REPORT

Palladium glutathione, N-acetylcysteine, D-penicillamine conjugation chemistry

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Abstract: The metalloelement Palladium has a number of potential Pharmaco-clinical advantages. Palladium compounds have antiviral, antibacterial, neuroprotective and antitumor properties. However studies have also indicated some mild to serious toxic effects of Palladium metalloelements. Biothiols are important antioxidants that provide protection against metals toxicity. The interaction of metalloelements with biothiols can provide valuable information about the level of toxicity of the metalloelements and about the protective role of biothiols thereof. In this piece of work the effect of salt and complexes of Palladium on the status of different thiols (GSH, NAC, and D-Pen) in aqueous medium, were examined, The thiol quantification was carried out using Elman's method through UV-visible spectrophotometry and ^{1}H-NMR. Results of the study performed in aqueous medium showed that level of different thiols depleted after the addition of the inorganic salts and organic complexes of Palladium. The mechanism of interaction of Palladium with thiols was examined using H-NMR. The results indicate that the depletion in the level of thiols may be due to 1:1 or 1:2 conjugation of Palladium with thiols. These conjugation reactions further suggest that the Palladium have xenobiotic nature causing oxidative stress and thiols play their role in detoxification and biotransformation of these metalloelements.

Keywords: Glutathione, N-Acetyl cysteine , D-penicillamine, palladium Nitrate, Bis-benzonitrile Palladium ii chloride.

INTRODUCTION

Oxidative stress promotes oxidative damage by reactive oxygen species (ROS) and is concerned with pathological processes for instance atherosclerosis, cancer, and neurodegenerative disorders (Moran *et al*., 2001). As a result of their capacity of being adequately oxidized, sulfhydryl groups are vulnerable to oxidative stress. This impairs the sulfhydryl-disulfide balance which is a key player in redox-sensitive processes (Winterboum & Hampton, 2008). Sulfhydryl groups occur as non-protein compounds (Glutathione and free cysteine) and, in protein such as thioredoxins, glutaredoxine and albumin, which is the chief protein constituent of blood plasma (Moran *et al*., 2001, Winterboum & Hampton, 2008 and Biswas *et al*., 2006). The total sulfhydryl group (TSH) content of a biological sample is a valuable indicator of oxidative stress and of oxidative protein damage, Quantification of protein thiols relies on use of thiol-reactive reagents, such as 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's Reagent) (Ying *et al*, 2007). DTNB is the most commonly used reagent for the measurement of total sulfhydryl groups spectrophotometrically due to its simplicity and the validity of obtained results (Elman's, 1959). Reaction between sulfhydryl groups and DTNB produces a mixed disulfide and a yellow colored thiolate, 5-thio-2-nitrobenzoic acid (TNB) with maximal absorbance at 412nm (Elman's, 1959; Hu, 1994). We have applied the DTNB-based assay to transition inorganic/organometalic complexes of Palladium and Vanadium format. This permits to study with reduced amount of sample and reagents with satisfactory results in short period of time. Transition metals act as catalysts in the oxidative deterioration of biological macromolecules, and therefore, the toxicities associated with these metals may be due at least in part to oxidative tissue damage (currie, 1995). Recent studies have shown that metals such as Palladium, Vanadium and other elements (Khan H *et al.,* 2010, 2011a, 2011b and 2012; Muktiar *et al., 2012 and* 2013; Shah *et al.,* 2013 & 2013a; Khan J *et al.,* 2012; Naseem *et al*., 2015; Hashmat *et al*., 2015, 2015a, 2016 and 2016a) exhibit the ability to produce reactive oxygen species, resulting in lipid per oxidation. Enhanced per oxidation of lipids may result in the damage to the cells, tissues and organs. The response of the biota to exposure to individual metals may differ from its response to other metals, as metals may interact with the biological system or may be chemically inert are low affinity for biological

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system resulting in severe toxicity or may be low toxic or showing no toxicity. Thus in the present study we intent to analyze and compare for the first time, the aqueous, the blood and hepatic antioxidant responses induced by different doses of two different toxic metals (Vanadium and Palladium) by treating alone with the thiols (Glutathione, N- Acetylcysteine and D-penicillamine) in aqueous medium in order to compare their toxicity. Physiological thiols vary substantially in their reactivity, and on this basis, thiol groups would be preferred cellular targets for various oxidants. In this paper we set out to determine different parameters relating to the oxidative status of various thiols like Glutathione N- Acetylcysteine and d-penicillamine verses Palladium and like concentration.

MATERIALS AND METHODS

Materials

All reagents were commercially obtained. Ellman's reagent (5, 5 di-thiobis 2 nitro benzoic acid i.e. DTNB), Bisbenzo-nitrile Palladium ii chloride, Palladium Nitrate, were purchased from Sigma Aldrich. Sodium Dihydrogen Phosphate (Merck) Sodium Hydroxide, L. Glutathione (GSH), N-Acetylcysteine, D-Pencillamine, and HCl 35% (Kolchlight) were purchased from (fluka), (10M Perchloric Acid 70% (fluka), Sodium chloride (Merck), Distilled Water (Double Refined) and Potassium Dihydrogen Phosphate (Merck). PH Meter (NOV-210, Nova Scientific Company Ltd. Korea), UV. Visible Spectrophotometer (Shimadzue, 1601Japan, Magnetic Stirrer, hot plate 400(England), Oven: Memmert Model U-30,854 Schwabach (Germany), Micropipettes 200 µl, 500 µl, 1000 µl (Socorex Swiss Finland), Siliconized Glass test tubes, Disposable rubber gloves.

Method

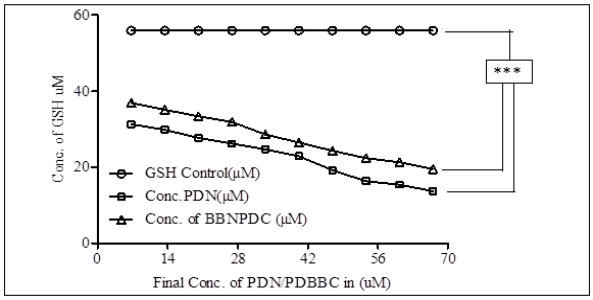
Preparation of stock solutions

0.1M Phosphate buffer PH 7.6 Stock Solution: - 200 ml of phosphate buffer pH 7.6 was prepared by first dissolving 27.218g of Monobasic Potassium Phosphate (KH_2PO_4) in 1000ml of distilled water to get 0.2M Monobasic Potassium Phosphate solution and then mixing 42.4ml of 0.2M NaOH solution with 50ml of already prepared 0.2M Monobasic Potassium Phosphate solution and diluted to 200ml with distilled water. The pH was then adjusted with 0.2M NaOH or 0.2M HCl solutions.

2mM Glutathione (GSH) Stock solution: - 30.75mg (Mol. weight 307.4) of Glutathione (GSH) was prepared by dissolving 30.75mg of Glutathione sufficient quantity of 0.1M Phosphate buffer in a 50ml volumetric flask, the final volume was adjusted to 50ml by adding further 0.1M Phosphate buffer pH7.6. This stock solution was then kept in refrigerator till further use.

2mM N- Acetylcysteine (NAC) Stock Solution: N- Acetyle cysteine (NAC) was prepared by dissolving 16.23mg mg of N-Acetylcysteine (Mol. weight 162.3 a.m.u) in sufficient quantity of 0.1M Phosphate Buffer in a 50ml volumetric flask, the final volume was adjusted to 50ml by adding further 0.1M Phosphate buffer PH 7.6. This stock solution was then kept in refrigerator till further use.

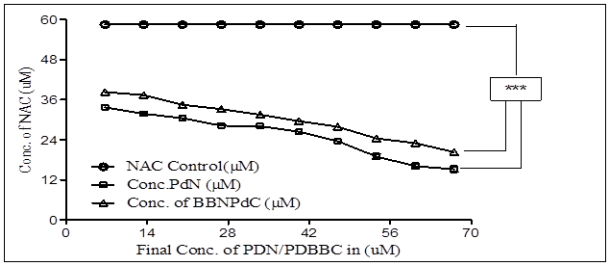
2mM D-pen Stock solution: - 2mM of D-pencillamine(D-pen) was prepared by dissolving 14.9mg of D-pencill amine (Mol. weight 149.2) in sufficient quantity of 0.1M phosphate Buffer pH 7.6 in a 50ml volumetric flask, the final volume was adjusted to 50ml by adding further 0.1M phosphate buffer pH 7.6. This stock solution was then kept in refrigerator till further use.



Values were compared statistically by using One way ANOVA followed by Dunnett test.

The error bars indicate the mean± SE. ***P, 0.001, versus control.

Fig. 1: Conc. dependent effect of (6.7 and 67 µM) of PDN/ BBNPC on the chemical status of GSH Results are the mean ±SE of n=3 experiments of GSH.



Values were compared statistically by using One way ANOVA followed by Dunnett test.

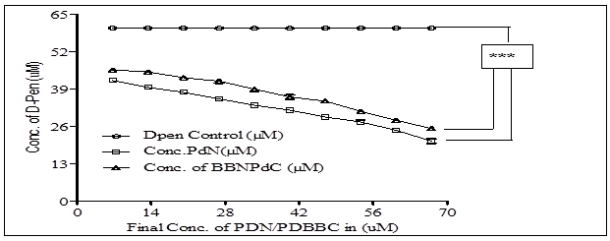
The error bars indicate the mean± SE. ***P, 0.001, versus control.

Fig. 2: Conc. dependent effect of (6.7 and 67µM) of PDN/ BBNPC on the chemical status of NAC Results are the mean ±SE of n=3 experiments of NAC.

1mM 5, 5-dithiobis (2-nitrobenzoic acid), DTNB/ Ellman's Reagent:-1mMof 5, 5-dithiobis, 2-Nitrobenzoic Acid (DTNB) (M.W 396.35) was prepared by dissolving 39.64mg (DTNB) in sufficient quantity of 0.1M phosphate buffer pH 7.6 to make 100ml of 1mM DTNB solution. This stock solution was then kept in refrigerator till further use.

0.1M (Perchloric acid 70%): 0.1M (Perchloric acid purity70%) was prepared by mixing 1ml of 10M

(Perchloric acid purity70%) in sufficient quantity of Distilled water to make the final volume 100ml.



Values were compared statistically by using One way ANOVA followed by Dunnett test.
The error bars indicate the mean± SE. ***P, 0.001, versus control.

Fig. 3: Conc. dependent effect of (6.7 and 67 µM) of PDN/ BBNPC on the chemical status of D-Pen Results are the mean ±SE of n=3 experiments of D-Pen.

2mM of Palladium Nitrate (PDN) stock solutions was prepared by dissolving 26.6 mg, of Palladium Nitrate (PDN Mol. weight 266.3 g/mol in sufficient quantity of distilled water to make the whole volume 50ml.

2mM Bis-benzonitrile Palladium II chloride: 2mM Bis-benzonitrile Palladium II chloride stock solution was prepared by dissolving 38.36 mg of benzonitrile Palladium II chloride (BNPDC, Mol. weight 383.57 g/mol in sufficient quantity of 0.1M (Perchloric acid 70%) to make final volume 50ml.

Estimation of GSH by Elman's method

Method of Elman's (1959) was followed to estimate thiols (GSH, NAC and D-pen). The colored complex formed by reaction of SH group of thiols blank and (mixtures of thiols with metals after treatment with different concentrations of Palladium and Vanadium salts and complexes) with Elman's reagent i.e.5, 5'dithio bis- 2 nitro benzoic acid (DTNB) in aqueous solution, which give absorbance at 412nm which intern shows the concentration of corresponding thiol.

Standard Calibration Curve

A series of five different dilution i.e. 0.2, 0.4, 0.6, 0.8 and 1mM GSH was prepared by adding further 0.1M Phosphate buffer PH 7.6 to the 2mM GSH stock solution.

Experimental protocol

Preparation of Reaction Mixture, Reading Sample for Either Palladium Nitrate with either Glutathione, or N-Acetylcysteine or D-Penicillamine for Aqueous Media 1.0ml of different concentrations (.2, .4, .6, .8, 1, 1.2, 1.4, 1.6, 1.8 to 2mM)of Palladium Nitrate, solution was added to 1.0 ml of 2mM Glutathione, solution taken in ten separate test tubes, shacked well. Final concentration of Glutathione in each of the above test tube was 1mM (1000µM) and that of Palladium Nitrate was 0.1mM (100µM), 0.2mM (200µM), 0.3mM (300µM), 0.4mM (400µM), 0.5mM (500µM) and 0.6mM (600µM), 0.7mM (700µM), 0.8mM (800µM), 0.9mM (900µM), 1mM (1000µM) respectively. Then a series of ten samples cuvettes were prepared by taking 0.5ml of 1mM DTNB from stock solution in 10 separate test tubes. To it added 0.2ml of Palladium Nitrate Glutathione mixture from each one of the above made test tubes, diluted with 2.3ml of phosphate buffer pH 7.6 and incubated for five minutes. The final concentration of Glutathione in each of these test tubes was 0.03333mM (33.33µM) and of Palladium Nitrate was (6.7µM 13.4µM, 20.1µM, 26.8µM, 33.5µM), 40.2µM (46.9µM), 53.6µM (60.3µM) and 67.0µM respectively. For control sample 0.2ml of 2mM Glutathione stock solution was added to a separate test tube already containing 0.5ml 1mm DTNB stock solution and the final volume was adjusted to 3ml by the addition 2.3 ml of phosphate buffer pH 7.6. The reaction mixture and reading sample for the other metals salts like bis-benzonitrile Palladium (II) chloride, with either Glutathione or N-acetylcysteine or d-penicillamine respectively were also prepared in similar way as for the preparation of reaction mixtures and reading samples for Palladium Nitrate and Glutathione. Absorbance for each of the above mentioned sample of all the metal salts and complexes were recorded on UV- visible spectrophotometer at fixed wavelength at 412nm.

Preparation of ^{1}H NMR samples

A series of mono and Bis thiolate-Palladium (Glutathione, D-penicillamine, N-acetylcysteine) solutions were prepared in-situ 0.1MKH2PO4 in D_2O at PH 7.4 using Palladium Nitrate dehydrate and either one or two equivalents of corresponding thiolate. By dissolving1.33mg of Palladium Nitrate in 5ml volumetric flask and 1.54mg of Glutathione, 0.082mg of NAC and 0.76mg of D-Penicillamine and then these solution were mixed in 1:2 and 1:1 Palladium Nitrate and thiols in 0.8ml NMR Test tube respectively and the 1H NMR spectra were obtained using a Bruker AVANCE 3 spectrometer operating at 400.12 MHz. Samples were maintained at 300 K during spectral acquisition. The free induction decay was generated by a 3.13µs pulse width corresponding to a 30^o pulse with a 2 sec delay between pulses. Each data set was collected in 32 K of memory. A 1Hz line broadening function was applied before Fourier transformation to reduce the effect of the baseline noise.

RESULTS

Effect of various Conc. (6.7 to 67µM) of PDN/BBNPDC on the Chemical Status Glutathione

Reduced Glutathione (GSH) of reaction mixture of either Palladium salts and/or its organometalic complex was measured in each tube by Ellman's method. The absorbances were recorded at 412nm on a UV-Visible spectrophotometer and were converted into concentrations. The unknown conc. of GSH in the

Table 1: Summary of Distribution of chemical shifts for the ^{1}H-NMR spectrum of Glutathione

S No	Chemical shift, ppm (δ)	No of protons	Assignment of species
1	4.6	2	Cys (CάH$_2$)
2	3.0	2	Cys (CβH$_2$)
3	3.91	2	Glu (CάH$_2$)
4	2.5	2	glu (C□H$_2$)
5	2.1	2	Glu (CβH$_2$
6	4.09	2	gly (CH$_2$)

Table 2: Summary of Distribution of chemical shifts for the 1H-NMR spectrum of N-acetylcysteine

S. No	Chemical shift, ppm	Number of protons	Assignment of specie
1	1.37	3	Actyl-CH3
2	2.9	2	Cystenyl-CH2
3	4.4	1	Carboxyl-CH

Table 3: Summary of Distribution of chemical shifts for the 1H-NMR spectrum of (D) –penicillamine

S. No	Chemical shift, ppm	Number of protons	Assignment
1	1.37	3	CH3
2	1.55	3	CH3
3	3.59	1	CH

Palladium and GSH mixture was then calculated using the above known standard curve. When GSH was exposed to Lowest and Highest concentrations of either Palladium Nitrate/or bis-benzonitrile Palladium (ii) chloride (6.7μM to 67μM) respectively in aqueous media, it was observed that thelevel of GSH was depleted significantly ($p<0.001$) as compared to control GSH from (44.1% to 75%) and (34.0 to 65.0 %) as shown in fig. 1.

Effect of various conc. (6.7μM to 67μM) of PDN/BBNPDC on the chemical status N- acetylcysteine

N- Acetylcysteine (NAC) was also measured in each tube by Ellman's method the absorbances were recorded at 412nm and were converted into concentration as mention in standard curve. When NAC was exposed to different concentrations (6.7μM to 67μM) of either Palladium Nitrate /or Bis-Benzonitrile Palladium (II) Chloride respectively in aqueous media, the level of NAC was also significantly decreased ($p<0.001$) as compared to control NAC from (42.39 % to 74%) and (37.5% to 67%) as shown in figure 2.

Effect of various Conc. (6.7 to 67uM) Conc. PDN/ BBNPDC on the Chemical Status D-Penicillamine and With Time (0 to 90 Minutes)

D-penicillamine (D-pen) was measured in each tube by Ellman's method, again when D-pen was exposed to different concentrations (6.7μM to 67μM) of either Palladium Nitrate / or Bis (benzonitrile) Palladium (II) chloride respectively in aqueous media. The level of D-penicillamine was decreased significantly ($p<0.001$) as compared to control D-pen from (32.6 to 66.56%) and (26% to 60%) by Palladium Nitrate and Bis (benzonitrile) Palladium (ii) chloride treatment respectively, as shown in fig. 3.

Interaction of palladium with glutathione

Palladium and Glutathione 1:2 and 1:1 Complexation of Palladium Nitrate with Glutathione were generated in situ and the ^{1}H-NMR spectras were generated on H-NMR 400Hz. The NMR Spectra of solutions of GSSG and Plane GSH in 7.4 pH 0.1M phosphate buffer was also generated in order to compare the structural differences in the reduced and oxidize Glutathione with thiolate Palladium mixture spectras. In H-NMR, the amino acids protons of the reduced Glutathione give signals at 6 different chemical shifts. The cysteine residue of the reduced Glutathione shows signals at 3.0ppm and 4.6 ppm in case of oxidized Glutathione, β-cysteinyl residue split in such away, that two identical quadrates are produced due to chiral center giving signals at (3 to 3.5ppm), while the ά-cysteinyl residue of oxidized Glutathione disappeared due to water of oxidation. In case of Palladium GSH complex the β-cysteinyl residue also split into two quadrate between (2.6ppm and 3.5ppm, but not identical like homoleptic disulphide of oxidized Glutathione as shown in table (1), and figure (4.1) and (4.2) From this finding it is suggested that Palladium combine with the cysteine residue of the Glutathione and Palladium cysteinyl complex is formed which bring changes in the resonances of the βcysteinyl residual proton environment and thus split into new peaks, which is different from both cysteine, residue of reduced and oxidized Glutathione, this finding is in agreement with another data stated by other author who showed that arsenic Glutathione complexes AS(GS)$_3$ displays a very

familiar distributed eight line pattern between (δ3.2 and δ3.35) indicative of two cysteinyl connected as a disulphide or cis-metal thiolate (Scott *et al*., 1993, Raab*et al*., 1993 & Percy *et al*., 2008). In 1:1 Palladium GSH reaction again we see splitting of signals of cysteinyl residue to some complex peak and also changes in the all other peaks of GSH, suggesting that Palladium may form some complex structure in which both sulfur as well as nitrogen of two different carbons are bonded which bring conformational changes in the environments of all the residual protons of the entire species figure (4.1). This study is also supported by literature that Palladium may form 1:2 as well as more complex structures with thiols (D-penicillamine) in which Palladium is attached to sulfur of carbon of one amino acid and nitrogen of the next in cluster form (Cervantes *et al*., 1998).

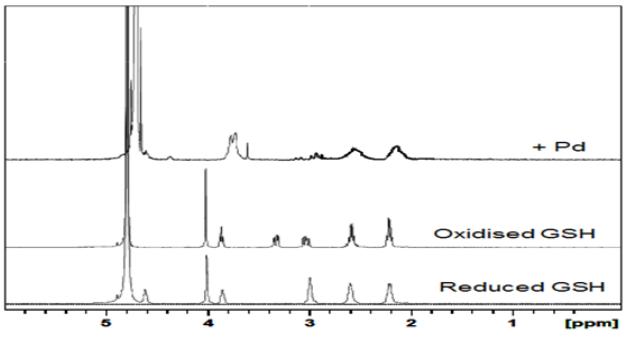


Fig. 4.1: The 400 MHz ^{1}H NMR spectra (ns =64) obtained by titrating solutions of Glutathione 0.1M KH_2PO_4 in $^{2}H_2O$ at pH 7.4) with a Pd $(NO_3)_2$ (forming Pd $(SGH)_2$ Complex in situ.

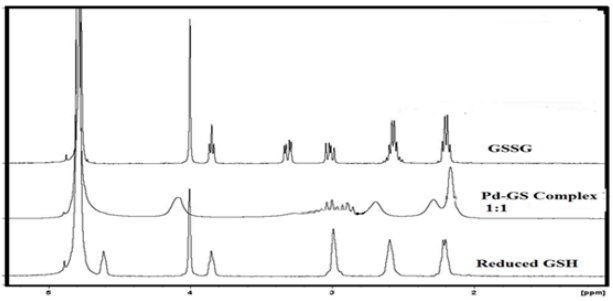


Fig. 4.2: The 400 MHz ^{1}H NMR spectra (ns = 64) obtained by titrating solutions of Glutathione 0.1M KH_2PO_4 in $^{2}H_2O$ at pH 7.4) with a Pd $(NO_3)_2$ (forming Pd $(SGH)_2$ Complex in situ

Interaction of palladium with N-acetylcysteine

The N-Acetylcysteine plane solution and 1:2 and 1:1 Complexation of Palladium Nitrate and N-Acetylcysteine spectras were also generated in phosphate buffer in D_2O pH 7.4 on a Bruker 400MHz H-NMR instrument. The resulting spectrum are shown in table (2) and Figures (4.3,) and (4.4) that showed that a singlet band was observed at chemical shifts of 1.37ppm integrated for three protons, of the methyl groups, attached to the carbon of N- acetyl group. A singlet at 4.4ppm, integrated for one proton, of the CH attached carboxylic function group. A singlet at 2.9 ppm, integrated for one proton, of the CH attached SH- functional group. Results showed that the Palladium 1:2 and 1:1 exhibited the same pattern of splitting of the cysteinyl residue of the N-acetylcysteine as in Glutathione because in 1:2 reaction the cysteinyl residue at 2.9ppm split into two quadrates and in the 1:1 Palladium N-Acetylcysteine reaction, a more complex peak was observed also the Acetyle peak showed changes due to complex structure, produced during 1:1 reaction of Palladium and N-Acetyle cysteine. From this finding it is suggested that Palladium form both 1:1 and 2:1 complex with N-acetylcysteine. The pattern of splitting of cysteinyl residue was almost similar as in Glutathione.

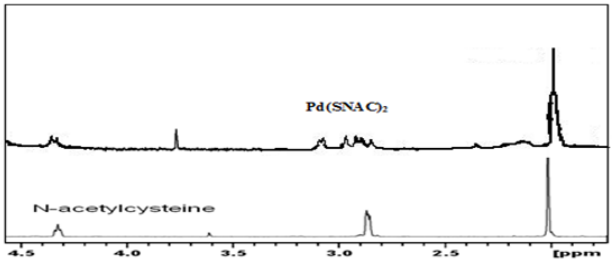


Fig. 4.3: The 400 MHz ^{1}H NMR spectra (ns = 64) obtained by titrating solutions of N- acetylcysteine 0.1 M KH_2PO_4 in $^{2}H_2O$ at pH 7.4) with a Palladium Nitrate (2:1) formed in-situ.

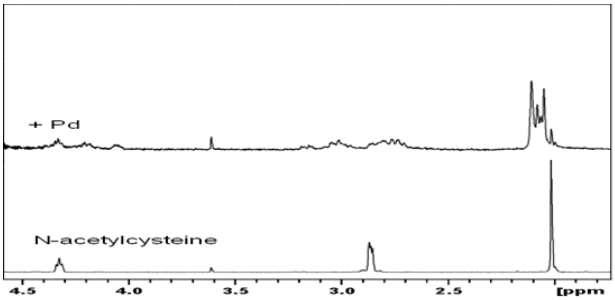


Fig. 4.4: The 400 MHz ^{1}H NMR spectra (ns = 64) obtained by titrating solutions of N- acetylcysteine 0.1M KH_2PO_4 in $^{2}H_2O$ at pH 7.4) with a Palladium Nitrate1:1 formed in-situ.

Interaction of palladium with (D)-penicillamine

The ^{1}H-NMR spectrum of (D) -penicillamine in D_2O Phosphate buffer pH7.4 was obtained on a Bruker 400 MHz instrument. The resulting spectrum is shown in table 3 and fig 4.5 and 4.6 that showed that two singlet bands were observed at chemical shifts of 1.37 and 1.55ppm, each of which integrated for three protons, of the two methyl groups, attached to the carbon of cysteinyl group. A singlet at 3.59ppm integrated for one proton, of the CH functional group. When the thiolate Palladium spectra was compared with the control D-pencillamine then a shifting of the resonances of CH_3 group from 1.37ppm to lower ppm and splitting of the resonance of CH_3 group attached to the SH group at 1.45ppm into two resonance

at (1.5 and 1.6ppm) occurs. Also the resonances of the CH group split into two resonance at 3.59 ppm into (3.7ppm, 2.2ppm) occur in both 1:1 and 1:2 reactions. When the 1:1 and 1:2 resonances were compared then there were differences of the shifting in the entire signal in both spectras of 1:1 and1:2 complexes, it is confirmed from these results that Palladium making both 1:1 and 1:2 complexation with D-pen. As the Pd (D-pen) and Pd (D-pen) $_2$ have different proton environment so the observed signals of both species were different. These results are also in agreement with other reports that showed that smaller thiol species like D-pencillamine form Pd (D-pen)1, Pd (D-pen)2 Pd-(D-pen)3 complexes with Palladium in which the S, N,O of D-penicillamine co-ordinates with Palladium resulting in a mono, di and tridentate species (Cervantes G *et al*.,1998).

DISCUSSION

Interactions between Pd (II) complexes with sulfur-containing biomolecules are very important from a biological and medical point of view. For instance Despite the clinical anti-cancer utility of cis-platin, carboplatin, oxaliplatin, and several other complexes in clinical trials, there is a continued interest in the design of new complexes that shows anti-tumor activities equivalent or better than these agents (Torshizi Mahboube I-Moghaddam *et al*., 2008 & Kwon *et al*., 2003). In the present study two different salts of Palladium were treated with biologically important low molecular thiols (Glutathione, and N-acetylcysteine) a synthetically active cystien containing residue D-penicillamine) spectrophotometrically in ordered to test which salts showing more toxicity toward biologically active ligand and also to explore new agent like D-pencillamine in reducing their toxicity while using these Metallo-element as a therapeutic agents, The results of our finding suggested that Palladium and Vanadium depleted the level of Glutathione in aqueous medium both in dose dependent manner, the depletion level of all the thiols (Glutathione, N- acetylcysteine and D-penicillamine) in aqueous media may be due to the Pd -(SR)2 or Pd(SR), conjugation .Our results also showed that the depletion level in Glutathione and NAcetylcysteine was almost the same while both showed more than from D-pencillamine. A possible explanation of the depletion deference among three thiols is that Glutathione and N- Acetyle cysteine are structure analog and thus showing nearly same affinity towards the metals while the lower depletion of the d-penicillamine may be due to steric hindrances because of the two methyl groups attached to the cysteinyl group of D-penicillamine. This study is also supported by literature elsewhere that the reactivity of the thiol nucleophile follows the sequence D-penicillamine <L-cysteine < Glutathione (Zivadin *et al*., 2001). In another study it is stated that Glutathione is considerably more reactive when treated with Pd^{+2} and Pt^{+2} complexes treated with thiols (Glutathione N-acetylcysteine) due to an appreciable anchimeric (neighbouring group) effect capable of reducing the activation barrier of the substitution reaction, arising from hydrogen bonding interactions between the acidic group located in a suitable position of the nucleophile (Wilkins, *et al*., 1991). The anchimeric (neighbouring group) effect has been reported for other reactions at Pt(II) complexes and is well known for organic reactions (Wilkins, *et al*., 1991). Compare to Glutathione, d-pencillamine showed strong steric interactions due its two methyl bulky groups on the carbon center near to the sulfur atom, making the entering thiols into its lower nucleophilicity (Wilkins, 1991).It was also observed from our results that inorganicsalts of palladium showed more of the thiols as compare to organic salt of the Palladium metal suggesting that the inorganic salt produced more toxicity compared to its organic complexes. This finding could be due to the rapid dissociation of metal inorganic salt into free radical formation in solution. And hence may easily form complexes with thiols sulphydryl group.

CONCLUSION

It is concluded from this finding that D-pencillamine and N- Acetylcysteineis aproven effective chelating agent in protecting the redox status of Glutathione in highly oxidative stress condition.

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