t*-BOOH is the latent donor of RO^\bullet alkoxyl radicals, and oxidative DNA damage induced by RO^\bullet radicals causes transitions and transversions of AT base pairs, *t*-BOOH could be used to increase *argE3*→ Arg^+ reversions (31). Since mutagen was plated together with cells on media containing vitamin C, performed experiments were of cotreatment type.

As expected from its antioxidative properties, vitamin C exhibited strong inhibitory potential against *t*-BOOH-induced mutagenesis in repair proficient strain, with no effects on cell viability. Inhibition of mutagenesis varied between 31 and 64 % for vitamin C concentrations ranging from 1 to 7.5 mM. Inhibitory potential of vitamin C against spontaneous mutagenesis was monitored in repair proficient and *mutS* strains. Inhibition of mutagenesis was obtained only in *mutS* strain (52 and 72 % for 2.5 and 5 mM, respectively) (Table 2). Strong reduction of mutagenesis obtained with vitamin C in MMR-deficient strain supports recent literature data indicating that MMR is involved in repair of oxidative DNA lesions (10,32,33).

In the present study, the potential of vitamin C to reduce microsatellite instability (MSI) was evaluated. Testing was performed on *E. coli* K12 strains, transformed with plasmid pAJ47 (Table 1). Plasmid contains microsatellite sequences, *i.e.* dinucleotide repeats placed out of frame within the coding region of the β -lactamase gene. Therefore, cells harboring the plasmid are sensitive to β -lactam antibiotics, such as carbenicillin (34). Microsatellite sequence is a +2 frame construct $(CA)_{11}$ and the mutation that restores the reading frame and provides resistance to carbenicillin is a 2 bp deletion, monitored in this test. The repair-proficient (IB122) and MMR-deficient (IB123 *mutH*) strains, transformed with plasmid, were used for monitoring *t*-BOOH-induced and spontaneous MSI, respectively. MMR is involved in the repair of short strand-slippage intermediates and the frequency of spontaneous frameshift mutations is higher in MMR-deficient cells (4,24). Test of microsatellite instability was performed as a posttreatment experiment, indicating the

Table 2. The effect of vitamin C on *t*-BOOH-induced mutagenesis in repair-proficient and MMR-deficient strain of the *E. coli* K12

Strain	<i>m(t-BOOH)</i>		<i>c(vitamin C)/mM</i>				
	$\mu\text{g/plate}$	0	0.5	1.0	2.5	5.0	7.5
SY252	0						
	Viable cells/plate ^a	93±1	92±2	90±2	93±1	79±3	78±4
	Arg^+ revertants/plate ^b	19±4	17±2	21±4	21±1	19±2	19±3
	150						
	Viable cells/plate ^a	51±2	52±4	50±2	53±3	57±3	48±5
	Arg^+ revertants/plate ^b	85±6	79±3	59±4*	50±7*	50±4*	31±1*
	<i>I</i> /% ^d		7	31	41	41	63
mutS	0						
	Viable cells/plate ^a	33±4	33±3	29±1	37±6	36±2	37±2
	Arg^+ revertants/plate ^c	253±13	275±23	270±20	259±11	121±15*	70±9*
	<i>I</i> /% ^d		-9	-7	-2	52	72

The presented values are the average of duplicate samples from three independent experiments

^aviable cells/mL=viable cells/plate·10⁷

^b Arg^+ revertants/mL= Arg^+ revertants/plate·10

^c Arg^+ revertants/mL= Arg^+ revertants/plate·20

^d $I/\%=(1-N_t/N_c)\cdot 100$; N_t - sample with vitamin C, N_c - control sample (0)

* $p<0.05$

efficiency of vitamin C to remove the already induced damage.

The obtained results indicate that the lowest concentrations tested (0.1 mM for MMR-deficient and 0.5 mM for repair-proficient strain) had minor inhibitory effect on spontaneous and *t*-BOOH-induced MSI. Higher concentrations stimulated it, especially in repair-proficient cells treated with *t*-BOOH, probably due to additional oxidative effects of vitamin C (Table 3). This result is in correlation with literature data that antioxidants as the redox-active substances may induce both antioxidative and prooxidative effects, depending on the applied concentrations and other experimental conditions (16,35,36). In our experiments, the amplification of target sequences favouring frameshifts due to multiple copies of plasmid pAJ47 could contribute to increased test sensitivity. The effect of vitamin C could also be considered as a hormesis phenomenon, observed for γ -rays and numerous other toxins, pollutants and environmental fluctuations. The possible explanation for this phenomenon is that lower concentrations could stimulate repair and other defense mechanisms, while higher concentrations saturate them (37).

E. coli WP2 reversion test

In the present study, we examined the effect of vitamin C in *E. coli* WP2 reversion assay, recommended for screening of oxidative mutagens (Table 1). Strain IC202, chosen for this study, is an *oxyR* mutant deficient in removing ROS, and thus more sensitive to detection of the antimutagenic potential of antioxidants. The OxyR pro-

tein is a redox-sensitive transcriptional activator of genes encoding antioxidative enzymes: catalase hydroperoxidase I, alkyl hydroperoxide reductase and glutathione reductase. Strain IC202 also contains plasmid pKM101, carrying *mucAB* that enhances SOS mutagenesis (24).

Since IC202 possesses constitutive antioxidative defense, it was necessary to induce oxidative stress with low doses of *t*-BOOH. Mutagenesis monitored was *trpE65*→Trp⁺ reversions. Mutation *trpE65* is ochre; it can be reverted by base substitutions at the AT base pairs in the *trpE65* site or at the extragenic ochre suppressor loci. As mentioned above, *t*-BOOH could cause base substitutions of AT pairs, and therefore induce *trpE6*→Trp⁺ reversions (29).

In IC202, vitamin C stimulated both spontaneous and *t*-BOOH-induced mutagenesis. The highest obtained values were 89 and 41 %, respectively (Table 4). Stimulation of mutagenesis could result from *oxyR* deficiency of the strain, probably promoting the prooxidative effect of vitamin C. Therefore, it seems that intact antioxidative defense enzymes are important to preserve antioxidative potential of vitamin C. In favour of this hypothesis, vitamin C did not have any effect on spontaneous mutagenesis in OxyR proficient control strain IC185 (data not shown).

S. cerevisiae D7 reversion test

In order to get preliminary information about antimutagenic potential of vitamin C in eukaryotic cells, we included *S. cerevisiae* D7 test system in our study (Table 1). The diploid strain D7 contains *ilv1-92* mutation on

Table 3. The effect of vitamin C on the microsatellite instability

Strain	$\gamma(t\text{-BOOH})$	<i>c</i> (vitamin C)/mM				
	$\mu\text{g/mL}$	0	0.5	1.0	2.5	
SY252/pAJ47	0					
Viable cells/plate ^a		265±15	271±8	266±12	278±7	
Carb ^R revertants/plate ^b		248±17	241±30	276±16	308±29	
<i>I</i> % ^c			3	-11	-24	
	225					
Viable cells/plate ^c		79±8	72±4	69±1	74±6	
Carb ^R revertants/plate ^d		185±6	176±13	195±17	268±15*	
<i>I</i> % ^e			5	-5	-45	
Strain	$\gamma(t\text{-BOOH})$	<i>c</i> (vitamin C)/mM				
	$\mu\text{g/mL}$	0	0.1	0.25	0.5	1
SY252mutH/pAJ47	0					
Viable cells/plate ^a		149±25	141±20	155±11	152±6	150±11
Carb ^R revertants/plate ^b		173±13	143±21	194±10	204±11	207±10
<i>I</i> % ^e			17	-12	-18	-20

The presented values are the average of duplicate samples from three independent experiments

^aviable cells/mL=viable cells/plate·10⁶

^bCarb^R revertants/mL=Carb^R revertants/plate·10³

^cviable cells/mL=viable cells/plate·10⁵

^dCarb^R revertants/mL=Carb^R revertants/plate·10²

^e*I*%=(1-*N*_t/*N*_c)·100; *N*_t – sample with vitamin C, *N*_c – control sample (0)

**p*<0.05

Table 4. The effect of vitamin C in the WP2 reversion test

Strain	<i>m(t-BOOH)</i>	<i>c(vitamin C)/μM</i>							
	μg/plate	0	50	75	100	125	250	500	750
IC202	0								
Trp ⁺ revertants/plate ^a		101±2	104±2	104±1	98±4	93±4	136±8*	150±13*	195±5*
<i>I</i> % ^b			-3	-3	3	8	-31	-44	-88
	25								
Trp ⁺ revertants/plate ^a		337±19	326±28	314±26	289±17	275±26	345±5	370±26	476±4*
<i>I</i> % ^b			3	7	14	18	-2	-9	-41

The presented values are the average of duplicate samples from three independent experiments

^aTrp⁺ revertants/mL=Trp⁺ revertants/plate·10

^b*I*%=(1-*N*_t/*N*_c)·100; *N*_t - sample with vitamin C, *N*_c - control sample (0)

**p*<0.05

both homologous chromosomes. Revertants to *I*lv prototrophy are formed by base substitutions at *ilv1-92* or specific suppressor loci (25). As in bacteria, oxidative mutagenesis was induced with *t*-BOOH. Obtained results indicate that vitamin C expressed moderate inhibitory potential against *t*-BOOH-induced *ilv-92*→*I*lv⁺ reversions, with no effect on spontaneous mutations (Table 5). The highest inhibition was 41 % for 2.5 mM of vitamin C.

S. cerevisiae comet test

The effect of vitamin C on DNA strand breaks was determined by using the alkaline comet assay on *S. cerevisiae* 3A strain (Table 1) (26). To induce single strand breaks, hydrogen peroxide (H₂O₂) was used. By testing the increasing concentration of H₂O₂, we established that the concentration of 250 μM induced the highest number of comets without any effect on cell viability (data not shown).

In preliminary experiments, different concentrations of vitamin C (0.01–10 μM) were tested. While concentrations of 0.01 and 0.05 μM of vitamin C did not have any effect on the number of comets, the concentration of 0.1 μM and higher induced DNA damage and significantly increased the number of comets (Fig. 1). This is in accor-

dance with reported data that vitamin C, although antioxidant in low concentrations, may act as prooxidant in high concentrations and induce DNA damage (16,35,36).

Since concentration of 0.05 μM did not affect the number of comets, it was applied to measure protective effects of vitamin C against H₂O₂-induced damage. Experiments of both pretreatment and cotreatment with vitamin C were done. Pretreatment could point at additional beneficial effects on antioxidant cell pool together

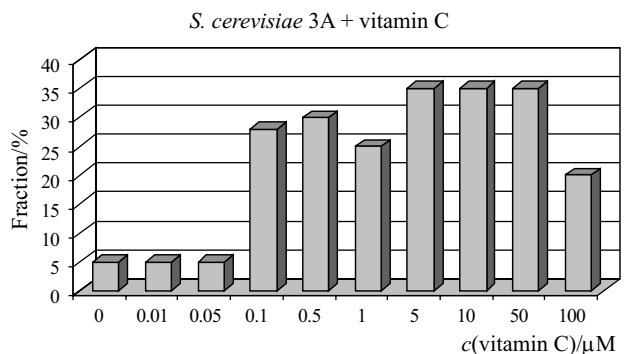


Fig. 1. The effect of vitamin C in *S. cerevisiae* comet assay

Table 5. The effect of vitamin C in the *S. cerevisiae* D7 reversion test

	<i>m(t-BOOH)</i>	<i>c(vitamin C)/mM</i>							
	μg/plate	0	0.25	0.50	0.75	1.00	1.50	2.00	2.50
	0								
Viable cells/plate ^a		120±5	120±13	118±5	106±9	119±5	112±3	125±2	112±4
<i>I</i> lv ⁺ revertants/plate ^b		16±1	19±4	18±3	20±3	19±2	10±1*	20±2*	14±2
	500								
Viable cells/plate ^a		110±4	121±15	115±7	121±3	117±4	117±12	111±8	107±6
<i>I</i> lv ⁺ revertants/plate ^b		54±4	58±1	52±6	37±4	39±3	34±4	32±5	32±3
<i>I</i> % ^c			-7	4	31	28	37	41	41

The presented values are the average of duplicate samples from three independent experiments

^aviable cells/mL=viable cells/plate·10⁶

^b*I*lv⁺ revertants/mL=*I*lv⁺ revertants/plate·10

^c*I*%=(1-*N*_t/*N*_c)·100; *N*_t - sample with vitamin C, *N*_c - control sample (0)

**p*<0.05

with scavenging action, while the cotreatment predominantly detects scavenging of ROS.

The obtained results showed strong protective capability of vitamin C. Reduction in the number of comets was 65 % for both pretreatment and cotreatment experiments (Fig. 2). The same inhibition of comets, obtained in both experimental conditions, suggested that antioxidative properties of vitamin C might be primarily based on ROS scavenging activities.

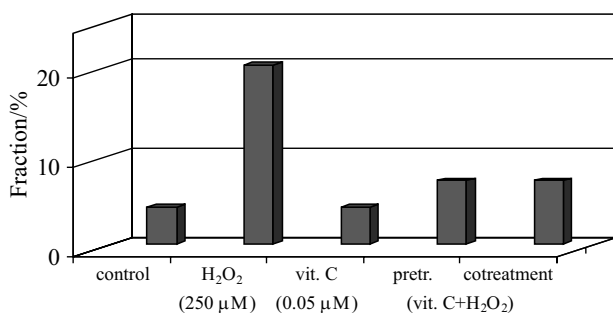


Fig. 2. The effect of vitamin C on the H₂O₂-induced comets in *S. cerevisiae* 3A

Conclusions

The effects of vitamin C on oxidative DNA damage and mutagenesis were evaluated with several antimutagenicity and antigenotoxicity tests. Antimutagenic potential of vitamin C was demonstrated in repair proficient and MMR deficient strains of the *E. coli* K12 reversion assay, as well as in the *S. cerevisiae* D7 reversion test. This protective effect of vitamin C could be attributed to its antioxidant properties, since *t*-BOOH, used as a mutagen, induces oxidative damage.

Weak mutagenic effects of vitamin C (stimulation of frameshifts in microsatellite sequences) were obtained in repair-proficient, as well as in MMR-deficient strains of the *E. coli* K12 assay, carrying microsatellite sequences on plasmid. Strong stimulation of mutagenesis was obtained in the *oxyR* strain of *E. coli* WP2, deficient in antioxidative defense enzymes. These mutagenic responses could be attributed to prooxidative behaviour of vitamin C, visualized in assays especially sensitive for detection of oxidative DNA damage.

The results of the comet assay on yeast confirmed that vitamin C promoted DNA damage at higher concentrations, but also could protect from H₂O₂-induced DNA damage if applied at lower concentrations. The same antigenotoxic potential of vitamin C detected in pretreatment and cotreatment experiments, pointed at scavenging activities of vitamin C as predominant mechanism of protection. The obtained results indicate that vitamin C can exhibit antigenotoxic or genotoxic effects, depending on the dose, genetic background and other experimental conditions.

In comparison with the concentration of vitamin C in human plasma (~50 μM for healthy persons), tested concentrations were higher in reversion assays. However, it is important to mention that this is an *in vitro* study on bacterial and yeast cells. In the comet assay the

significantly lower concentration (0.05 μM) was applied, but previous study had shown considerably higher sensitivity of comet assays performed on yeast than on mammalian cells (26). In order to extrapolate these results on humans, further *in vivo* study on mammals as well as on human cultured cells should be done.

Acknowledgements

This research was supported by the Ministry of Science and Environmental Protection of the Republic of Serbia (Project »Plant antimutagens« No. 1502).

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