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Sixth Conference

"Biochemistry and Interdisciplinarity: Transcending the Limits of Field"

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"***Biochemistry and Interdisciplinarity: Transcending the Limits of Field***"

Foreword

Dear Colleagues

It is my distinct pleasure to welcome you to the 6th Conference of the Serbian Biochemical Society, entitled "Biochemistry and Interdisciplinarity: Transcending the Limits of Field". It is an honor for me to be selected as the Editor of Proceedings of the Conference. I am grateful to the Steering Committee of Serbian Biochemical Society for giving me this opportunity to shape the premiere forum in biochemistry in the region. We have been tremendously fortunate to have Mihajlo B. Spasić as the first Editor. He nurtured this Conference (and Society) through its re-starting years as it grew in quality and relevance. Clearly, following in his footsteps is a challenge.

We have invited Djuro Josić from the University of Rijeka and eight experts from four major universities in Serbia to give lectures at the 6th Conference. The visit of our dear colleague from Croatia is a part of an initiative for closer collaboration within FEBS3+ (Croatia, Hungary, Slovenia, and Serbia) Meeting Programme that was established by FEBS in 2010. We have also invited students at the final years of PhD studies to present their work in our Proceedings as Abstracts. Official languages at the Conference will be Serbian, Croatian, and English.

I would like to express my gratitude to the members of the Scientific Board who suggested lecturers and to all respected colleagues who accepted the invitation.

Editor of the Proceedings
Ivan Spasojević

Investigation of vitamin C effects on DNA damage during enzymatic decolorization

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Introduction

DNA damage is partially mediated by reactive oxygen species (ROS) which can lead to mutations and development of cancer [1]. Complex azo colorants can be generators of endogenous ROS by: dye itself, during reductive biotransformation or by products obtained after oxidation [2]. Horseradish peroxidase (HRP) is an oxidoreductase which can catalyze degradation of numerous aromatic substrates, such as dye molecules, in the presence of hydrogen peroxide (H_2O_2) [3]. However, the effect of enzymatic decolorization should take into account the toxicity of degradation products as well as the percentage of color removed [4]. Since vitamin C is one of most studied antioxidants with demonstrated *in vitro* potential inprevention of oxidation of important biological molecules, we aimed to investigate the protective effects during enzymatic decolorization. We have applied medium throughput comet assay to measure DNA damage in lymphocytes of healthy adults treated with azo dyes before and after decolorization by HRP.

Materials and methods

Orange II (OR2, λ_{max} 490 nm) and Amido Black 10B (AB, λ_{max} 600 nm) were dissolved in 20 mM bicarbonate buffer pH 9.0 (BB). Vitamin C (L-ascorbic acid) was freshly prepared as stock 113 mM solution in water. Decolorization assay were done as follows. Dye solution of 100 μg mL^{-1} was incubated with 20 μL of 3 U ml^{-1} HRP and 170 μM H_2O_2. In parallel, decolorization reaction was prepared with addition of 50 μM vitamin C. Reaction mixtures were incubated 2 h at room temperature in the dark. Afterwards, decolorization of the dyes were calculated based on the formula: $D\ (\%) = [(A_i - A_d)/A_i] \times 100$, where A_i is the absorbance of the dye prior decolorization at λ_{max} and A_d is the absorbance after decolorization treatment.

Peripheral human lymphocytes were isolated from venous blood of healthy adults by centrifugation over Lymphoprep following manufacturer's instructions. The lymphocytes were embedded in 1% LMP agarose and treated with 30 μL of reaction mixture for 30 min.

Detection of DNA damage was done using the comet assay. Medium throughput comet assay was done by using the 12-gel comet chamber as described by Janovic et al. [4].

Results

HRP showed high efficiency in decolorization of OR2 and AB. In case of OR2 degradationwas 91% and vitamin C showed negligible effects on decolorization (90%). When AB was treated with HRP 72% of dye was decolorized. Interestingly, removal of AB in the presence of vitamin C was less efficient, where 7% less dye was removed under the same experimental conditions (Figure 1a).

Treatment with BB had no effects on lymphocytes, while some DNA damage was observed in the presence of vitamin C (Figure 1b). DNA damage was 32% and 21% for OR2 and AB, respectively. After the HRP treatment, DNA damage hasdecreased in both cases, but some percentage of tail DNA was present (Fig. 1c; HRP treated). This could be partially mediated by fast decolorization and residual H_2O_2 in the reaction mixture. Nevertheless, when the same decolorization reaction took place in the presence of vitamin C, DNA damage was slightly influenced (Figure 1c; HRP treated + vitamin C). These results indicate minor pro-oxidant effects of the vitamin C under the experimental conditions applied. Similar, but yet more pronounced, effects were observed in the case of double azo dye, AB, than the single azo dye OR2.

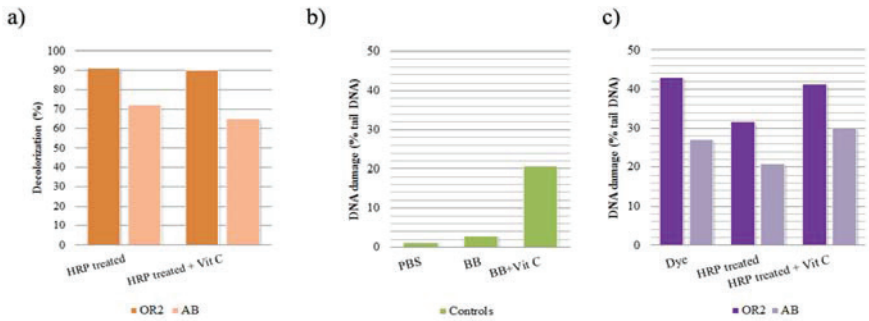

Figure 1. Effects of vitamin C (50 μM) on: a) decolorization of dyes by HRP; b) DNA damage in the negative controls (PBS – phosphate buffered saline solution, BB – bicarbonate buffer); c) DNA damage before and after HRP decolorization.

Conclusions

Using the comet assay we have investigated the possible protective effect of vitamin C against DNA damage caused by excess or remaining H_2O_2during or after decolorization of azo dyes. The potential of HRP for dye decolorization was showed in terms of reduction of genotoxicity potential of dyes tested. Vitamin C showed no effects in protecting DNA against potential damage occurring during oxidoreductase reaction.

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