Bioorganic Chemistry 38 (2010) 132–137

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

5-Hydroxy-1,4-naphthoquinone (juglone) and 2-hydroxy-1,4-naphthoquinone (lawsone) influence on jack bean urease activity: Elucidation of the difference in inhibition activity

Mirosława Kot [a,*], Waldemar Karcz [b], Wiesława Zaborska [a]

[a] Jagiellonian University, Faculty of Chemistry, Ingardena 3, 30-060 Krakow, Poland
[b] Silesian University, Faculty of Biology, 40-032 Katowice, Jagiellońska 28, Poland

ARTICLE INFO

Article history:
Received 6 December 2009
Available online 13 February 2010

Keywords:
Urease
Inactivation
Inhibition
5-Hydroxy-1,4-naphthoquinone
2-Hydroxy-1,4-naphthoquinone
Juglone
Lawsone
Redox cycling

ABSTRACT

The aim of this study was elucidation of the difference in inhibition influence of 5-hydroxy-1,4-naphthoquinone (juglone) and 2-hydroxy-1,4-naphthoquinone (lawsone) on jack bean urease activity. It was found that juglone acted as a strong, time and concentration dependent inactivator of urease. On the contrary, lawsone showed an inconsiderable inhibition influence. The reactivation of juglone modified urease showed the participation of reversible and irreversible contribution in the inactivation. In the presence of an excess of DTT, urease inactivated by juglone regained 70% of its activity. The reversible inactivation was attributed to oxidation of the essential urease thiols by reactive oxygen species (ROS) realizing during reduction of juglone to seminaphthoquinone. Presence of hydrogen peroxide in the incubation system was proved by direct determination and by application of catalase. The irreversible contribution in the inhibition was assumed as an arylation of urease thiol groups by juglone. The insignificant urease inhibition by lawsone was concluded as an effect of a low hydrogen peroxide generation and lawsone resistance for reaction with protein thiols. It was found that lawsone well reacted with L-cysteine, poorly with glutathione and hardly with urease thiols. The observed sequence was arranged according the rule the more complex thiol the less susceptible for reaction with lawsone. On the other hand, juglone displayed an excellent reactivity towards both thiols and urease. Thus, this indicated a significance of a steric hindrance which appeared when the hydroxyl group changing position from 5 in juglone (5-hydroxy-1,4-naphthoquinone) to 2 in lawsone (2-hydroxy-1,4-naphthoquinone).

1. Introduction

Urease (urea amidohydrolase, EC 3.5.1.5) is an enzyme catalyzing the hydrolysis of urea: $CO(NH_2)_2 + H_2O \xrightarrow{urease} 2NH_3 + CO_2$. The enzyme has been found in plants, algae, fungi and bacteria [1–3]. The plant urease from jack bean is the homohexameric molecule (α_6). Its active site contains two nickel ions directly involved in binding of substrates and inhibitors [4,5]. Urease is a thiol rich enzyme. The total amount of thiols is equal 15 per jack bean urease subunit. However, only 6 of 15 cysteines are accessible to the thiol reagent (without denaturation of the enzyme). One of them, cysteine-592 plays critical role in the catalytic activity [6,7].

Beside the unquestionable environmental positive role of urease, some effects are damaging for human and animal health, as well as for nature. The prevention from harmful activity of the enzyme requires effective inhibitors which have been sought among numerous organic and inorganic compounds [3,8]. The specific group of examined chemicals are quinones [9–11]. Quinones have been extensively studied for their potent redox power. This ability involves them in enzymatic and nonenzymatic redox cycling with their corresponding semiquinone radicals. The side effect is the production of ROS (reactive oxygen species) such as superoxide radicals, hydrogen peroxide, and, in the presence of metal ions, hydroxyl radicals. The ROS as a strong oxidizing agents may be responsible for damage of macromolecules. Quinones also can covalently modify cellular nucleophiles such as glutathione or cysteine residues [12–16].

The quinones selected for this study, juglone and lawsone, are chemicals of the natural origin. Juglone (5-hydroxy-1,4-naphthoquinone) is a brown dye isolated from fruits, shells and leaves of walnut trees (*Juglans*). The main source of lawsone (2-hydroxy-1,4-naphthoquinone) is a henna herb *(Lawsonia inermis L.)*. The henna powder is used as a traditional pigment for dyeing hair, skin and nails. Juglone is known as an inhibitor of several enzymes [17–19]. Both naphthoquinones have been studied as the toxic factors of isolated hepatocytes [20–22]. D'Arcy Doherty and Rogers found that lawsone and juglone caused cytotoxity proceeded by

* Corresponding author. Fax: +48 12 634 05 15.
E-mail address: kot@chemia.uj.edu.pl (M. Kot).

0045-2068/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2010.02.002

depletion of intracellular glutathione. Juglone directly reacted with glutathione in contrast to lawsone which reactivity was thought to be enzyme-mediated [20]. Öllinger and Brunmark studied hydroxysubstituted naphthoquionones and elucidated the influence of the substituent position on the toxicity to rat hepatocytes [22]. The low toxicity of lawsone was attributed to its incapacity to undergo electrophilic addition and redox cycling (as a result of its very low one-electron reduction potential) [17]. However, the evidence of lawsone mediated redox cycling was found [23,24].

Despite a great number of investigations of juglone and lawsone cytoxicity, there is still lack of clear explanation of their activity mechanism. The inhibitors were mainly studied in biological complex systems with a large amount of interactions. System complexity generates difficulties in monitoring specific relations. Thus, the purpose of this work was study juglone and lawsone action in the system with the reduced number of by-interactions. The presented work describes the kinetics and mechanism of urease inhibition by 5-hydroxy-1,4-naphthoquinone and elucidates the lack of the inhibitory effect of 2-hydroxy-1,4-naphthoquinone. The protective effect of catalase as well as the reversibility of the inactivation were investigated. The production of hydrogen peroxide in the systems with the naphthoquinone were quantified. The role of redox cycling and thiol interaction was elucidated.

2. Materials and methods

2.1. Materials

Jack bean urease, Sigma type III, specific activity 16 U mg^{-1} solid, the total content of reducing agents 0.5 μg U^{-1}, urea (Molecular Biology Reagent), dithiothreitol (DTT), ascorbic acid, L-cysteine, glutathione, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), catalase (from bovine liver), activity 1340 U mg^{-1} solid were purchased from Sigma. 5-hydroxy-1,4-naphthoquinone (juglone) and 2-hydroxy-1,4-naphthoquinone (lawsone), were from Aldrich. Colorimetric hydrogen peroxide assay kit from Cayman Chemical Company (Ann Arbor, MI). Other chemicals were obtained from POCh, Gliwice, Poland. All reagents used were of analytical grade. Phosphate buffer 50 mM, pH 7.7, was prepared by adjusting pH of phosphoric(V) acid with NaOH. EDTA (2 mM) was added to all enzyme-containing solutions.

2.2. Standard urease activity assay

The standard assay mixture (25 cm^3) consisted of 50 mM urea in 50 mM phosphate buffer, pH 7.7 and 2 mM EDTA. The reactions were initiated by the addition of small aliquots of the enzyme-containing solution (0.5 cm^3), and the urease activity was determined by measuring ammonia concentration after a 5 min reaction. Ammonia was determined by the spectrophotometric, phenol-hypochlorite method. The absorbance was registered at 630 nm [25]. The measurements were performed at ambient temperature. The activity of uninhibited urease was accounted as the control activity of 100%.

2.3. Inactivation of urease by 5-hydroxy-1,4-naphthoquinone (juglone) and 2-hydroxy-1,4-naphthoquinone (lawsone)

The solution of urease was incubated with the solution of 5-hydroxy-1,4-naphthoquinone in the absence of urea. The incubation mixture contained 1.0 mg cm^{-3} of urease, 50 mM phosphate buffer, pH 7.7, 2 mM EDTA and juglone in the range 5–40 μM and lawsone in the range 0.25–0.5 mM. The time when the enzyme and the inhibitor were mixed was marked as zero time of incubation. After appropriate period of time, aliquots from the incubation mixture were withdrawn and transferred into the standard assay mixtures for urease residual activity determination.

2.4. Reactivation of urease inactivated by juglone

The reactivation of juglone inactivated urease was studied by using DTT. The incubation mixture contained 1.0 mg cm^{-3} urease, 20 μM juglone, 50 mM phosphate buffer, pH 7.7. After 60 min incubation DTT was added. The DTT concentration in the incubation mixture was equal to 250 μM. The activity of urease was determined before and after DTT addition. After appropriate period of time, samples of the incubation mixture were withdrawn and transferred into the standard assay mixture and urease residual activity was determined. The experiment was triply repeated.

2.5. Protection influence of catalase on urease inactivation by juglone

Catalase was incubated with urease and inactivator for 20 min. The final mixtures contained 1 mg cm^{-3} urease, 200 U cm^{-3} catalase, 50 mM phosphate buffer, pH 7.7 and inactivator: 20 and 40 μM juglone, 90 mM hydrogen peroxide, respectively. After incubations, aliquots were withdrawn from the incubation mixture and were transferred into the standard assay mixtures for urease activity determination.

2.6. Quantitative determination of hydrogen peroxide generation

Hydrogen peroxide was assayed using a colorimetric hydrogen peroxide assay kit from Cayman Chemical Company (Ann Arbor, MI). The quantitative analysis was based on Fenton reaction [26]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^-$$
$$Fe^{3+} + XO \rightarrow Fe^{3+} - XO$$

where XO is xylenol orange which forms with Fe^{3+} a stable colored complex. Hydrogen peroxide was determined in assays contained 0.1 mM juglone and lawsone (in 50 mM phosphate buffer, pH 7.7) in the absence and presence of 0.6 and 1.2 mM DTT, respectively. The absorbance was measured at 595 nm.

2.7. DTNB determination of urease-naphthoquinone and naphthoquinone-thiol interaction

1. Urease was incubated with the naphthoquinone (lawsone and juglone) for 20 and 40 min, respectively. The incubation mixture contained: 1 mg cm^{-3} urease, 25 μM naphthoquinone, 50 mM phosphate buffer, pH 7.7, 2 mM EDTA. After incubation, the mixture urease-naphthoquinone (2.5 cm^3) was transferred to a cuvette (light path 5 cm) and mixed with 2.5 cm^3 0.15 mM DTNB (prepared in 50 mM phosphate buffer, pH 7.7). The absorbance was measured at 412 nm into continuous mode for 5 min according to Ellman's protocol [27]. The control measurements of absorbance of the used mixtures were performed: urease and DTNB-naphthoquinone in the proportions corresponding to the final reaction mixture at 412 nm into continuous mode for 5 min. The recorded control absorbances were subtracted from the absorbances recorded for the reaction mixtures: urease-DTNB-naphthoquinone.

The activity of urease before and after the incubation with naphthoquinone was tested in the standard experiment.

2. Thiol (L-cysteine and glutathione, respectively) was incubated with the naphthoquinone (juglone and lawsone, respectively) for 10 min. The incubation mixture contained: 0.05 mM thiol, 25 μM naphthoquinone, 50 mM phosphate buffer, pH 7.7. The continuation of the procedure is described above at the point 1. The control measurements of absorbance of the used mixtures were performed

for the thiol and DTNB-naphthoquinone in the proportions corresponding to the final reaction mixture.

3. Results and discussion

3.1. Urease inactivation: kinetics elucidation

The inactivation progress curves as a dependence of the urease activity vs incubation time of urease with lawsone and juglone are presented in Fig. 1. The obtained data showed that lawsone insignificantly inhibited urease. In contrast to lawsone, juglone was found to be very effective inhibitor. Juglone inhibited urease in a time and concentration dependant manner. Under the experimental conditions obeyed terms of pseudo-first-order reaction, urease was completely inactivated within 60 min by 40 μM juglone. The Kitz–Wilson equation was applied for analysis of the obtained curves: $1/k_{app} = (K_i/k_{inact})(1/[juglone]) + (1/k_{inact})$ [28,29]. The value K_i corresponds to the dissociation constant of urease–juglone intermediate complex and k_{inact} is the limiting rate of inactive urease formation. Transfer of the inactivation data into semilogarithmic scale (Fig. 1, inset) revealed that the system followed the monophasic, pseudo-first-order kinetics for approximately double half-time of the reaction. The double reciprocal plot of the pseudo-first-order constant k_{app} as a relation of juglone concentration is presented in Fig. 2. The system produced straight line passing through the origin. This result indicated that inactivation followed the simple bimolecular reaction: urease + inactivator → inactive urease. (Since action of juglone on urease is a complex process the name of the inactivator cannot to be specified). The presence of intermediate complex urease-inactivator was undetectable because of the fast inactivation rate relative to the urease–inactivator complex creation. The second order rate constants $k'(k' = k_{inact}/K_i)$ equal to 0.0039 $(\mu M\ min)^{-1}$ was obtained.

Fig. 3 depicts the decrease of the enzymatic activity as a function of juglone concentration for two incubation times equal to 20 min and 40 min. Obtained relationships were used for determination of the parameter IC_{50} equal to 13.5 μM and 4.8 μM for a 20 and 40 min incubation, respectively.

IC_{50} of 1,4-naphthoquinone (at the same experimental conditions, for a 20 min incubation) was found to be equal 20 μM. Thus, 1,4-naphthoquinone is approximately three times stronger urease inhibitor than juglone. The characteristics of naphthoquinones as urease inhibitors are listed in Table 1.

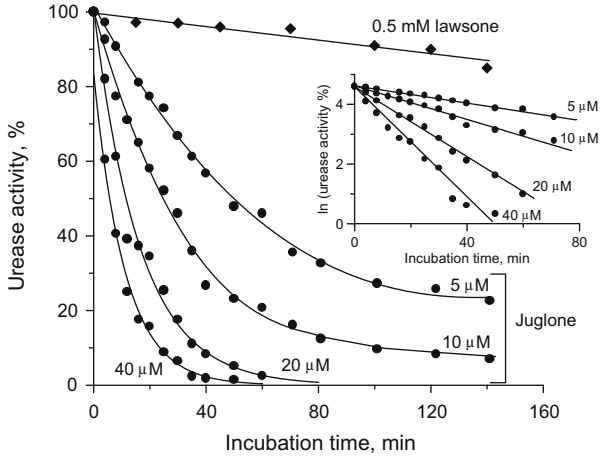


Fig. 1. Inactivation progress curves as a dependence of urease activity vs. incubation time for different juglone concentrations (open symbols) and for 0.5 mM lawsone (full symbols). (Inset) Dependence of urease activity vs incubation time in semilogarithmic system for juglone. Concentrations are given numerically.

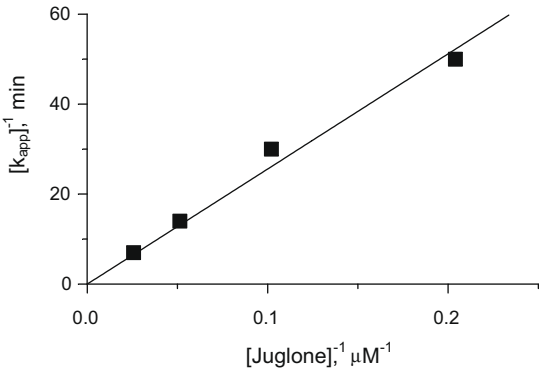


Fig. 2. Dependence of k_{app} vs. juglone concentration in a double reciprocal system.

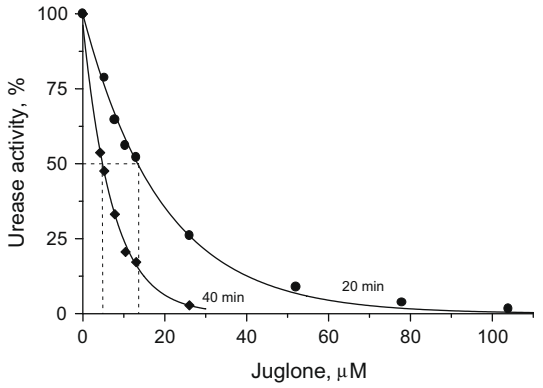


Fig. 3. Urease activity as a function of juglone concentration for a 20 and 40 min incubation.

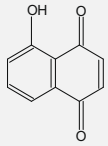
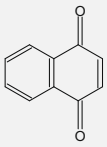
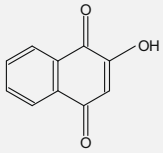
3.2. Reactivation of juglone modified urease

The reactivation of juglone inactivated urease was carried out by DTT application. Urease was incubated with 20 μM juglone until the reactivity of the enzyme decreased to 5% (after a 60 min incubation), then DTT was added into the incubation mixture. The enzyme quickly (1–2 min) regained 70 ± 5% of its activity. Obtained results are presented in Fig. 4. The experiment indicated the contribution of two modes to urease inactivation. The first resulted in reversible inactivation after DTT application. The next caused irreversible urease modification.

3.3. Protection of urease against juglone inactivation in the presence of catalase: determination of hydrogen peroxide in the incubation systems

The protective influence of catalase was tested after a 20 min incubation of urease with juglone and catalase. The results were compared with the data of unprotected system (urease with juglone). The coexistence of catalase in the incubation mixture prevented urease against the juglone inhibition (Fig. 5). Furthermore, it was observed that urease retained almost two times more active after incubation with juglone and catalase, than without catalase. This result was obtained for both studied concentrations of juglone. However, it should be highlighted that the protection was not total. Thus, the inhibition was not the simple hydrogen peroxide influence on urease. Urease inactivated just by hydrogen peroxide stayed 100% active in the presence of catalase because of the complete decomposition of the inactivator. This case was shown for 90 mM hydrogen peroxide as urease inactivator (Fig. 5). The catalase protection experiment proved the presence of hydrogen

Table 1
Characteristics of naphthoquinones as urease inhibitors at 20 mM phosphate buffer, pH 7.7 after a 20 min incubation.

Inhibitor	IC_{50} (mM)	Regained activity with DTT (%)	Single-electron reduction potential (mV)	Ref.
 5-hydroxy-1,4-naphthoquinone (juglone)	0.025	70	−0.09	This paper
 1,4-naphthoquinone	0.030	40	−0.15	[11]
 2-hydroxy-1,4-naphthoquinone (lawsone)	–	–	−0.41	This paper
Hydrogen peroxide	13	100		[30]

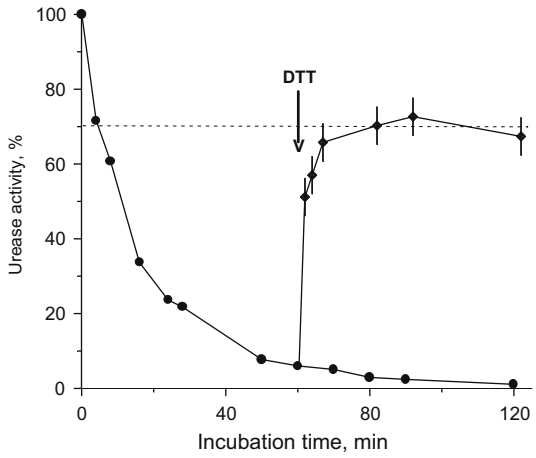


Fig. 4. Reactivation of juglone inactivated urease by DTT addition. Activity of urease inactivated by juglone (●) and after adding DTT (◇). Urease was inactivated by 20 μM juglone, in 60th min of the incubation a small aliquot of DTT was added. DTT concentration in the system was equal to 250 μM.

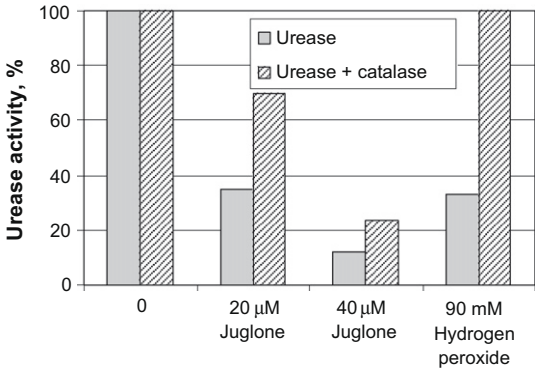


Fig. 5. Catalase influence on urease inactivation by 20 μM and 40 μM juglone, and 90 mM hydrogen peroxide, after a 20 min incubation.

peroxide in the incubation mixture and indirectly showed that the action of hydrogen peroxide was not the only way of inactivation. Moreover, the amount of hydrogen peroxide production was determined for the naphthoquinones in the absence and presence of DTT as a reduction factor (Table 2). DTT efficiently increased the creation of hydrogen peroxide by both naphthoquinones. However, juglone-provoked hydrogen peroxide generation was more effective than in lawsone system. The data presented in Fig. 5 revealed that urease inactivation by 90 mM hydrogen peroxide and 20 μM juglone (without catalase) were comparable in spite of the significant difference in concentrations. This confirmed the fact that hydrogen peroxide is a very weak urease inactivator. Moreover, this indicated how much should be generated hydrogen peroxide by juglone in order to efficiently inactivate urease. The determined juglone-provoked hydrogen peroxide generation (Table 2) was below 1.2 mM for 0.1 mM juglone (five time higher concentration than that presented in Fig. 5) with addition of DTT. Such low hydrogen peroxide concentration was not sufficient to significantly inhibit urease. This fact suggested that the real inhibition factor must be different and much more effective than hydrogen peroxide.

Quinones in the presence of reducing agent undergo redox cycling with their corresponding semiquinone radical and as a result generate superoxide anion radicals. The reaction of hydrogen peroxide, formed by spontaneous dismutation of superoxide anion radicals, with trace amounts of iron or other transition metals gives hydroxyl radicals [26]. Since our enzymatic investigations were

Table 2
Hydrogen peroxide production by 0.1 mM juglone and lawsone (in 20 mM phosphate buffer, pH 7.7) after a 60 min incubation in the absence and presence of 0.6 and 1.2 mM DTT, respectively.

DTT	Hydrogen peroxide (mM)	
	Juglone	Lawsone
0	0.085	0.02
0.6 mM	0.52	0.18
1.2 mM	1.08	0.37

carried out in the presence of EDTA, the influence of metal ions should be excluded (but nickel ions in the urease active site). The effectiveness of urease inhibition by juglone with reference to the quantity of generated H_2O_2 was assumed that the inactivation was caused by other ROS factor than hydrogen peroxide.

3.4. Urease-naphthoquinone and naphthoquinone-thiol interaction

The interactions were determined by the use of DTNB [27]. The method showed the quantity of thiol groups (in urease or thiols) which had not reacted with the naphthoquinone. The DTNB procedure was successfully applied for studies of urease inhibition by 1,4-benzonquinone, tetrachloro-1,4-benzoquinone and 1,4-naphthoquinone [11,30]. The data presented in Fig. 6A pointed out that urease after a 40 min incubation with 25 μM lawsone possessed almost equal amount of unmodified thiol groups to that before the incubation. The tested urease activity after incubation with lawsone proved the fact that the enzyme did not lost the activity (Fig. 1).

The curves obtained for urease–juglone system showed the significant decrease of thiol groups in urease accessible for DTNB after incubation with 25 μM juglone. The decrease was more significant for longer incubation time. The urease activity after a 20 min and 40 min incubation with juglone was equal to 28% and 5%, respectively. Fig. 6B depicted the interaction between 0.05 mM L-cysteine and 25 μM juglone and 25 μM lawsone, respectively. The covered absorbance curves obtained for both systems testified that affinity of juglone and lawsone towards L-cysteine was similar. The next experiment tested the affinity of the naphthoquinones towards cysteine bonded in tripeptide – glutathione (γ-Glu-Cys-Gly). It was found that glutathione interaction with juglone was much more significant than with lawsone (Fig. 6C). The curve related to glutathione-lawsone system was situated close to the glutathione curve. Thus, just a little lawsone reacted with glutathione. However, the juglone interaction with L-cysteine and glutathione was observed on the similar high level.

4. Conclusion

Quinones represent a class of chemicals known for their cytotoxicity, immunotoxicity and carcinogenesis. However, there is a big differentiation of their effectiveness [12,31]. Some of quinones (e.g. lawsone) revealed a toxic influence only in specific conditions, others are highly toxic (e.g. 1,4-naphthoquinone, LD_{50} = 25–100 mg kg^{-1} for mice [10]). The way by which quinones caused harmful effects can be complex. Two general mechanisms have been elucidated. The first is a direct interaction between enzyme and quinone which leads to covalent modification of protein thiols and generation of thioethers (arylation process). The result is irreversible inhibition that cannot be reversed by DTT application. The next mechanism is indirect influence on essential protein residues. The mediators are reactive oxygen species (ROS), e.g. H_2O_2, $O_2^{\cdot-}$ liberated while quinone induced redox cycling. ROS can oxidize the catalytic thiols to sulfenic, sulfinic or sulfonic acids. Sulfenic acid is susceptible for externally implicated DTT and can be reduced back to the initial thiol group. A low concentration of hydrogen peroxide is conducive to modification thiols to sulfenic acids while a higher concentrations (above 1 mM) could irreversibly oxidize sulfur to higher oxidation states [32].

The studies reported in this paper shown significant urease inhibition by juglone in contrast to its isomer lawsone. The inves-

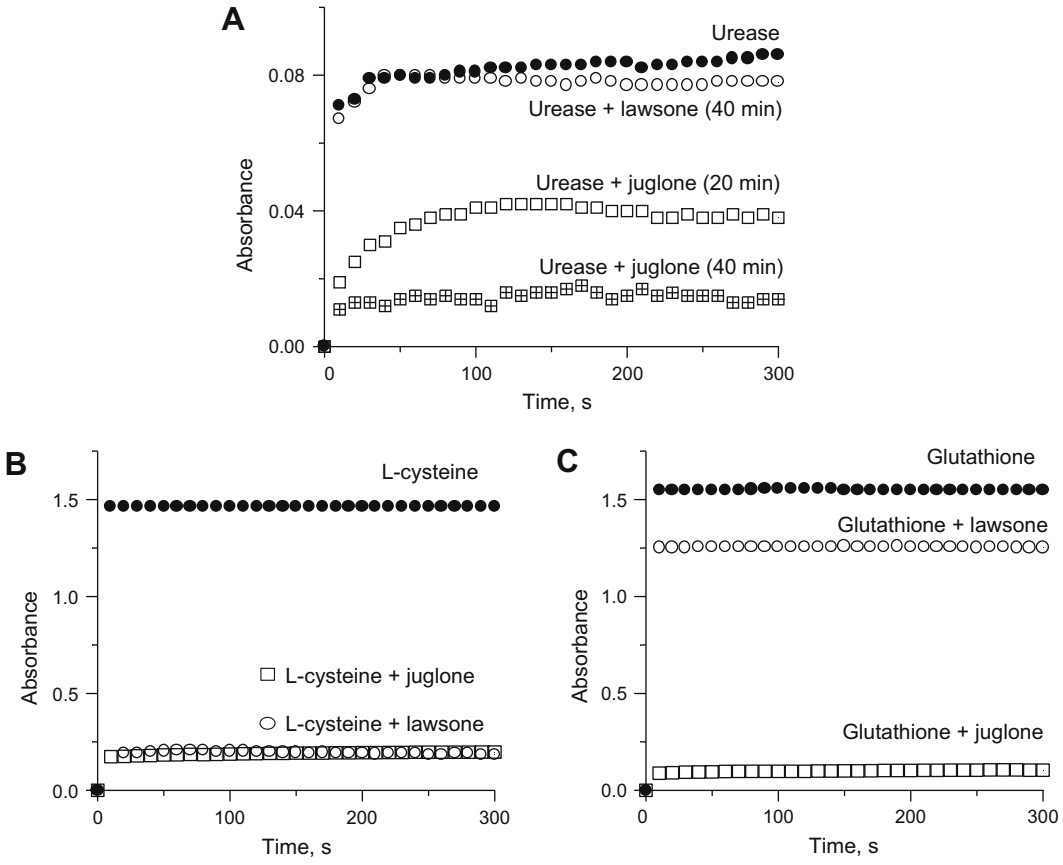


Fig. 6. Absorbance curves of DTNB reaction with thiol groups in the systems: (A) urease, urease modified by 25 μM lawsone and 25 μM juglone, respectively, after incubation (incubation times are given in brackets), (B) 0.05 mM L-cysteine, 0.05 mM L-cysteine modified by 25 μM lawsone and 25 μM juglone, respectively, after a 10 min incubation and (C) 0.05 mM glutathione, 0.05 mM glutathione modified by 25 μM lawsone and 25 μM juglone, respectively, after a 10 min incubation.

tigations of inhibition reversibility indicated double mode of urease modification by juglone. The reversible modification was attributed to ROS action on urease thiols. The presence of H_2O_2 in the system was confirmed by catalase protection influence and the direct determination of H_2O_2. However, the measured amount of H_2O_2 was estimated as not sufficient to result in such effective inhibition (Fig. 5). This suggested the presence and action of other ROS which affected urease more effectively than hydrogen peroxide.

The aim of the study was also explanation why lawsone so poorly inhibited urease. Lawsone was reported to be a non-redox cycling compound because of a low one-electron potential (−410 mV) [17]. In our experiment we observed the weak generation of hydrogen peroxide in lawsone system. However, the amount of hydrogen peroxide liberated in lawsone system was 2.5 times lower than in juglone system (Table 2). This univocally suggests weaker inhibition power of lawsone comparing to juglone. The next point of the research was explanation of lack of arylation process while lawsone and urease interaction. Determination of thiols in urease modified by juglone displayed that there was appreciable decrease of thiols accessible for thiol specific agent DTNB. On the contrary, DTNB test showed that the number of unmodified urease thiols before and after incubation with lawsone did not change. This disclosed lack of lawsone-urease interaction. Interestingly, juglone and lawsone reacted on the same level with L-cysteine but the degree of interaction with glutathione was different. Glutathione reacted significantly with juglone and much weaker with lawsone (Fig. 6). The thiol arylation of naphthoquinones results in substitution of thiol anion RS^- in position 2. This position is free in juglone quinoid ring and is accessible for arylation. In lawsone position 2 is taken by hydroxyl group which limiting the space for a new substituent. The substituent can occupy site 3, between one of quinone oxygens and hydroxyl group. Small molecules as L-cysteine easily can reach the site but bigger, with side chains like glutathione meet with hindrance. This site is totally inaccessible for protein thiols. Inactivation of urease requires modification of a critical for enzymatic activity cysteine-592, which is located on the mobile flap closing the active site of the enzyme. Localization of cysteine-592 and the size of urease molecule prevent it from binding with lawsone. On the contrary, the position of hydroxyl group in aromatic ring of juglone do not create any obstacle.

References

[1] H.L.T. Mobley, R.P. Hausinger, Microbiol. Rev. 53 (1) (1989) 85–108.
[2] A. Sirko, R. Brodzik, Acta Biochim. 47 (2000) 1189–1195.
[3] B. Krajewska, J. Mol. Catal. B 59 (2009) 9–21.
[4] S. Benini, W.R. Rypniewski, K.S. Wilson, S. Miletti, S. Ciurli, S. Mangani, Structure 7 (1999) 205–216.
[5] P.A. Karplus, M.A. Pearson, Accounts Chem. Res. 30 (1997) 330–337.
[6] R. Norris, K. Brocklehurst, Biochem. J. 159 (1976) 245–257.
[7] G. Mamiya, K. Takishima, M. Masakuni, T. Kayumi, K. Ogawa, T. Sekita, Proc. Jap. Acad. Ser. B 61 (1985) 395–398.
[8] H.L.T. Mobley, M.D. Island, R.P. Hausinger, Microbiol. Rev. 59 (1995) 451–480.
[9] L.G. Bundy, J.M. Bremner, Soil Biol. Biochem. 5 (1973) 847–853.
[10] A. Ashiralieva, D. Kleiner, FEBS Lett. 555 (2003) 367–370.
[11] W. Zaborska, B. Krajewska, M. Kot, W. Karcz, Bioorg. Chem. 35 (2007) 233–242.
[12] J.L. Bolton, M.A. Trush, T.M. Penning, G. Dryhurst, T.J. Monks, Chem. Res. Toxicol. 13 (2000) 135–160.
[13] J.L. Cape, M.K. Browman, D.M. Kramer, Phytochemistry 67 (2006) 1781–1788.
[14] P.J. O'Brien, Chem. Biol. Interact. 80 (1991) 1–41.
[15] A. Brunmark, E. Cadenas, Free Radical Biol. Med. 7 (1989) 435–477.
[16] C.E. Rodriguez, M. Shinyashiki, J. Yu, R.C. Froines, J.M. Fukuto, A.K. Cho, Toxicology 201 (2004) 185–196.
[17] N. Cenas, H. Nivinskas, Z. Anusevicius, J. Sarlauskas, F. Lederer, E.S.J. Arner, J. Biol. Chem. 279 (2004) 2583–2592.
[18] W. Knecht, J. Hanseling, M. Loffler, Chem. Biol. Interact. 124 (2000) 61–76.
[19] L. Henning, C. Christner, M. Kipping, B. Schelberg, K.P. Rücknagel, R. Grabley, G. Küllertz, G. Fischer, Biochemistry 37 (1998) 5953–5960.
[20] A. d'Arcy Doherty, A. Rodgers, G.M. Cohen, J. Appl. Toxicol. 7 (1987) 123–129.
[21] A. Kumbhar, S. Padhye, D. Ross, Biometals 9 (1996) 235–240.
[22] K. Öllinger, A. Brunmark, J. Biol. Chem. 266 (1991) 21496–21503.
[23] R. Sauriasari, D.-H. Wang, Y. Takemura, K. Tsutsui, N. Masuoka, K. Sano, M. Horita, B.-L. Wang, K. Ogino, Toxicology 235 (2007) 103–111.
[24] A.M. Osman, P.C.M. van Noort, J. Appl. Toxicol. 23 (2003) 209–212.
[25] M.W. Weatherburn, Anal. Chem. 39 (1967) 971–974.
[26] L. Deiana, C. Carru, G. Pes, B. Tadolini, Free Radical Res. 31 (1999) 237–244.
[27] G.L. Ellman, Arch. Biochem. Biophys. 82 (1959) 70–77.
[28] R. Kitz, I.B. Wilson, J. Biol. Chem. 237 (1962) 3245–3249.
[29] R.B. Silverman, Meth. Enzymol. 249 (1995) 240–283.
[30] B. Krajewska, W. Zaborska, Bioorg. Med. Chem. 15 (2007) 4144–4151.
[31] A.M. Clark, T.M. Jurgens, C.D. Hufford, Phytother. Res. 4 (2006) 11–14.
[32] M.P. Bova, M.N. Mattson, S. Vasile, D. Tam, L. Holsinger, M. Bremer, T. Hui, G. McMahon, A. Rice, J.M. Fukuto, Arch. Biochem. Biophys. 429 (2004) 30–41.