PHARMACOLOGY AND TOXICOLOGY

Role of Cu^{2+} in Free Radical Oxidation of Human Serum Albumin and L-Tyrosine Dipeptide with Multicomponent Metal-Containing Xenobiotic

S. V. Tkachev and A. A. Ushkov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 9, pp. 291-294, September, 2005
Original article submitted October 26, 2004

Cu^{2+} entering the composition of multicomponent metal-containing xenobiotic *in vitro* initiated free radical oxidation of human serum albumin and L-tyrosine dipeptide. Oxidative modification was accompanied by the formation of derivatives of amino acids tyrosine and tryptophan and structural changes in human serum albumin.

Key Words: *copper; protein oxidation; reactive oxygen species*

Cu^{2+} compounds are the most hazardous and widespread environmental contaminants [5]. Cu^{2+} excess in the body above physiological levels is accompanied by a toxic effect. Toxicity of Cu^{2+} is associated with its ability to catalyze Fenton's reaction with generation of reaction oxygen species (ROS) [5,11]. ROS induce oxidation of lipids, proteins, and DNA [1].

Biological consequences of free radical processes initiated by Cu^{2+} depend on oxidative modification of protein molecules. Oxidative degradation is followed by inactivation of various enzymes, including glutathione peroxidase, hexokinase, lactate dehydrogenase, trypsin, superoxide dismutase, carboxypeptidase, and alcohol dehydrogenase [3,6,7]. Protein oxidation is accompanied by aggregation, fragmentation of protein molecules, and modification of amino acid residues [6,7].

Our previous experiments on rats showed that intoxication with technogenic xenobiotics containing considerable amounts of Cu^{2+} led to accumulation of oxidative protein degradation products (carbonyl derivatives of amino acids; and derivatives of tryptophan and dityrosine) [3]. It should be emphasized that the test preparations include compounds of Cd^{2+} and Zn^{2+} in low concentrations. The presence of these elements probably contributes to toxic activity of Cu^{2+}. To test this hypothesis, we compared *in vitro* oxidation of human serum albumin (HSA) and L-tyrosine dipeptide (Tyr-Tyr) in the presence of $CuSO_4$ and complex mainly consisting of Cu^{2+} compounds and trace amounts of Zn^{2+} and Cd^{2+} compounds.

MATERIALS AND METHODS

Experiments were performed with a copper-containing solution (CCS) including water-soluble complexes of Cu^{2+} and ammonia. Atomic absorption spectroscopy was performed on an AAS Vario 6 atomic absorption spectrophotometer (Analytik Jena AG). Cu^{2+}, Zn^{2+}, and Cd^{2+} were shown to be the major constituents of CCS (105.4, 0.178, and 0.038 g/liter, respectively). CCS was obtained during conversion of Cu^{2+}-containing galvanic waste into fungicides and plant-protecting preparations.

We used purified lyophilized HSA and Tyr-Tyr (Sigma). HSA and Tyr-Tyr were oxidized for 1 h in a medium containing $CuSO_4$ or CCS in various concentrations. The concentration of $CuSO_4$ and CCS varied from 25 to 250 μM (by Cu^{2+} concentration). The concentration of HSA and Tyr-Tyr in control and treated samples was 1 mg/ml. The incubation medium included ascorbic acid and H_2O_2 in final concentrations of

Department of Toxicology, Republican Research-and-Technology Center of Hygiene, Minsk. ***Address for correspondence:*** sergtk@rambler.ru. S. V. Tkachev

0007-4888/05/1403-0309

2 and 100 mM, respectively. The reagents were dissolved in 0.01 M Tris-HCl/0.15 M NaCl buffer (pH 7.4).

Oxidative modification of HSA was recorded by accumulation of dityrosine and decrease in fluorescence of tryptophan residues. Oxidative transformation of Tyr-Tyr was evaluated by the formation of dityrosine. Fluorescence of dityrosine was measured at the excitation and emission wavelengths of 325 and 16 nm, respectively. Fluorescence of tryptophan was measured at excitation and emission wavelengths of 297 and 336 nm, respectively [2].

Structural changes in HSA were studied using 1-anilino-8-naphthalene sulfate ammonium salt (ANS, Fluka) in a concentration of 5 μM. Fluorescence spectra of ANS were recorded at an excitation wavelength of 380 nm.

Fluorescence was measured on a SFM 25 spectrofluorometer (Kontron). Emission spectra of ANS were recorded using a SM 2203 spectrofluorometer (Solar).

Experimental data are expressed as the means of 4 independent measurements.

RESULTS

Addition of $CuSO_4$ and CCS in minimum concentration (25 μM Cu^{2+}) to the incubation medium sharply decreased fluorescence of HSA tryptophanyl (by 60-70%, Fig. 1). Tryptophanyl fluorescence progressively decreased with increasing the concentration of preparations in the protein solution. Tryptophanyl fluorescence reached minimum (~25% of the control level) in the presence of $CuSO_4$ and CCS at a concentration equivalent to 250 μM Cu^{2+}.

Addition of $CuSO_4$ and CCS in a concentration equivalent to 25-75 μM Cu^{2+} was followed by an increase in the concentration of dityrosine (by 133 and 118%, respectively, compared to the control) in the solution of HSA. Further increase in Cu^{2+} concentration in HSA solution led to a decrease in the amount of dityrosine. After treatment with $CuSO_4$ and CCS in a concentration equivalent to 250 μM Cu^{2+} the content of dityrosine exceeded the control by 27 and 43%, respectively.

Incubation of Tyr-Tyr with CCS and $CuSO_4$ was accompanied by a dose-dependent increase in the concentration of dityrosine. Addition of $CuSO_4$ and CCS in maximum concentration increased dityrosine fluorescence by 230 and 285%, respectively, compared to the control (Fig. 2).

Recording of the ANS emission spectrum showed that CCS and $CuSO_4$ cause structural modification of HSA (Fig. 3). Oxidation of HSA affected different spectral characteristics of ANS (*e.g.*, intensity and position of the fluorescence maximum). The test substances in a concentration of 50 μM (Cu^{2+}) sharply decreased ANS fluorescence and shifted fluorescence maximum by 60 nm toward a long-wavelength range. A 2-fold increase in Cu^{2+} concentration in the solution of HSA produced further decrease in the intensity of ANS fluorescence.

Previous studies [10] of the mechanisms for protein oxidation in the presence of transition metals (Fe^{2+} and Cu^{2+}) showed that metal-catalyzed oxidation of proteins is a site-specific process. This process starts from fixation of the transition metal ion to a metal—binding protein surface. The metal—protein complex generates $HO^{\bullet}$ in the presence of reducing agents. $HO^{\bullet}$ causes oxidative modification of amino acid residues at the metal-binding protein site. Products of amino acid residue oxidation are formed in several consecutive reactions. Free radical attack on the polypeptide backbone is initiated by $HO^{\bullet}$-dependent release of hyd-

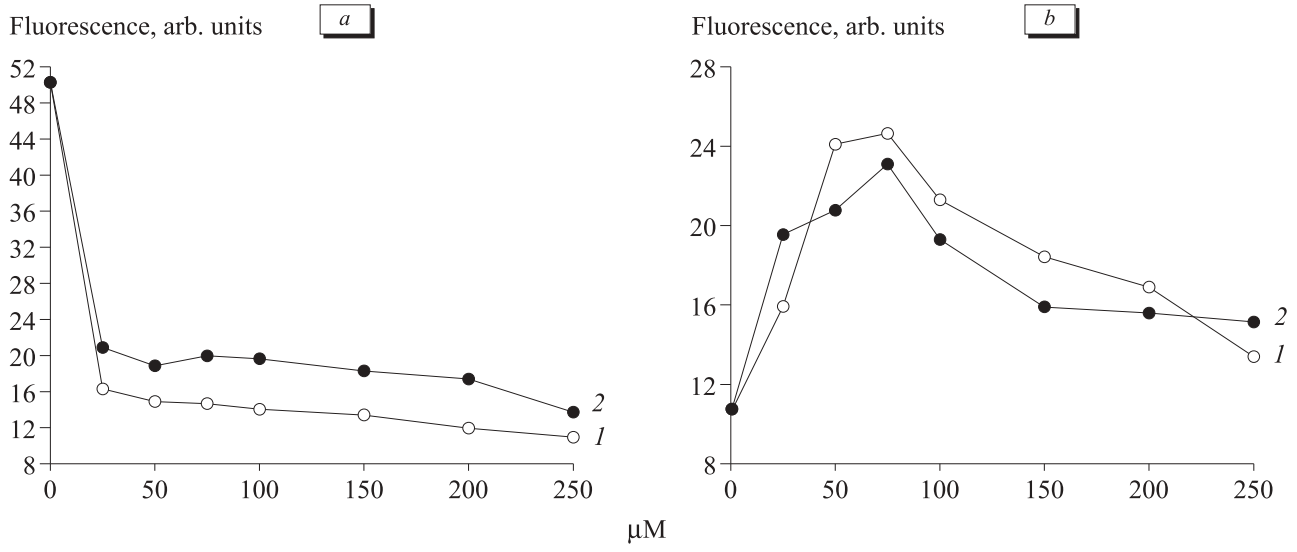

Fig 1. Oxidation of tryptophan (*a*) and tyrosine residues (*b*) of human serum albumin as a function of concentrations of $CuSO_4$ (*1*) and copper-containing solution (*2*). Here and in Fig. 2: abscissa, concentrations of $CuSO_4$ and copper-containing solution (by Cu^{2+}).

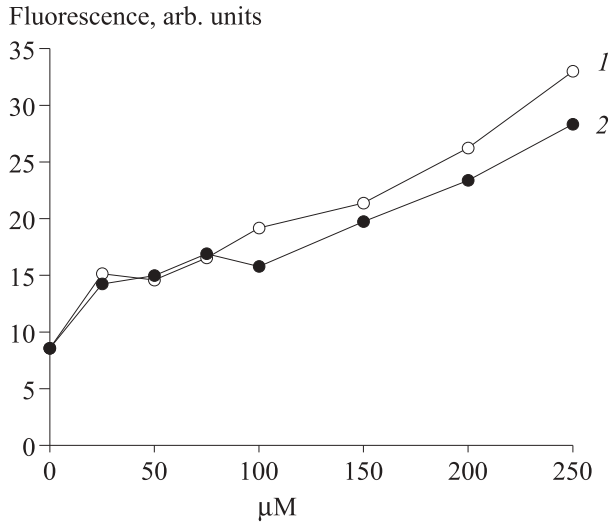

Fig. 2. Dityrosine formation in the solution of dipeptide tyrosine at different concentrations of $CuSO_4$ (*1*) and copper-containing solution (*2*).

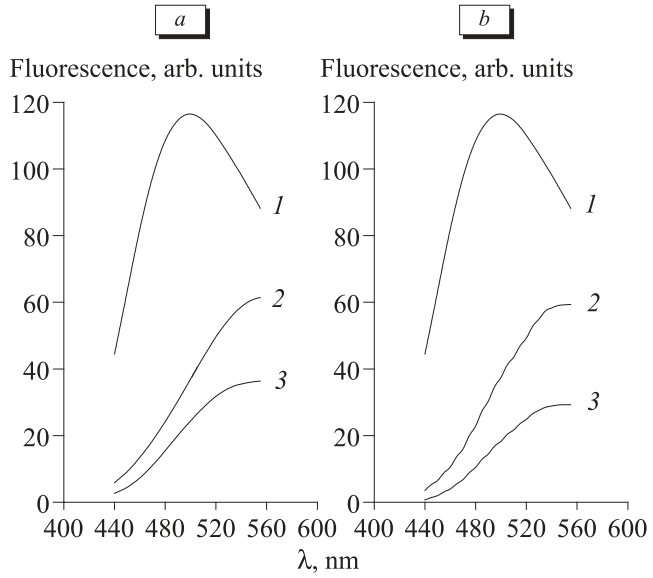

Fig. 3. Florescence spectra for 1-anilino-naphthalene-8-sulfonate in human serum albumin oxidized in the presence of $CuSO_4$ (*a*) and copper-containing solution (*b*). Native albumin (1 mg/ml, *1*); $CuSO_4$ and copper-containing solution (50 μM by Cu^{2+}, *2*); $CuSO_4$ and copper-containing solution (100 μM by Cu^{2+}, *3*).

rogen from the α-carbon atom, which leads to the formation of alkyl radicals. Alkyl radical interacts with oxygen, which results in the formation of alkyl peroxyl, alkyl peroxide, and alkoxyl radicals and hydroxyl protein derivatives [6].

Free radical oxidation of HSA in the presence of ascorbic acid and Cu^{2+} is accompanied by cleavage of amino acid backbone and formation of typical protein fragments (50, 47, 22, 18, and 3 kDa). Destruction of HSA is also accompanied by a decrease in tryptophanyl fluorescence [8].

Nearly all amino acid residues of proteins are oxidized with ROS. For example, tryptophan undergoes conversion to 5-hydroxytryptophan, 7-hydroxytryptophan, kynurenine, 3-hydroxykynurenine, and formylkynurenine. Oxidation of tyrosine is followed by the formation of dityrosine. This covalent biphenol is formed after transformation of tyrosine radicals [6, 7,10]. Our results are consistent with published data [7,10] that oxidation of proteins is accompanied by a decrease in tryptophan fluorescence and formation of intra- and intermolecular dityrosine bonds. Dityrosine formation can be blocked by the superoxide anion radical, which reduces tyrosyl radical [7]. Reduction of tyrosyl radical with aloxyl, alkoxyl, and alkyl peroxide radicals of amino acid residues probably contributes to the decrease in dityrosine formation in HSA solution during oxidation with CCS and $CuSO_4$.

Long-wavelength shift in the ANS spectrum reflects spatial reconfiguration of HSA (*e.g.*, changes in the position of ANS-binding regions in the protein polypeptide chain and polarity of the probe environment). Structural modification of HSA probably increases probe accessibility to fluorescence-quenching collisions with molecules of H_2O_2 and Cu^{2+}.

Our results show that CCS strongly stimulates oxidative modification of HSA and Tyr-Tyr. Similar concentration dependences for dityrosine formation and tryptophan oxidation were revealed in experiments with CCS and $CuSO_4$. Moreover, CCS and $CuSO_4$ produced the same structural changes in HSA. These data indicate that Cu^{2+} *in vitro* and *in vivo* plays a key role in oxidative damage to proteins with CCS.

We are grateful to V. A. Kostyuk for his participation in discussion of the results.

This work was supported by the Foundation for Basic Research (Belarussian Academy of Sciences, grant No. 151-B03, April 14, 2003).

REFERENCES

1. Yu. A. Vladimirov, O. A. Azizova, A. I. Deev, *et al*., *Itogi Nauki Tekhniki. Ser. Biof*., **29**, 7-9 (1991).
2. E. E. Dubinina, S. V. Gavrovskaya, E. V. Kuz'mich, *et al*., *Biokhimiya*, **67**, No. 3, 413-421 (2002).
3. E. E. Dubinina and I. V. Shugalei, *Usp. Sovr. Biol*., **113**, No. 1, 71-81 (1993).
4. S. V. Tkachev, *Belorusskii Med. Zh*., No. 1, 84-86 (2004).
5. D. G. Barceloux, *Clin. Toxicol*., **37**, No. 2, 217-230 (1999).
6. S. B. Berlett and E. R. Stadtman, *J. Biol. Chem*., **272**, 20,313-20,316 (1997).
7. R. T. Dean, Sh. Fu, R. Stocker, and M. J. Davies, *Biochem. J*., **324**, 1-18 (1997).
8. G. Marx and M. Chevion, *Ibid*., **236**, 397-400 (1985).
9. E. Pigeolet, P. Corbisier, A. Houbion, *et al*., *Mech. Ageing Dev*., **51**, No. 3, 283-297 (1990).
10. E. R. Stadtman, *Free Radic. Biol. Med*., **9**, 315-325 (1990).
11. S. J. Stohs and D. Bagchi, *Ibid*., **18**, No. 2, 321-336 (1995).