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Determination of Glutathione Reductase Activity Changes Exposed to Some 2-Aminothiazole Derivatives

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Abstract. In this work, effects of concentrations ranging from 0 to 500 mg/L of some 2-aminothiazole derivatives such as 4,4'-(disulfanediylbis(methylene))bis(thiazol-2-amine) dihyrochloride (DMTA) and 2-amino-4-(chloromethyl)thiazole hydrochloride (ACT) on glutathione reductase from baker's yeast *(Saccharomyces cerevisiae)* (GR) were investigated. With exposure of 25, 50, 100, 250 and 500 mg/L concentrations, % GR activity changes were calculated as -5.29 ; -3.85 ; -2.40 ; -6.73 and -10.58 in DMTA applications, while these changes were calculated as +0.98 ; 0.00 ; -0.49 ; -2.45 and 0.00 in ACT applications, respectively. This work indicated that there was a slight decrease in GR activity with the increase of DMTA concentrations and there was no significant change in GR activity with the increase of ACT concentrations. But according to control activities, no statitistical changes were observed in GR activities with exposure of these 2-aminothiazole derivatives ($p > 0.05$, n=3).

***Keywords*:** Glutathione Reductase, 2-Aminothiazole, 4,4'-(disulfanediylbis(methylene)) bis(thiazol-2-amine) dihyrochloride, 2-amino-4-(chloromethyl)thiazole hydrochloride.

Bazı 2-aminotiazol Türevlerine Maruz Kalmış Glutatyon Redüktaz Aktivitesindeki Değişimlerinin Belirlenmesi

Özet. Bu çalışmada, 2-Aminotiazol türevleri olan 4,4'-(disulfanediylbis(methylene))bis(thiazol-2-amine) dihyrochloride (DMTA) ve 2-amino-4-(chloromethyl)thiazole hydrochloride (ACT)'in 0 dan 500 mg/L ye değişen derişimlerinin ekmek mayası *(Saccharomyces cerevisiae)* glutatyon redüktazı (GR) üzerine olan etkileri araştırılmıştır. 25, 50, 100, 250 ve 500 mg/L derişimlere maruz bırakılma ile % GR aktivitesindeki değişimler, DMTA uygulamalarında sırasıyla -5,29 ; -3,85; -2,40 ; -6,73 ve -10,58 olarak hesaplanırken, ACT uygulamalarında sırasıyla +0,98; 0,00; -0,49; -2,45 ve 0,00 olarak hesaplanmıştır. Bu çalışma, DMTA derişimlerinin artışı ile GR aktivitesinde hafif bir düşüş olduğunu ve ACT derişimlerinin artışı ile GR aktivitesinde önemli bir değişimin olmadığını göstermiştir. Ancak kontrol aktivitelerine göre, bu 2-aminotiazol türevlerinin etkileştiği GR aktivitelerinde herhangi bir istatistiksel değişim gözlemlenmemiştir ($p > 0,05$, n = 3).

***Anahtar Kelimeler*:** Glutatyon Redüktaz, 2-Aminotiazol, 4,4'-(disulfanediylbis(methylene)) bis(thiazol-2-amine) dihyrochloride, 2-amino-4-(chloromethyl)thiazole hydrochloride.

1. INTRODUCTION

2-Aminothiazole derivatives have a heterocyclic ring system and have antiviral [1], antimicrobial [2], anticancer [3] and anti-inflammatory [4] activities. Recent research has shown that 2-aminothiazole derivatives act as inhibitors against kynurenine-3-hydroxylase and cyclin-dependent kinase enzymes [5].

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Glutathione reductase (EC 1.8.1.7) (GR) acts an antioxidant. GR converts oxidized glutathione (GSSG) to form reduced glutathione (GSH) in the presence of NADPH (β-Nicotinamide adenine dinucleotide 2'-phosphate reduced) [6].

$$GSSG + NADPH + H^{+} \xrightarrow{GR} 2\,GSH + NADP^{+}$$

GSSG contains disulfide bridge (-S-S-) in its structure. DMTA contains disulfide bridges such as GSSG, which is the substrate of the GR enzyme. DMTA has the potential to make inhibition due to this feature. If inhibition occurs, GSH will not occur. GSH has important functions in metabolism. GSH plays a key role in maintaining proper functions in human cells and preventing oxidative stress. It neutralizes hydroxyl radicals, singlet oxygen and various electrophiles [7]. ACT is a 2-aminothiazole derivative without disulfide bridge. In this study, we investigated whether 2-aminothiazole derivative compounds containing disulfide bridge and no disulfide bridge affect on GR.

2. MATERIALS AND METHODS

2.1. Chemicals

4,4'-(disulfanediylbis(methylene))bis(thiazol-2-amine) dihyrochloride (Fig.1a) was received from Dr. Cumhur KIRILMIŞ [8]. 2-Amino-4-(chloromethyl)thiazole hydrochloride (SYX00295) (Fig.1b), L-Glutathione oxidized (G4501), β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (N1630), Glutathione reductase from baker's yeast *(S. cerevisiae)* (G3664) were received from Sigma-Aldrich. Other chemicals used were analytical grade.

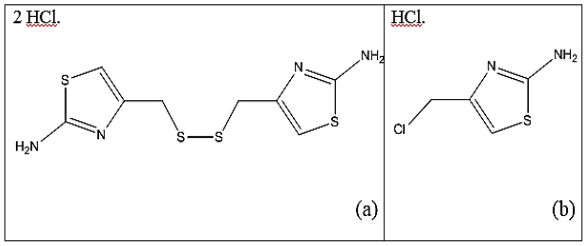

Figure 1. Structures of 4,4'-(disulfanediylbis(methylene))bis(thiazol-2-amine) dihyrochloride (a) and 2-amino-4-(chloromethyl)thiazole hydrochloride (b).

2.2. Protein determination

The protein concentration of GR was measured spectrophotometrically at 750 nm [9]. Bovine serum albumin was used as standard for the determination of GR protein concentration. For determination of protein concentration, four solutions were prepared. 1. Solution (A): 0.5 g CuSO4.5 H2O and 1 g sodium citrate dihydrate were dissolved at distilled water and completed to 100 mL by distilled water. 2. Solution (B): 2 g Na2CO3 and 0.4 g NaOH were dissolved at distilled water and completed to 100 mL by distilled water. 3. Solution (C): 2 mL solution A was added to 100 mL solution B. 4. Solution (D): 20 mL Folin-Ciocalteu was added to 20 mL distilled water. After preparation of these four solutions, 2.5 mL solution C was added to 0.5 mL of GR solution, shaked, waited for 10 minutes at room temperature, then added 0.25 mL of solution D, shaked, waited for 30 minutes and read at 750 nm for determination of GR concentration.

2.3. Glutathione reductase activitiy

The enzyme activity was measured spectrophotometrically by reading the changes in absorbance at 340 nm during oxidation of NADPH to $NADP^{+}$ by GSSG at 37 °C at incubated UV-1800 UV-VIS Spectrophotometer (Shimadzu Scientific Instruments) [10]. The reaction solution was contained: 1.0 mM GSSG, 0.12 mM NADPH, 0.10 M potassium phosphate buffer (pH 7.6). The oxidation of 1 µmol of NADPH/minute under these conditions was used as a Unit (U) of GR activity. Milimolar extinction coefficient of β- NADPH at 340 nm was used as 6.22. The specific activity of GR was indicated as U/mg protein.

2.4. Effect of 2-aminothiazole derivatives on enzyme activity

Solutions of 5000 mg/L DMTA and ACT in distilled water were prepared. After that, arrangement of 0, 25, 50, 100, 250 and 500 mg/L DMTA and ACT with distilled water and 700 µL GR solution were done [11]. At control or 0 mg/L, 300 µL distilled water and 700 µL GR solution were used. Solution volume of enzyme and

distilled water and 2-aminothiazole derivative was 1 mL. The mixture of GR and distilled water and 2-aminothiazole derivative was waited at room temperature for 10 minutes. Then activities of GR were determined.

2.5. Value analysis

The obtained values were shown as mean ± standard deviation. For the statistical analyses, one-way analysis of variance (ANOVA) was used, followed by the Student Newman-Keul's test using the IBM SPSS version 22 statistical software (SPSS Inc. Chicago, IL, USA). Differences were considered as significant if $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of 4,4'-(disulfanediylbis(methylene))bis(thiazol-2-amine) dihyrochloride on glutathione reductase activity

GR activities exposed to solutions of DMTA from 0 to 500 mg/L were measured. Mean of enzyme activity and standard deviation values were given in Table 1. Activity-concentration graph was shown in Fig. 2. When Table 1 and Fig.2 were examined, it was observed that there was a slight decrease in GR enzyme activity while DMTA concentration increased. However, no statistically significant changes were observed when compared to control group ($p > 0.05$, $n = 3$). The percent changes in GR enzyme activities exposed to 25, 50, 100, 250 and 500 mg/L of DMTA were calculated as -5.29; -3.85; -2,40; -6.73 and -10.58 respectively.

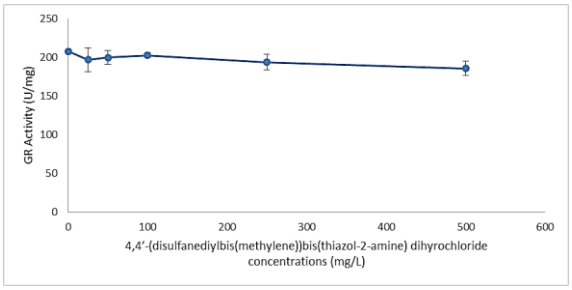

Figure 2. Effect of DMTA on GR activity.

3.2. Effect of 2-amino-4-(chloromethyl)thiazole hydrochloride on glutathione reductase activity

GR activities exposed to solutions of ACT from 0 to 500 mg/L were measured. Mean of GR activity and standard deviation values were given in Table 1. Activity-concentration graph was shown in Fig. 3. When Table 1 and Fig.3 were examined, it was seen that there were no statistically significant changes in GR enzyme activities when compared to control group while ACT concentration increased ($p > 0.05$, $n = 3$). The percent changes in GR activity by exposure of GR to 25, 50, 100, 250 and 500 mg/L ACT were calculated as +0.98; 0.00; -0.49; -2,45 and 0.00 respectively.

Table 1. Effect of DMTA and ACT 2-aminothiazole derivatives concentrations on GR activity.

2-Aminothiazole Derivatives Concentration (mg/L)	GR activity ± standart deviation (U/mg) for DMTA	GR activity ± standart deviation (U/mg) for ACT
0	208±3a	204±8a
25	197±15a	206±12a
50	200±9a	204±11a
100	203±3a	203±6a
250	194±10a	199±5a
500	186±9a	204±3a

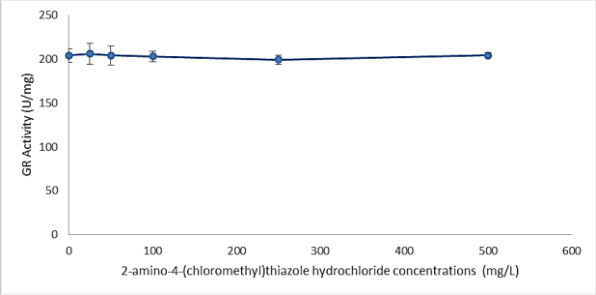

Figure 3. Effect of ACT on GR activity.

When we look at the literature, we did not find any direct studies on the effects of DMTA and ACT on GR activity. However, studies on the effects of 2-aminothiazole derivatives or thiazole derivatives on other enzyme activities were found. Such as, 2-aminothiazole derivatives act as inhibitors against kynurenine-3-hydroxylase and cyclin-dependent kinase enzymes [5]. Also, the 2-aminothiazole-4-carboxamide compound was a novel class of inhibitors of serine / threonine protein kinase (CHK1) [12]. In another study, (4 - ((4- (4-chlorophenyl) -2-thiazolyl) amino) phenol compound at a concentration of 10 µM was a moderate inhibitor (15-25% inhibition) in the experimental conditions for sphingosine kinase [13]. These result was similar like our findings about DMTA which DMTA caused a moderate inhibition (10.58 % inhibition at 500 mg/L). Another study, 3- (5- (4- (benzyloxy) -3-methoxyphenyl) -1- (4- (4-bromophenyl) thiazol-2-yl) -4,5-dihydro-1H-pyrazol-3-yl) -2H -chromen-2-one was shown to be a potential tyrosinase inhibitor [14].

4. CONCLUSION

DMTA is similar to GSSG in that it contains the disulfide bridge. DMTA may compete with GSSG to influence GR. DMTA has slightly inhibited GR (10.58 % inhibition at 500 mg/L). ACT did not have any effect on GR because it did not contain disulfide bridge. As a result, we found that DMTA caused a moderate inhibition and ACT did not cause any inhibition. But ultimately, we didn't find any statistically significant changes on GR activities in the our work.

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