

Spectrophotometric Study of the Biocoordination Chemistry of Strontium Nitrate and Glutathione

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(Received: 27 February 2012;

Accepted: 4 January 2013)

AJC-12650

The effect of strontium nitrate on the status of glutathione in aqueous media was examined spectrophotometrically. The wavelength of 412 nm was used for the determination of thiol concentration in samples in accordance with a well known Elman's method. During the experiments the pH was maintained at 7.6 by phosphate buffer which was proposed to be suitable for performing *in vitro* experiments on thiols by Evans. When glutathione was exposed to different concentrations of strontium nitrate in aqueous solution, there was a significant decrease in the concentration of glutathione which means that strontium nitrate causes chemical modulation of glutathione and converts this molecule to its oxidized or disulfide form or glutathione-strontium complex. Time dependent, temperature and pH effect of strontium nitrate on the chemical status of glutathione was also examined. It was observed that there was a decrease in concentration of glutathione with these parameters. This study was carried out in *in vitro* condition to establish the model of *in vivo* reaction. The exact mechanism of reaction between metal and glutathione still need to be investigated but formation of strontium-glutathione complex may be proposed.

Key Words: Strontium nitrate, 5,5-Dithiobis,2,nitrobenzoic acid, Ellman's method, Glutathione.

INTRODUCTION

Glutathione (GSH) is one of the main non protein tripeptide thiol-compounds in mammalian-cells and has bio-reducing activity¹. Inside the cells, GSH is present in two forms *i.e.*, reduced state (GSH) and oxidized state (GSSG). Inside cells glutathione is present in reduced state (GSH) and is more than 95 % of the total (GSH + GSSG) content. Intra-cellular oxidized glutathione (GSSG) is present in small quantity but may increase with oxidative-stress or pathological-conditions and reduced glutathione depletion occurs at the same time². GSH and GSSG act as a thiol redox-couple and have role in gene regulation and intra-cellular signal transduction³. Glutathione in reduced state (GSH) has important roles in cell division, immune-response, signal processes control, programmed cell-death, some xenobiotics and heavy metals detoxification^{4,5}. Glutathione has antioxidant activity and a cofactor for enzymatic reactions that need readily available electron pairs⁶. Glutathione is very reactive from physico-chemical point of view and conjugate to other molecules including the heavy metal ions because of its sulfhydryl moiety^{7,8}. GSH/GSSG ratio gives an early indication of oxidative stress or risk of disease². Metals toxicity

includes geno-toxicity or carcinogenicity, neuro-toxicity⁹ and this reaction with GSH has attracted the attention of biomedical scientists.

In present study the effect of strontium nitrate on the chemical status has investigated spectrophotometrically in aqueous medium of strontium nitrate on the metabolism of biologically active molecules like glutathione. Therefore it was of interest to see the chemical effect of this metal strontium nitrate on the chemical and metabolic status of glutathione in aqueous solution.

EXPERIMENTAL

L-Glutathione (GSH) (Fluka), strontium nitrate (Merck), 5,5-dithiobis,2-nitrobenzoic acid (DTNB) (Sigma), sodium hydroxide (Fluka AG), potassium dihydrogen phosphate (Merck), HCl 37 % (Kolch light), distilled water (double distilled). UV/visible 1601 spectrophotometer: (Shimadzu), pH Meter: NOV-210 (Nova scientific company Ltd. Korea), Oven: Memmert Model U-30,854 Schwabach (Germany), Magnetic stirrer (England), hot plate: 400 (England), sensitive weighing balance, Sortorius. Micropipettes (200, 500 and 1000 μ L) Socorex Swiss (Finland).

RESULTS AND DISCUSSION

Effect of different concentration (0.0001, 0.001, 0.01, 0.1, 1, 2 mM) of strontium nitrate on the chemical status of glutathione (1 mM): 2 mL of different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 2 mM) of strontium nitrate solutions were added separately to 2 mL of 1.0 mM glutathione taken in six separate test tubes, shaken well. Final concentration of glutathione in each of the above test tube was 0.50 mM (500 μ M) and that of strontium nitrate were 0.00005 mM (0.05 μ M), 0.0005 mM (0.5 μ M), 0.005 mM (5 μ M), 0.05 mM (50 μ M), 0.5 mM (500 μ M) and 1 mM (1000 μ M), respectively.

0.2 mL from each one of the above test tubes was taken followed by the addition of 2.3 mL of phosphate buffer pH 7.6 and 0.5 mL of 1 mM DTNB solution. The final concentration of glutathione in the test tubes was 0.03333 mM (33.33 μ M) and of strontium nitrate were 0.000003 mM (0.003 μ M), 0.00003 mM (0.03 μ M), 0.0003 mM (0.33 μ M), 0.003 mM (3.33 μ M), 0.0333 mM (33.33 μ M) and 0.06666 mM (66.66 μ M), respectively.

Control solution (glutathione blank) containing 2 mL of 1 mM glutathione solution and 2 mL of phosphate buffer having pH of 7.6 was added. The final concentration of glutathione in control solution (glutathione blank) was also 0.5 mM (500 μ M), as in the sample. 0.2 mL was taken from this sample and add to it 2.3 mL of phosphate buffer pH. 7.6 followed by the addition of 0.5 mL of (1 mM) DTNB. The ultimate concentration of glutathione in control sample will be 0.03333 mM (33.33 μ M).

The absorbences were taken after 5 min at 412 nm against reference cell containing 2.8 mL phosphate buffer pH 7.6 and 0.2 mL of 1 mM glutathione solution. The effect of strontium nitrate on the chemical status of glutathione was studied in terms of determination of the absorbences which were then converted into concentration of glutathione in mixtures by a well known Elman's method¹⁰. The concentrations of glutathione (determined from the glutathione standard curve) left after treatment with strontium nitrate was plotted against the concentration of strontium nitrate in mixture samples (Fig. 1).

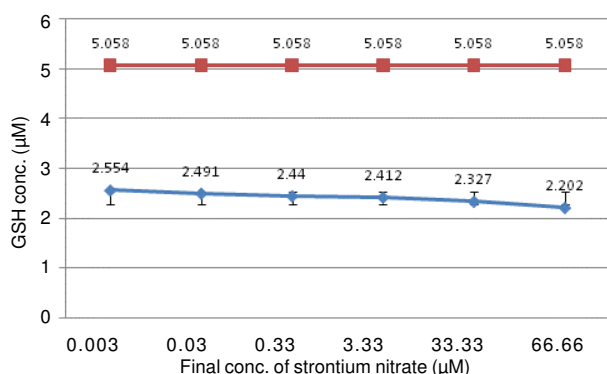


Fig. 1. Effect of different concentration of Sr(NO₃)₂ on the status of GSH. ■ Control GSH (without metal). ◆ Sr(NO₃)₂ + GSH (0.003-66.66) μ M. Results are the mean \pm SE of 3 experiments

Effect of different concentration (0.0001, 0.001, 0.01, 0.1, 1 and 2 mM) of strontium nitrate on the chemical status

of glutathione (1 mM) with time: 2 mL of different concentrations (0.0001, 0.001, 0.01, 0.1, 1 and 2 mM) of strontium nitrate solutions were added to 2 mL of 1 mM glutathione and the chemical status of GSH was examined.

The absorbences were noted at 0, 20, 40, 60, 90 and 120 min and the concentration of glutathione (determined from the glutathione standard curve) left after treatment with strontium nitrate was plotted against the time interval and are shown in Figs. 2-6.

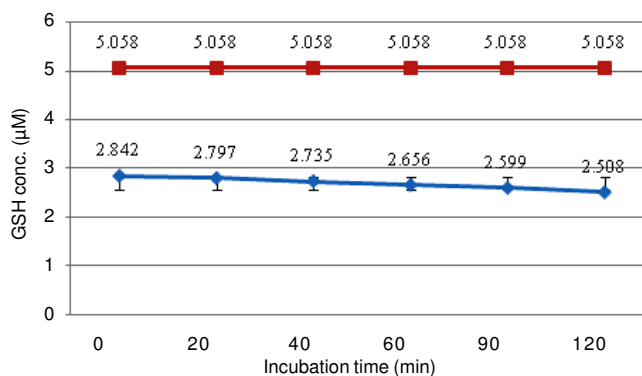


Fig. 2. Effect of strontium nitrate (0.003 μ M) with time, incubation period (0-120 min). ■ Control GSH (without metal). ◆ Sr(NO₃)₂ + GSH. Results are the mean \pm SE of 3 experiments

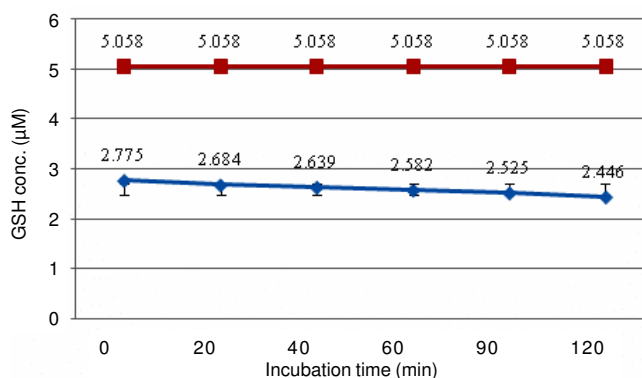


Fig. 3. Effect of strontium nitrate (0.03 μ M) with time, incubation period (0-120 min). ■ Control GSH (without metal). ◆ Sr(NO₃)₂ + GSH. Results are the mean \pm SE of 3 experiments

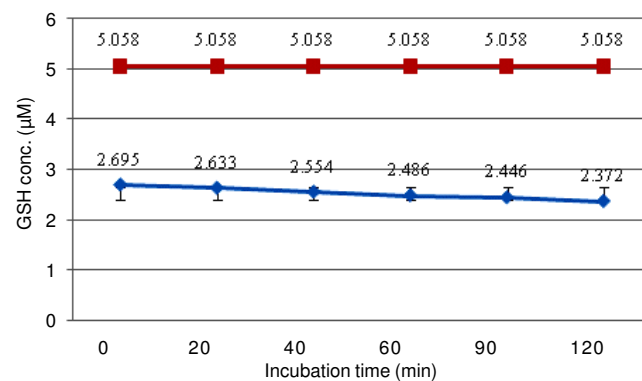


Fig. 4. Effect of strontium nitrate (0.33 μ M) with time, incubation period (0-120 min). ■ Control GSH (without metal). ◆ Sr(NO₃)₂ + GSH. Results are the mean \pm SE of 3 experiments

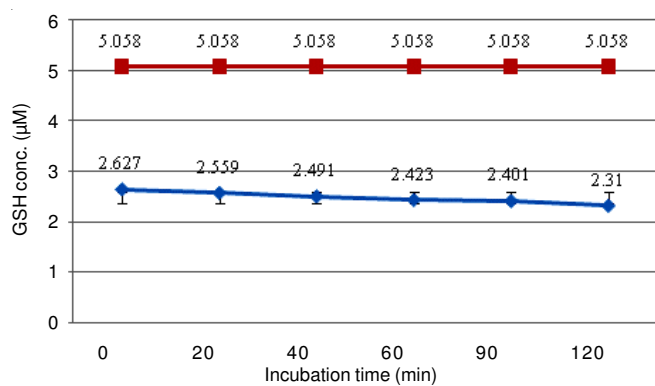


Fig. 5. Effect of strontium nitrate (3.33 μM) with time, incubation period (0-120 min). ■ Control GSH (without metal). ◆ Sr(NO₃)₂ + GSH. Results are the mean \pm SE of 3 experiments

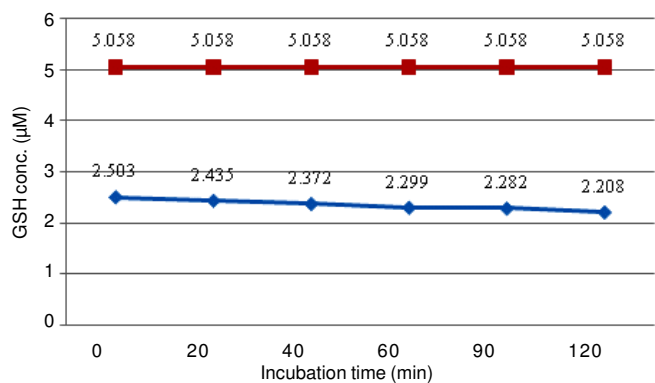


Fig. 6. Effect of strontium nitrate (66.66 μM) with time, incubation period (0-120 min). ■ Control GSH (without metal). ◆ Sr(NO₃)₂ + GSH. Results are the mean \pm SE of 3 experiments

Effect of different pH (6.5, 7.5, 8.5, 9.0, 9.5, 10) buffer on the chemical status of glutathione solution in the absence and presence of strontium nitrate:

2 mL of 1 mM glutathione was mixed with 2 mL of 1 mM strontium nitrate in test tube. Shake well and left for 5 min. The final concentration of glutathione and strontium nitrate in reaction mixture was calculated to be 0.5 mM (500 μM). Sample cuvettes were prepared by taking 0.2 mL from above mixture in six test tube separately and 2.3 mL of each buffer solution having pH (6.5, 7.5, 8.5, 9.0, 9.5, 10) was added in six test tube, followed by the addition of 0.5 mL of 1 mM DTNB. The final concentration of glutathione and strontium nitrate in sample mixture was 0.03333 mM (33.33 μM). Control solution was prepared by mixing 2 mL of 1 mM glutathione and 2 mL of each buffer solution having pH (6.5, 7.5, 8.5, 9.0, 9.5, 10) separately. Left for 5 min, 0.2 mL from above mixture was taken and mixed with 2.3 mL of each buffer solution having pH (6.5, 7.5, 8.5, 9.0, 9.5, 10) followed by the addition of 0.5 mL of 1 mM DTNB.

Absorbencies were taken after 5 min at 412 nm against reference cell containing 2.8 mL of respective phosphate buffer pH (6.5, 7.5, 8.5, 9.0, 9.5, 10) and 0.2 mL of 1 mM glutathione. The effect of strontium nitrate on the chemical status of glutathione in different pH (6.5, 7.5, 8.5, 9.0, 9.5, 10) buffer was studied in terms of determination of the absorbencies which were then converted into concentration of glutathione in mixtures by a well known Elman's method¹⁰, as mentioned

in standard curve for glutathione. Finally, the concentrations of glutathione (determined from the glutathione standard curve) left after treatment with strontium nitrate was plotted against the final concentration of strontium nitrate in mixture samples (Fig. 7).

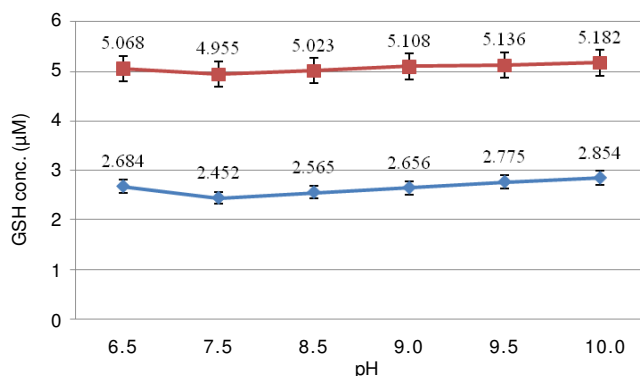


Fig. 7. Effect of strontium nitrate (27.3 μM) on GSH (27.3 μM). ■ Control GSH (without metal). ◆ Sr(NO₃)₂ + GSH. Results are the mean \pm SE of 3 experiments

Effect of different temperature (at 25, 35 and 45 °C)

on the chemical status of glutathione (0.1 mM): 2 mL of 0.1 mM strontium nitrate solution was added separately to 2 mL of 0.1 mM glutathione taken in three separate test tubes, shaken well. These test tubes were kept in water bath for 10 min to maintain the temperature of (25, 35 and 45 °C). Final concentration of glutathione and strontium nitrate in each of the above test tube was 0.05 (50 μM), respectively.

Samples cuvettes were prepared by taking 0.2 mL of strontium nitrate plus glutathione mixture from each one of the previously made test tubes and diluted with 2.3 mL of phosphate buffer pH 7.6 and then 0.5 mL of 1 mM DTNB stock solution was added in to each test tube. The final concentration of glutathione and strontium nitrate in the sample test tubes was 0.0033 mM (3.33 μM), respectively.

Control solution (glutathione blank) for glutathione was also prepared by taking 2 mL of 0.1 mM glutathione stock solution in a test tube to which 2 mL of phosphate buffer having pH of 7.6 was added. The final concentration of glutathione in control solution (glutathione blank) was also 0.05 mM (50 μM) as in the sample. 0.2 mL was taken from this sample and add to it 2.3 mL of phosphate buffer pH. 7.6 followed by the addition of 0.5 mL of (1 mM) DTNB. The ultimate concentration of glutathione in control sample will be 0.0033 mM (3.33 μM).

Absorbencies were taken after 5 min at 412 nm against reference cell containing 2.8 mL phosphate buffer pH 7.6 and 0.2 mL of 0.1 mM glutathione solution. The effect of strontium nitrate on the chemical status of glutathione at different temperature was studied in terms of determination of the absorbencies which were then converted into concentration of glutathione in mixtures by a well known Elman's method, as mentioned in standard curve for glutathione. Finally the concentrations of glutathione (determined from the glutathione standard curve) left after treatment with strontium nitrate was plotted against the final concentration of strontium nitrate in mixture samples (Fig. 8).

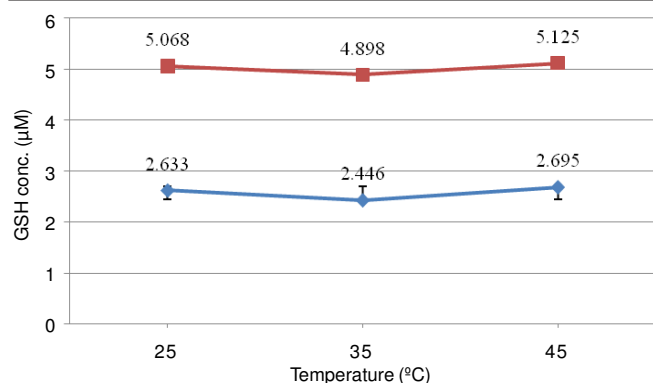


Fig. 8. Effect of strontium nitrate (3.33 μM) on GSH (3.33 μM). ■ Control GSH (without metal). ◆ Sr(NO₃)₂ + GSH. Results are the mean \pm SE of 3 experiments

Glutathione (GSH) is an endogenously produced tripeptide thiol, which plays key role in intra and extra cellular antioxidants defense¹¹. Glutathione has several interesting and important physiological chemical, pharmacological and toxicological implications, which suggest the role of GSH in the detoxification of electrophile, metabolite of chemicals or/and drugs¹².

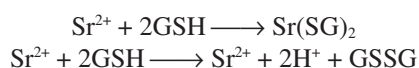
Strontium has many biological uses and is used to treat patients suffering from meta static prostate cancer¹³, osteoporosis¹⁴, as an anti-allergic¹⁵ and to prevent dental caries¹⁷. As GSH can undergo oxidation, so GSH acts as electron donor¹⁸.

Since GSH and strontium metal have many important pharmacological and biological uses, so it was interesting to investigate the interaction between GSH and strontium metal. There are reports on the interaction of divalent Sr²⁺ ion with thiol (SH) group¹⁹.

The effect of strontium nitrate, on the status of reduced glutathione (GSH) in aqueous media was examined spectrophotometrically. Study performed suggests that as the concentration of strontium nitrate increases GSH concentration decreases, when interaction time between GSH and metal increases, concentration of GSH also decreases.

Maximum interaction between GSH and metal was observed at pH of 7.5 and temperature of 35 °C. The results of present study indicated that there might have some interaction between strontium metal and GSH but the exact mechanism of action of strontium nitrate on GSH metabolic status in this study is not known. However there are two proposed possibilities of conversion of GSH by strontium nitrate to: Conversion of reduced glutathione (GSH) to oxidized form GSSG. Conversion of GSH to strontium-glutathione complex Sr-SG.

Such proposed reaction can be written in equation form as follow.



Conclusion

In the above experiments, glutathione was exposed to different concentration of strontium nitrate and the main

interest was to observe the effect of strontium nitrate on the chemical status of glutathione in aqueous media. When glutathione was exposed to different dilutions of strontium nitrate in aqueous media, there was significant decrease in the concentration of reduced glutathione. In means that strontium nitrate causes an increase reduction in reduced form of glutathione and converts this multi functional bio thiol molecule to its oxidized or disulfide form. During this study time dependent effect of different concentrations of strontium nitrate on the chemical status of glutathione was also observed and it was found that there was gradual depletion of reduced glutathione *i.e.*, GSH as the time passed from 0-120 min. The decrease becomes more significant particularly after 1 h exposure to the action of strontium nitrate. It means that strontium nitrate plays important role in the conversion of reduced (GSH) to its disulfide form (GSSG) or Sr-SG complex. Similarly during this study the interaction of strontium nitrate and glutathione at various pH was also observed and results showed that strontium nitrate causes maximum decrease in GSH level at a pH of 7.5. The effect of temperature on the chemical status of GSH was also studied and result revealed that at a temperature of 35 °C a maximum reaction takes place between strontium nitrate and glutathione, due to which a sufficient decrease in glutathione level occur.

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