Short Communication

Chelation of lanthanum (La^{3+}) by various thiols - an *in vitro* study

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ABSTRACT

In the present study the interaction of various thiols (L-glutathione, L-cysteine, *N*-acetyl cysteine and D-penicillamine) with $LaCl_3$ was studied *in vitro* by modified Ellman's method. Experiments carried out at various concentrations inferred gradual decrease in thiol concentration with the increase in La^{3+} concentration in aqueous solutions. Maximum interaction between thiols and La^{3+} was observed at pH 7.5 and 35 °C as indicated by the lowest values of residual thiol concentration. The order of reactivity was DPA>GSH>NAC>Cys, where reactivity increased with increasing pK_a of their thiol group. The possible products of reaction are proposed to be $La(RS)_3$ complex or oxidized form with disulfide bond (RSSR).

Key words: Chelation, Ellman's modified method, lanthanum, thiols.

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INTRODUCTION

Thiols are organosulfur compounds that contain carbon-bonded sulfhydryl (R-SH) group, which is a strong reducing agent. Thiols are readily oxidized, rapidly regenerated and play an essential role in biochemical and pharmacological processes.[1] The most abundant intracellular thiol, glutathione (GSH) is an antioxidant that protects important cellular components from reactive oxygen species (ROS) such as free radicals and peroxides.[2] Another thiol, Cysteine (Cys), forms disulfide bridges that result in cystine to stabilize tertiary structure of proteins that act as enzymes. Likewise, *N*-acetylcysteine (NAC) improves GSH production and contributes in scavenging of hydroxyl radicals and reduced H_2O_2 production.[3] The sulfhydryl group of thiols forms a fairly stable chelator-metal complex, which results in excretion and reduction of toxic effects of heavy metals.[4] Cys-rich metallothionein tightly binds Hg, Pb, and Cd[5], NAC chelates Pd from drugs or precursors synthesized by coupling reactions [6], and D-penicillamine (DPA), an acid degradation product of β-lactam antibiotics, is recommended for removal of excess Cu in patients with Wilson's disease, treatment of arsenic poisoning[7] and rheumatoid arthritis.[8]

Lanthanum (La) is the most basic element of trivalent Lanthanides found in some rare-earth minerals. It is used in glass, lighters, and hybrid automobile batteries and has industrial applications. La^{3+} has pharmacological effects on various receptors and ion channels. Boldyreva[9] reported that La^{3+} increased open channel time and decreased desensitization in a subunit configuration dependent manner, thus acts as positive allosteric modulator on native and recombinant γ-amino butyric acid (GABA) receptors. Lanthanum carbonate was approved as medication to absorb excess phosphate to treat renal failure or hyperphosphatemia.[10] Citta et al[11] mentioned that La^{3+} significantly inhibited thioredoxin reductase (TrxR) activity in human ovarian carcinoma cells. In clinical pharmacology, interaction of metalloelements

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with thiols is receiving significant attention as a biomarker of detoxification. The present study investigates La^{3+} chelation by GSH, Cysteine, NAC and DPA *in vitro* as a function of time, pH and temperature, suggesting possible reaction and products to act as model for studies *in vivo*.

MATERIALS AND METHODS

Chemicals

GSH, Cysteine, NAC, DPA, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and $LaCl_3$ were purchased from Sigma-Aldrich, MO. All other chemicals were of analytical grade, purchased from Merck, NJ.

Solution preparation

Stock solutions (1×10^3 µM) of thiols and DTNB, and DTNB blank solution (167 µM) were prepared in 0.1M phosphate buffer (pH 7.6). $LaCl_3$ stock solution (2×10^3 µM) was prepared in deionized water and 100, 10, 1.0 and 0.1 µM solutions were prepared by serial dilution. All the solutions were kept at 4 °C till use.

Determination of thiol concentration

Ellman's DTNB modified method[12] was used to determine thiol concentration in aqueous solution after treatment with various concentrations (0.1, 1, 10, 1×10^2, 1×10^3, 2×10^3 µM) of $LaCl_3$. In brief, 2 ml each of various concentrations of $LaCl_3$ and thiol stock solution were mixed in test tubes and left for 10 min at room temperature. 0.2 ml of this mixture was added to 2.3 ml of phosphate buffer (0.1 M, pH 7.6) followed by the addition of 0.5 ml DTNB (1×10^3 µM) and again left for 5 min. Decrease in thiol concentration was determined spectrophotometrically (UV-1601, Shimadzu, Japan) at 412 nm against reference solution containing 2.8 ml of phosphate buffer (0.1M, pH 7.6) and 0.2 ml of thiol stock solution.

In another experiment, absorbance (A_{412}) was recorded at various time intervals (0 –120 min) when 33.33 µM thiol reacted with various concentrations (0.1, 1, 10, 1×10^2, 1×10^3, 2×10^3 µM) of $LaCl_3$. Thiol controls were prepared by mixing 2 ml each of thiol stock solution and phosphate buffer (0.1M, pH 7.6). The tubes were shaken well and left for 10 min at room temperature before recording A_{412}. All the experiments were conducted thrice and the data were the mean of triplicate analysis. Microsoft Excel was used for calculation of summary statistics and graphical representation using scatter plots.

Effect of pH and temperature

To determine the effect of pH, 0.2 ml each of $LaCl_3$ and thiol solutions (2:1) were taken into six tubes. Buffer solutions (2.3 ml) of pH 6.5, 7.5, 8.5, 9.0, 9.5 and 10 were added to each tube followed by the addition of DTNB (167 µM). Reference solution contained 2.8 ml of buffers and 0.2 ml of thiol stock solution. Rest of the procedure was same as described above. To determine the effect of temperature, thiol determination was conducted at 25, 30, 35, 40 and 45 °C at pH 7.5. To maintain temperature, tubes were placed in TW2 water bath (Julabo, Germany). All the experiments were conducted thrice and the data were the mean of triplicate analysis.

RESULTS

Thiol concentration decreased with the increase in $LaCl_3$ concentration. Below 3.33 µM $LaCl_3$, thiol concentration rapidly decreased while after 3.33 µM there was a gradual decrease in concentration. This effect was more prominent for DPA with 1.85 µM residual concentration at 66.66 µM $LaCl_3$ as compared to Cys with 2.75 µM residual concentration (Figure 1).

Figure 2 more clearly represents this decrease at various time intervals when 1×10^3 µM thiols reacted with $LaCl_3$. Again, lowest residual concentration was observed for DPA (2.03 µM) at 120 min as compared to 2.21,

Figure 1: Effect of $LaCl_3$ on residual thiol concentration at 30 °C and pH 7.6

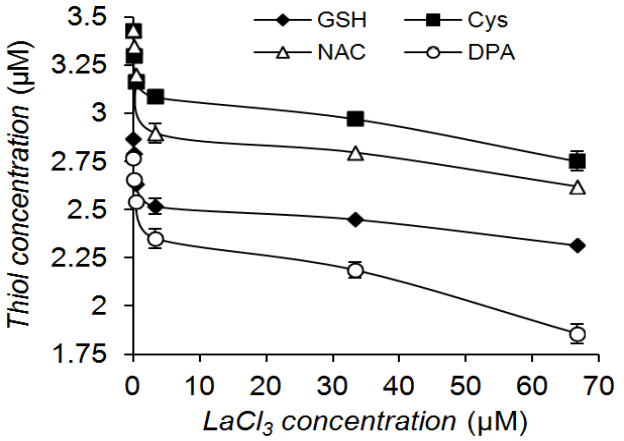
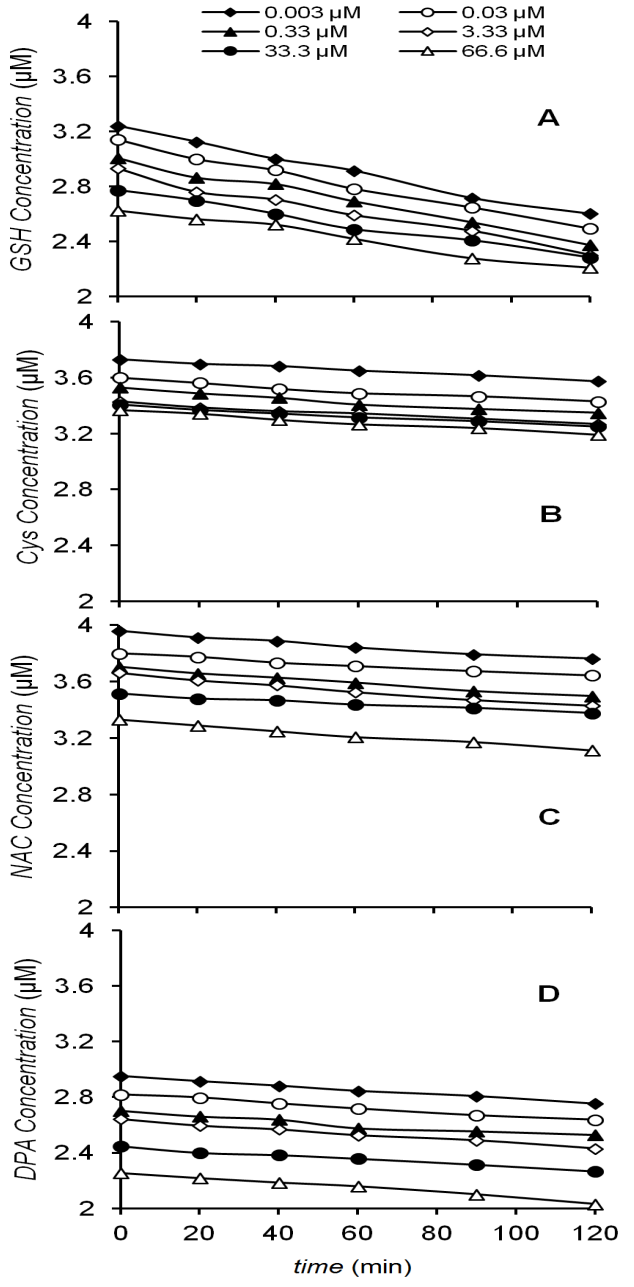


Figure 2: Effect of various concentrations of $LaCl_3$ on thiols studied at various time intervals. [A-GSH; B-Cys; C-NAC; D-DPA]



3.193, and 3.111 µM for GSH, Cys, and NAC, respectively.

Maximum interaction between thiols and $LaCl_3$ was observed at pH 7.5 (Figure 3) and 35 °C was found optimum temperature for reaction (Figure 4), as indicated by the lowest values of residual thiol concentrations. For both pH and temperature, lowest values were obtained for DPA, with the trend DPA<GSH<NAC<Cys.

Figure 3: Effect of pH on reaction of La^{3+} with thiols

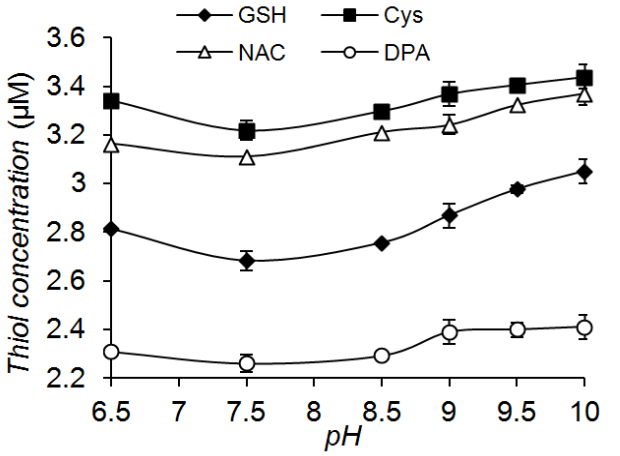
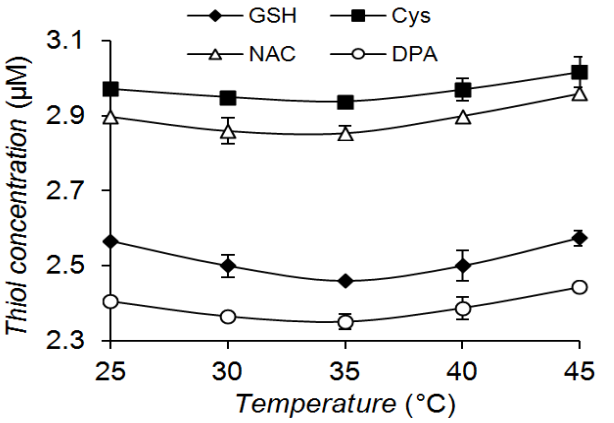


Figure 4: Effect of temperature on reaction of La^{3+} with thiols



DISCUSSION

Intracellular thiols act as antioxidants to protect important cellular components from ROS, form disulfide bridges to stabilize tertiary structure of proteins, and form chelator-metal complex to reduce toxic effects of heavy metals. Like various elements, trivalent La interacts with thiols to affect enzymatic activity. As mentioned, residual thiol concentration decreased with increasing La^{3+} concentration.

The lowest values were recorded for DPA among other thiols.

Rapid reactivity of thiols is due to their sulfhydryl (–SH) groups, which in turn depend on the adjacent functional groups. According to chemical structures, –SH group is attached to 5th carbon (C5) of GSH while 3rd carbon (C3) of Cys, NAC, and DPA. Two –H groups are also attached to the same carbon in GSH, Cys and NAC. However, in DPA structure, these two –H are replaced with two methyl ($–CH_3$) groups. Noticeably, the –H group is regarded as having no effect while $–CH_3$ group is electron-donating in the situation where C is more electronegative than H. As a result, C acquires a slight negative charge by pulling nearby bonded electrons and correspondingly H acquires slight negative charge. Thus, C will push electronic charge towards the group to which it is linked. In contrast, –SH group has electron-withdrawing inductive effect. The net effect on DPA due to these groups could possibly be responsible for its highest reactivity.

If RSH represents multi-functional biothiol molecule, two possible products of the reaction are La-RS complex and oxidized (disulfide) form. The proposed reactions are:

$$La^{3+} + 3\,RSH \rightarrow La(RS)_3 + 3H^+$$

$$La^{3+} + 2\,RSH \rightarrow La^{3+} + 2H^+ + RSSR$$

As shown in the results, DPA is the most reactive thiol with $LaCl_3$ among others. The order of reactivity was: DPA>GSH>NAC>Cys (–SH group pK_a 10.5, 9.65, 9.52 and 8.18, respectively). Thus, the reactivity of thiols with $LaCl_3$ increased with increasing pK_a of their thiol group. Conversely, reactivity decreased with increasing pK_a of amino ($–NH_3^+$) group (pK_a 7.90, 8.75, 9.52 and 10.28, respectively). Winterbourn and Metodiewa[13] reported that reactivity of thiols is inversely proportional to their pK_a values.

The formation of disulfide-bonded form (RSSR) or $La(RS)_3$ complex might have clinical implications. Keeping in view the therapeutic applications of thiols, this *in vitro* study will possibly act as a model for studies *in vivo* and will open up new horizons to study chelator-metal complex in future.

In conclusion, trivalent lanthanum interacts with thiols favorably at pH 7.5 and 35 °C, where order of reactivity was DPA>GSH>NAC>Cys. The reactivity increased with increasing pK_a of the thiol group. The possible products of reaction are proposed to be metal-chelator complex, $La(RS)_3$, or oxidized form with disulfide bond (RSSR).

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REFERENCES

1. Dickinson DA, Forman HJ. Cellular glutathione and thiols metabolism. Biochem Pharmacol 2002;64:1019-26.
2. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. The changing faces of glutathione, a cellular protagonist. Biochem Pharmacol 2003;66:1499-503.
3. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of *N*-acetyl cysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med 1989; 6:593-7.
4. Carageorgiou H, Tzotzes V, Pantos C, Mourouzis C, Zarros A, Tsakiris S. *In vivo* and *in vitro* effects of cadmium on adult rat brain total antioxidant status, acetylcholinesterase, (Na^+, K^+)-ATPase and Mg^{2+}-ATPase activities: protection by L-cysteine. Basic Clin Pharmacol Toxicol 2004; 94:112-8.
5. Bump EA, Brown JM. Role of glutathione in the radiation response of mammalian cells *in vitro* and *in vivo*. Pharmacol Ther 1990; 47:117-36.
6. Garrett CE, Prasad K. The art of meeting palladium specifications in active pharmaceutical ingredients produced by Pd-catalyzed reactions. Adv Synth Catal 2004; 346:889-900.

7. Gaffney D. Fell G. O'Reilly D. Wilson's disease: acute and presymptomatic laboratory diagnosis and monitoring. J Clin Pathol 2000;53:807-12.
8. Bonta IL, Parnham MJ, Vincent JE, Bragt PC. Anti-rheumatic drugs: present deadlock and new vistas. Prog Med Chem 1980; 17:185-273.
9. Boldyreva AA. Lanthanum potentiates GABA-activated currents in rat pyramidal neurons of CA1 hippocampal field. Bull Exp Biol Med 2005;140:403-5.
10. Citta A, Folda A, Scutari G, Cesaro L, Bindoli A, Rigobello MP. Inhibition of thioredoxin reductase by lanthanum chloride. J Inorg Biochem 2012; 117:18-24.
11. Kostova I, Manolov I, Karaivanova M. Synthesis, physicochemical characterization, and cytotoxic screening of new zirconium complexes with coumarin derivatives. Arch Pharm 2001;334:157-62.
12. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.
13. Winterbourn CC, Metodiewa D. Reactivity of biologically important compound with superoxide and hydrogen peroxide. Free Radic Biol Med 1999; 27:322-8.

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