INTRODUCTION

Glutathione (GSH) is one of the main non protein tripeptide thiol-compounds in mammalian-cells and has bio-reducing activity. 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 time. GSH and GSSG act as a thiol redox-couple and have role in gene regulation and intra-cellular signal transduction. Glutathione in reduced state (GSH) has important roles in cell division, immune-response, signal transduction, some xenobiotics and heavy metals detoxification. Glutathione has antioxidant activity and a cofactor for enzymatic reactions that need readily available electron pairs. 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. GSH/GSSG ratio gives an early indication of oxidative stress or risk of disease. Metals toxicity includes geno-toxicity or carcinogenicity, neuro-toxicity 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, Sor torius. Micropipettes (200, 500 and 1000 µL) 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 µM) and that of strontium nitrate were 0.00005 mM (0.05 µM), 0.0005 mM (0.5 µM), 0.005 mM (5 µM), 0.05 mM (50 µM), 0.5 mM (500 µM) and 1 mM (1000 µM), respectively.

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 µM) and of strontium nitrate were 0.000003 mM (0.003 µM), 0.0003 mM (0.03 µM), 0.003 mM (0.33 µM), 0.003 mM (3.33 µM), 0.0333 mM (33.33 µM) and 0.06666 mM (66.66 µM), 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 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 0.03333 mM (33.33 µM).

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 method. 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(NO$_3$)$_2$ on the status of GSH. ■ Control GSH (without metal). ◆ Sr(NO$_3$)$_2$ + GSH (0.003-66.66) µM. Results are the mean ± 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 µM) with time, incubation period (0-120 min). ■ Control GSH (without metal). ◆ Sr(NO$_3$)$_2$ + GSH. Results are the mean ± SE of 3 experiments

Fig. 3. Effect of strontium nitrate (0.03 µM) with time, incubation period (0-120 min). ■ Control GSH (without metal). ◆ Sr(NO$_3$)$_2$ + GSH. Results are the mean ± SE of 3 experiments

Fig. 4. Effect of strontium nitrate (0.33 µM) with time, incubation period (0-120 min). ■ Control GSH (without metal). ◆ Sr(NO$_3$)$_2$ + GSH. Results are the mean ± SE of 3 experiments
was mixed with 2 mL of 1 mM strontium nitrate in test tube.

The effect of strontium nitrate on the chemical status of glutathione (0.1 mM) was studied in terms of determination of the absorbencies 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).

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.

Absorrencies 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 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).
Glutathione (GSH) is an endogenously produced tripeptide thiol, which plays key role in intra and extra cellular antioxidants defense\(^1\), osteoporosis\(^4\), as an anti-allergic\(^5\) and to prevent dental caries\(^6\). As GSH can undergo oxidation, so GSH acts as electron donor\(^7\).

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 Sr\(^{2+}\) ion with thiol (SH) group\(^8\).

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.

\[
\text{Sr}^{2+} + 2\text{GSH} \rightarrow \text{Sr(GSH)}_2
\]

\[
\text{Sr}^{2+} + 2\text{GSH} \rightarrow \text{Sr}^{2+} + 2\text{H}^+ + \text{GSSG}
\]

**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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