Involvement of Trace Metals in the Reduction of Disulfide Bonds by Glutathione

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Glutathione (GSH) and its derivatives were used as reducing agents to evaluate the effects of trace metal elements on the reduction of oxidized proteins containing cysteine in their structures, using zinc finger protein as a substrate for the reduction reaction. As the thiol group of the cysteine engaged in the coordination bond of Zn(II) may be involved in reversible or irreversible redox reactions in vivo, zinc finger protein is considered as a suitable substrate for this experimental system. We generated oxidants of the GAGA zinc finger derived from Drosophila transcription factors and examined the change in the reduction reaction over time as well as the effects of the cofactor metal ions such as Zn(II), Co(II), and Ni(II). As a result, it was found that the metal ion is significantly involved in the reactivity of the disulfide reduction by coordination binding to the reaction intermediate, the GSH-mono adduct.

Key words : biological trace metal, cooperative reduction, glutathione (GSH), metal binding, zinc finger

1. Introduction

Metal ions play an important role in many biological processes [1]. Therefore, it is important to evaluate the interactions of metal ions with biomolecules such as DNA, peptides, and proteins as a model system to understand the fundamental chemical and physical properties involved in biochemical reactions and catalysis. Particularly, peptides and proteins have been reported to interact with various metal ions through Lewis base sites on their amino acid residues [2,3]. Typical examples are metalloproteins that have metal ion cofactors, whose dysfunction is involved in the development of various pathologies such as cancer and neurodegeneration. Glutathione (GSH), a ubiquitous tripeptide, is the major low-molecular weight thiol compound present in millimolar concentrations (0.5–10 mM) in plants and animals.

GSH plays an important role in cellular metabolism, including the repair of oxidative damage in red blood cells. This peptide is also suitable for use as an intracellular thiol "redox buffer" to maintain a constant redox potential of thiols/disulfides *in vivo*. In addition, GSH is an important binding factor in trace metal homeostasis and detoxification of heavy metals such as Cd(II) and Pb(II). Many researchers are interested in

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studying the coordination and binding properties of GSH-metal complexes due to their biological and pharmaceutical importance [4]. In this study, we investigated the involvement of trace metal elements such as Zn(II), Co(II), and Ni(II) in reducing the oxidized GAGA zinc finger protein using GSH and its derivatives as reducing agents (Fig. 1). The GSH γ-Glu and Gly residues contain Lewis base moieties such as carboxylic acid and amine groups in their side chains, and can, thus, participate in metal binding. To clarify how the structures and metal-binding capacities of Glu and Gly in the GSH structure are involved in the reduction reaction, we prepared two GSH analogs by deleting the amino acid residues around the Cys residue. Here, we propose a mechanism for the cooperation of *in vivo* trace metals in the GSH reduction reaction.
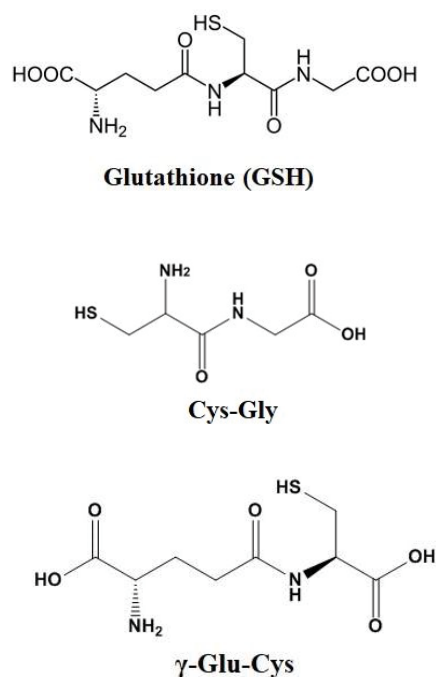
by oxidizing the reduced GAGA with diamide (N, N-dimethylformamide), a disulfide bond-selective oxidant.

Oxidized GAGA (40 μM) was treated with GSH (1 mM) in 50 mM Tris-HCl buffer (pH 7.5) in the absence or presence of 100 μM Zn(II). The reduction reaction was monitored using reversed-phase high-performance liquid chromatography (Fig. 2), and the reaction products were analyzed using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry.
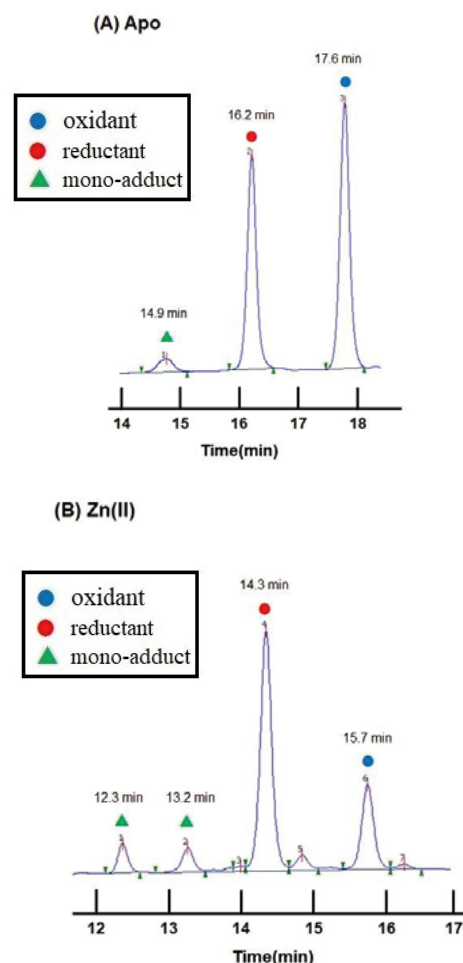

Fig. 1. Structure of GSH and its analogs.

2. Results and Discussion

Reduced GAGA and GSH analogs were synthesized on a Shimadzu PSSM-8 (Kyoto, Japan) using Fmoc chemistry. Oxidized GAGA was prepared

Fig. 2. Reduction of oxidized GAGA with 1 mM GSH in the absence (A) and presence (B) of 100 μM Zn(II) for 5 min. The oxidized, reduced, and GSH-mono adduct forms of GAGA are labeled as ●, ●, and ▲, respectively.

In the apo form of GAGA without metal

coordination, in addition to the oxidant peak (17.6 min), two new product peaks corresponding to the reducing agent (16.2 min) and mono-GSH adduct (14.9 min) were observed (Fig. 2A). In the presence of Zn(II), three new product peaks corresponding to the reducing agent (14.3 min) and mono-GSH adducts (12.3 and 13.2 min) were observed (Fig. 2B). Based on the mass spectrometry results, the GSH-mono adduct was considered to have resulted from the addition of GSH to one sulfur atom of the intramolecular disulfide bond of oxidized GAGA, resulting in formation of an intermolecular disulfide bond. Compared to the apo form, addition of Zn(II) increased the amount of GSH-mono adducts formed and further accelerated the reduction reaction.

Subsequently, we investigated the effect of various metal ions such as Zn(II), Co(II), and Ni(II) on the reduction of GSH (Fig. 3). The relative reduction rates due to Zn(II) and Ni(II) were much faster than those due to apo form and Co(II). In the case of Zn(II), reduced GAGA (approximately 80% yield) and GSH and GSH-mono adduct (approximately 5% yield) were produced after 30 min. After 120 min, an approximately 100% reduction of GAGA was obtained. These results indicate that the metal ions coordinate to GSH and are involved in the reduction reaction.
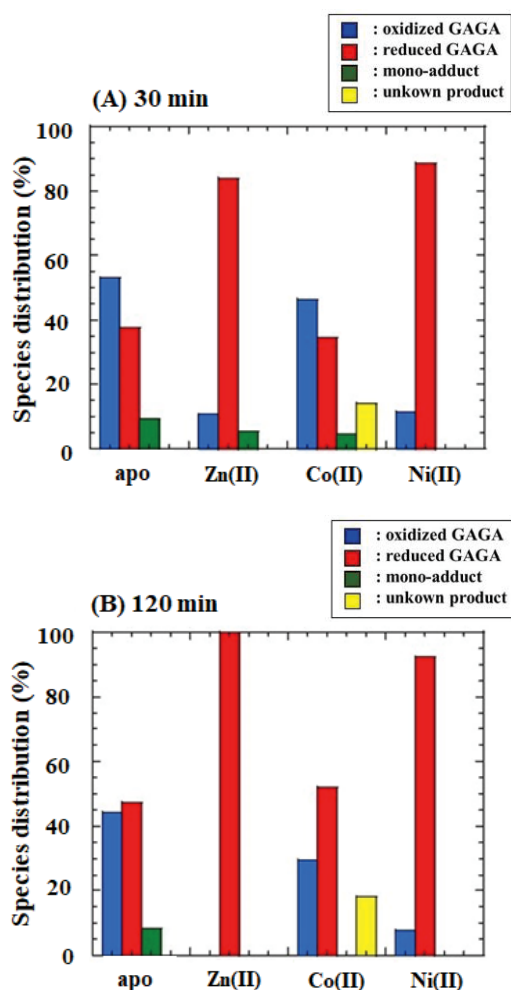

Fig. 3. Reduction of oxidized GAGA with 1 mM GSH in the absence or presence of 100 mM divalent metal ions at 30 min (A) and 120 min (B) in 50 mM Tris-HCl buffer (pH 7.5) containing 50 mM NaCl.
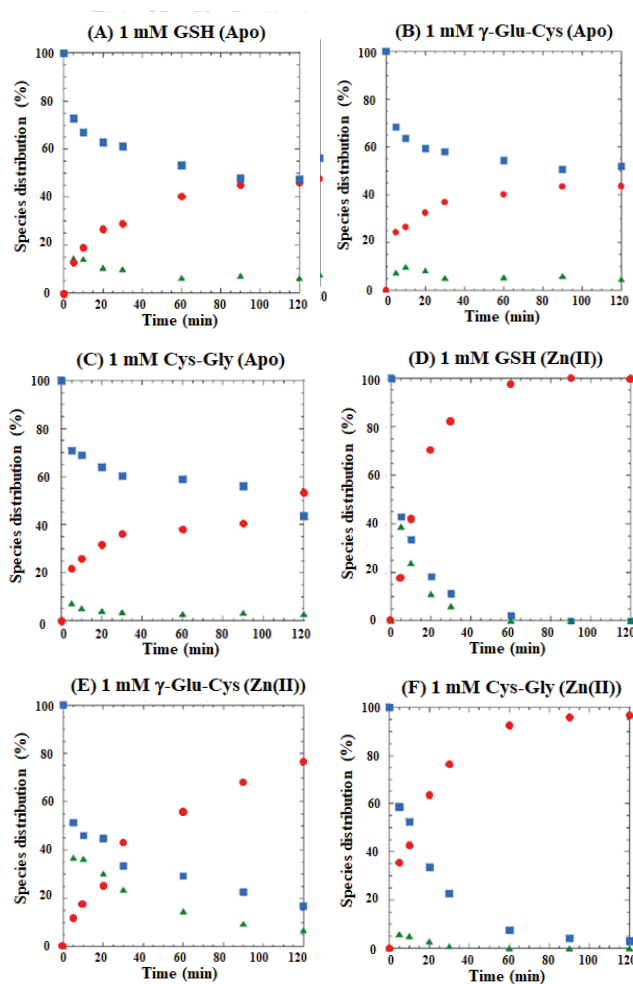

Fig. 4. Reduction of oxidized GAGA with 1 mM reductant in the absence or presence of 100 μM Zn(II) over a 2-h period in 50 mM Tris-HCl buffer (pH 7.5) containing 50 mM NaCl. The oxidized, reduced, and mono-GSH adduct forms of GAGA are labeled ■, ● and ▲, respectively.

To clarify the interaction between metal ions and GSH in the reduction reaction, we compared the reduction of GSH analogs, namely Cys-Gly and γ-Glu-Cys, in the absence and presence of Zn(II) (Fig. 4). In the case of the apo form, all reduction reactions proceeded slowly towards equilibrium, and thus the yield of reduced GAGA did not reach 100%. In contrast, in the presence of Zn(II), the reduction reaction proceeded more efficiently than in the apo form. In the case of GSH, the reduction reaction proceeded rapidly, and the formation rate of reduced GAGA reached 100% within 100 min. In the initial stage of the reaction, the yield of the GSH-mono adduct (40%) was higher than that of the reduced form, suggesting that the GSH mono-adduct was stabilized by coordination with Zn(II) as a reaction intermediate. For γ-Glu-Cys in the presence of Zn(II), the rate at which the reduced form was generated was lower than that of GSH. However, the yield of the GSH-mono adduct was much higher than that of GSH. The reduction reactivity of Cys-Gly was similar to that of GSH but minimal formation of the GSH-mono adduct was observed, indicating that the Cys-Gly GSH-mono adduct weakly interacted with Zn(II). Furthermore, the different formation rates of GSH-mono adducts between Cys-Gly and γ-Glu-Cys in the presence of Zn(II) suggest that the Lewis base site on the side chain of the γ-Glu residue may be important for the interaction of GSH-mono adducts with Zn(II).

3. Summary

The reactivity of the GSH reduction may depend on the interaction between the GSH-mono adduct and Zn(II). Considering these results, we propose a scheme for the reduction reaction in the absence and presence of Zn(II) (Fig. 5). In the case of the apo form, the reaction reached equilibrium, suggesting that an equilibrium exists between the oxidant (A), GSH-mono adduct (B), and reducing agent (C). By contrast, the amount of GSH-mono adducts increased in the presence of Zn(II),

suggesting that Zn(II) forms complexes with GSH-mono adducts. Therefore, two additional processes of forming Zn(II) complexes for both the GSH-mono adduct (B') and reducing agent (C') in the presence of Zn(II) may be necessary. The stability of the GSH-monoadduct was the rate-limiting step in the reduction of oxidized GAGA by GSH, strongly affecting the rate of the reduction reaction. Furthermore, our results suggest that the stability of GSH-mono adducts is controlled by the action of Zn(II) on the Lewis base sites of GSH, especially the side chain sites of γ-Glu, to form coordination bonds. Our results suggest a new reduction mechanism for GSH involving metal ions.
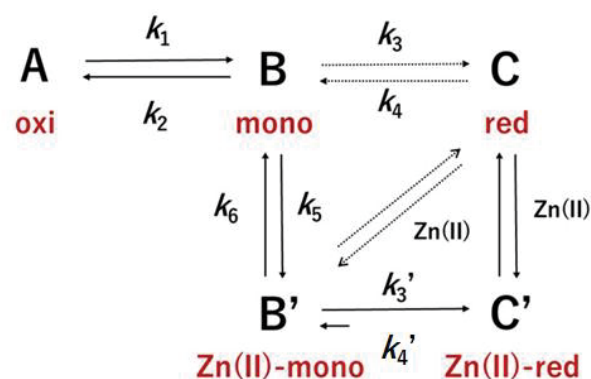

Fig. 5. Plausible reaction scheme of reduction of oxidized GAGA with GSH in the absence and presence of Zn(II).

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