ANTIINFLAMMATORY REACTIVITY OF COPPER(I)-THIONEIN

RALF MIESEL, HANS-JÜRGEN HARTMANN, and ULRICH WESER

Anorganische Biochemie
Physiologisch-Chemisches Institut der Universität Tübingen
Hoppe-Seyler Strasse 4, 7400 Tübingen, Federal Republic of Germany

Abstract—In unseparated human blood the reactivity of yeast copper (I)-thionein on TPA-activated polymorphonuclear leukocytes was evaluated and compared with low Mr copper chelates exerting Cu2Zn2 superoxide dismutase mimetic activity. Cu, 18μM, in the form of Cu-thionein was sufficient to inhibit the superoxide production of activated human blood phagocytes by 50%. Furthermore, the scavenging of hydroxyl radicals and singlet oxygen by Cu(I)-thionein was determined, using the 2-deoxyribose fragmentation assay induced by decaying K2CrO4 and the NADPH oxidation caused by UVA illuminated psoralen, respectively. The inhibitory reactivity of Cu-thionein in both assays was compared with that of serum proteins including albumin, ceruloplasmin, transferrin, and ferritin. The galactosamine/endotoxin-induced hepatitis in male NMRI mice was used to evaluate the antiinflammatory reactivity of Cu-thionein in vivo. The serum copper, superoxide dismutase, and sorbitol dehydrogenase concentrations, as well as the activity of polymorphonuclear leukocytes in unseparated blood seemed most appropriate to quantify the protective capacity of Cu-thionein in the course of an oxidative stress-dependent liver injury. The intraperitoneal application of 32.5 μmol/kg thionein-Cu limited this damage to 45%.

INTRODUCTION

Metallothioneins are ubiquitous proteins of approximately 6 kDa (1). They are characterized by an unusually high content of cysteine (> 30 mol%) and can be induced by transition metals, stress-related hormones, interferons, or bac-

1 Abbreviations: SOD, Cu2Zn2 superoxide dismutase (EC 1.15.1.1); PMN, polymorphonuclear leukocyte; TPA, 12-O-tetradecanoylphorbol-13-acetate; CuPu(Py)2, \{[N,N'-bis(2-pyridylmethylene)-1,4-butanediamine][N', N'', N''', N''''\]}-copper(II); CuPu(Im)2, \{[1,8-di(2-imidazolyl)-2,7-diazaoctadiene-1,7-\}-(N', N'', N''', N''''\}]-copper(II), Cu(Sal)2, copper salicylate; TBA, 2-thiobarbituric acid; DTPA, diethylenetriaminepentaacetic acid; SDH, sorbitol dehydrogenase (EC 1.1.1.14); BSA, bovine serum albumin.

2 To whom correspondence should be addressed.

471
terial endotoxins (2–6). Under physiological conditions, mainly copper and zinc are bound to metallothionein. In the course of inflammatory processes the biochemistry of copper is markedly affected (7). It is not known whether copper-thioneins are involved in inflammation. It was of interest, therefore, to evaluate the reactivity of Cu-thionein on TPA-activated human polymorphonuclear leukocytes. PMNs are the first cells that invade inflamed areas, where their NADPH-oxidase-dependent production of superoxide mediates the cooperative interactions with all other blood cells, known to be associated with the many inflammatory events (8, 9). As synthetic low Mr copper chelates display a pronounced superoxide dismutase mimetic reactivity (10–13) and are beneficial in the treatment of rheumatic diseases (14), we examined the SOD-like activity of Cu-thionein in vitro by simulating an increased oxidative stress-dependent flux of oxygen free radicals. For that purpose, the earlier described K$_3$CrO$_8$ decay (15, 16) as well as the UVA-psoralen–NADPH assay (17, 18) seemed most appropriate. Additionally, the in vivo reactivity of Cu-thionein was investigated using the galactosamine/endotoxin-induced hepatitis (19) in male NMRI mice. The breakdown of the Cu-thiolate chromophore caused by activated oxygen species (20–22) results in a transient Cu coordination that allows a Cu(I)/Cu(II) redox cycling necessary to catalyze superoxide dismutation. SOD-like activities protect inflamed tissues against an O$_2^·$ -dependent damage. Reactive oxygen species are known to degrade biopolymers including hyaluronic acid and DNA. At the same time they may induce lipid peroxidations (23). Usually ·OH radicals, originating from a transition metal catalyzed Fenton reaction, or singlet oxygen are of a more pronounced reactivity compared to that of superoxide (24). The simultaneous scavenging of ·OH radicals and singlet oxygen, combined with a SOD-like activity by one single low Mr copper compound should, therefore, reveal antiinflammatory reactivity, both in vitro and in vivo.

**MATERIALS AND METHODS**

*Chemicals.* The deionized water used was quartz-distilled and additionally purified with a Millipore water purification system. The conductivity was less than 0.05 μS. Cu$_2$Zn$_2$ superoxide dismutase from bovine erythrocytes, imidazole-2-aldehyde, putrescine, pyridine-2-aldehyde, 12-O-tetradecanoylphorbol-13-acetate (TPA), lucigenin, psoralen, trypan blue, ceruloplasmin (human), galactosamine, *Salmonella abortus equi* endotoxin (lipopolysaccharide), and lyticase from *Arthrobacter luteus* were purchased from Sigma, Munich. 2-Thiobarbituric acid (TBA), 2-deoxyribose, xanthine, diethyleneetriaminepentaacetic acid (DTPA), bovine serum albumin, and HEPES were from Serva, Heidelberg; xanthine oxidase from cow milk and ferritin from horse spleen from Boehringer, Mannheim. Transferrin was purchased from Behring, Marburg.

*Preparations.* Copper(I)$_x$-thionein was isolated from *Saccharomyces cerevisiae* strain X 2180-1Aa as earlier described (25). The incubation with lyticase (50 units/ml) increased the spontaneous release of Cu-thionein into the medium. Cu-thionein remaining inside the spheroplasts was