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Protective effects of resveratrol against oxidative/nitrative modifications of plasma proteins and lipids exposed to peroxynitrite

Beata Olas*, Pawel Nowak, Joanna Kolodziejczyk, Michal Ponczek, Barbara Wachowicz

Department of General Biochemistry, Institute of Biochemistry, University of Lodz, 90-237 Lodz, Poland

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Abstract

The protective effects of resveratrol (3, 4′, 5-trihydroxystilbene; present naturally in different plants) against the oxidative/nitrative damage of human plasma proteins induced by peroxynitrite ($ONOO^-$) were studied and compared with those of deferoxamine (DFO; a natural siderophore isolated from *Streptomyces pilosus*), which is a typical and well-known antioxidant. We also studied the effect of $ONOO^-$ on plasma lipid peroxidation and the role of tested antioxidants in this process. $ONOO^-$ at the used concentrations (0.01–1 mM) showed toxicity to human plasma components. Exposure of plasma to $ONOO^-$ (0.1 mM) resulted in an increase of the level of carbonyl groups and nitrotyrosine residues in plasma proteins (approximately 4-fold and 76-fold, respectively) and in a distinct augmentation of lipid peroxidation (approximately 2-fold). In the presence of 0.1-mM resveratrol, a distinct decrease of carbonyl group formation and tyrosine nitration in plasma proteins caused by 0.1-mM $ONOO^-$ was observed (by approximately 70% and 65%, respectively). Addition of 0.1-mM DFO to plasma also distinctly reduced the level of carbonyl groups and nitrotyrosines caused by 0.1-mM $ONOO^-$ (by approximately 50% and 60%, respectively). Moreover, these antioxidants also inhibited plasma lipid peroxidation induced by $ONOO^-$ (0.1 mM). The obtained results indicate that in vitro resveratrol, like well-known antioxidant DFO, has inhibitory effects on $ONOO^-$-mediated oxidation of proteins and lipids in human plasma.

Keywords: Plasma; Peroxynitrite; Tyrosine nitration; Carbonyl groups; Resveratrol; Deferoxamine

1. Introduction

The generation of reactive oxygen species (ROS) may occur in a large number of physiological and nonphysiological processes that include their generation as by-products of normal cellular metabolism. ROS may induce oxidative stress and damage to all types of biologic molecules. Different ROS, including superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$) and peroxynitrite ($ONOO^-$), lead to protein oxidation [1]. Oxidative stress causes damage to proteins by an adduction of products of glycoxidation and/or lipid peroxidation and by a direct oxidation of protein side chains [2,3]. ROS may oxidize amino acid residue side chains into ketone or aldehyde derivatives (carbonyl groups) [4]. Histidine, arginine and lysine are the most susceptible amino acids for ROS-mediated carbonyl formation [5]. It has been demonstrated that exposure of protein to $ONOO^-$ results in the oxidation of tryptophan, cysteine and methionine; nitration of tyrosine; formation of dityrosine; carbonyl group formation; and protein fragmentation [6–10]. The rate of $ONOO^-$ formation depends on the concentrations of $O_2^{\cdot-}$ and $^{\cdot}NO$, and even a relatively small increase of their concentrations may be responsible for a remarkable increase of $ONOO^-$ generation and its cytotoxic effects. It is hypothesized that, in vivo, the flux of $O_2^{\cdot-}$ is more critical to the rate of $ONOO^-$ generation than nitric oxide due to $^{\cdot}NO$ being usually in a large molar excess. Modification of proteins induced by $ONOO^-$ and its intermediates may lead to functional alterations of proteins. Our earlier studies showed that incubation of human blood platelets with $ONOO^-$ results in the changes of low-molecular-weight thiols (glutathione, cysteine and cysteinylglycine) [11] and in the depletion of free protein thiols [11] concomitant with an increase of nitrotyrosine amount in platelet proteins [12].

The defense mechanisms against $ONOO^-$ action are very important for biologic functions of human plasma components. Arteel et al. [13] and Klotz and Sies [14] suggest that defense against $ONOO^-$ action on cell structures may be at

* Corresponding author. Tel.: +48 42 6354484; fax: +48 42 6354484.
E-mail address: olasb@biol.uni.lodz.pl (B. Olas).

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the level of prevention of its formation, at the level of interception or repair of damage resulting from $ONOO^-$ reactions. Various agents were often tested for the ability to protect different molecules (lipids and proteins) from $ONOO^-$-induced changes in their structure and functions. Human diet is rich in a great variety of micronutrients with antioxidant properties. Among these, polyphenolic compounds widely distributed in fruits, vegetables and beverage such as tea, beer and wine hold an important role. However, the role of exogenous antioxidant resveratrol (3, 4′, 5-trihydroxystilbene; present naturally in grapes, fruits and a variety of medicinal plants) in the defense against $ONOO^-$ action in human plasma is still unknown. Therefore, the aim of our study was to estimate the effects of resveratrol on $ONOO^-$-induced changes in plasma proteins and lipids. Moreover, we compared the action of resveratrol with that of deferoxamine (DFO; a natural siderophore isolated from *Streptomyces pilosus*), which is a typical and well-known antioxidant. We determined lipid oxidation and protein oxidation/nitration in human plasma in the presence of antioxidants resveratrol and DFO. Our earlier studies indicate that resveratrol, like DFO, may protect proteins of blood platelet against oxidation caused by $ONOO^-$ or its intermediates [15].

2. Materials and methods

2.1. Materials

$ONOO^-$ was synthesized according to the method of Pryor and Squadrito [16]. Freeze fractionation (−70°C) of the $ONOO^-$ solution formed a yellow top layer, which was retained for further studies. The top layer typically contained 80–100 mM of $ONOO^-$ as determined spectrophotometrically at 302 nm in 0.1 M of NaOH ($\varepsilon_{302\ nm}$=1679 M/cm). Some experiments were also performed with decomposed $ONOO^-$, which was prepared by allowing $ONOO^-$ to decompose at neutral pH (7.4) in 100 mM of potassium phosphate buffer (15 min, room temperature). Resveratrol, DFO (mesylate salt), rabbit anti-dinitrophenylhydrazine (DNP) antibodies, anti-rabbit antibodies and anti-goat/sheep antibodies coupled with peroxidase were purchased from Sigma (St Louis, MO, USA). Stock solutions of resveratrol were made in 50% dimethylsulfoxide at a concentration of 25 mg/ml and kept frozen.

Sheep anti-nitrotyrosine polyclonal antibodies were from Oxis (Portland, OR, USA). Biotinylated anti-goat/mouse/rabbit antibodies and streptavidin-biotinylated horseradish peroxidase were from DAKO (Glostrup, Denmark). All other reagents were of analytical grade and were provided by commercial suppliers.

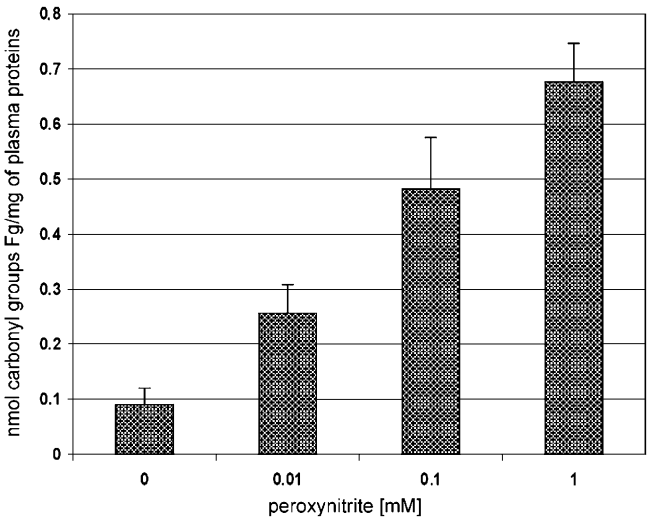

Fig. 1. DNP-reactive carbonyl formation following treatment of human plasma proteins with $ONOO^-$ (0.01–1 mM). The protein oxidation was measured by ELISA method. The results are expressed as nanomoles of carbonyl groups-Fg per milligram of plasma proteins. The results are representative of six independent experiments and are expressed as mean±S.D. The effects of three concentrations of $ONOO^-$ were statistically significant according to one-way ANOVA (P<.02). Correlation coefficient r=.835 (P<.005).

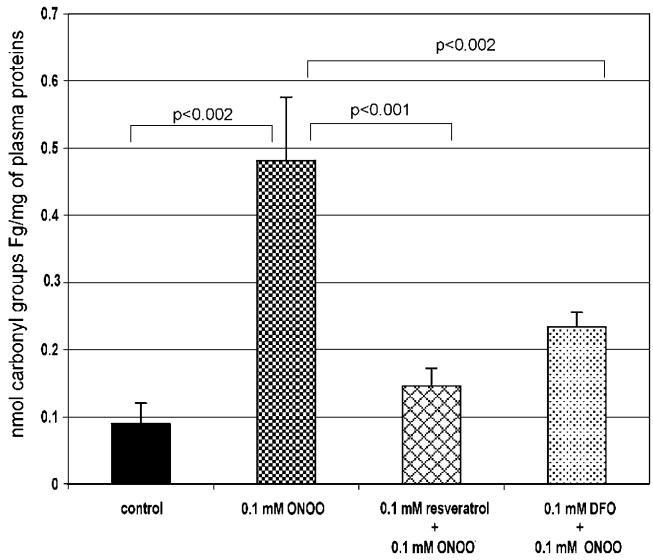

Fig. 2. The effects of resveratrol and DFO (0.1 mM) on carbonyl group formation (protein oxidation) induced by $ONOO^-$ (0.1 mM). The protein oxidation was measured immunologically using ELISA method. The results are expressed as nanomoles of carbonyl groups-Fg per milligram of plasma proteins. The results are representative of six independent experiments and are expressed as mean±S.D. The presented results are three distinct paired comparisons. The effects were statistically significant according to the paired Student's t test, ($ONOO^-$-treated plasma vs. control; resveratrol/DFO+$ONOO^-$-treated plasma vs. $ONOO^-$-treated plasma).

2.2. Incubation of plasma with antioxidants and $ONOO^-$

Human blood from healthy volunteers was collected into sodium citrate (5 mmol/L final concentration) and immediately centrifuged (3000×g, 15 min) to get plasma. $ONOO^-$ was added to plasma as a bolus to the final concentration of 0.01–1 mM and the samples were immediately vigorously mixed. Some samples of human plasma were preincubated (2 min at room temperature) with tested antioxidants