The Inhibition of Glutathione Reductase by Quinones

Daiva A. Bironaitė, Narimantas K. Čėnas, Juozas J. Kulys
Institute of Biochemistry, Lithuanian Academy of Sciences, 232600 Vilnius, Lithuania

Alexander G. Medentsev, Vasiliy K. Akimenko
Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, 142292 Pushchino, USSR

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Fully substituted quinones including some naturally occurring oxyquinones acted as inhibitors of yeast glutathione reductase (EC 1.6.4.2). They were competitive, mixed or uncompetitive inhibitors for NADPH, possessing K_i in the range of 1–200 μM and uncompetitive inhibitors for glutathione. Rhein (4,5-dioxy-9,10-anthraquinone-2-carbonic acid) and 9,10-phenanthrenequinone were the most effective inhibitors. It is concluded that certain quinones can bind to the NADP(H)-binding site and to the heteroaromatics binding site at the interface domain (P. A. Karplus, E. F. Pai, and G. E. Schulz, Eur. J. Biochem. **178,** 693–703 (1989)) of the enzyme.

Introduction

Glutathione reductase (EC 1.6.4.2) contains FAD and redox active disulfide in the active center [1–3] and catalyzes the reversible oxidation of NADPH by GSSG. This enzyme is responsible for the maintenance of sufficiently high concentrations of GSH and is one of the key enzymes in the protection against the "oxidative stress" – an increased concentration of radicals and other activated forms of oxygen [4]. Therefore its inhibitors and inactivators are often used as antitumour, antiparasitic or antimalarial agents [5–7]. It is supposed that heteroaromatic inhibitors of glutathione reductase bind to the interface domain at the site which is distinct from NADP(H) and GSSG binding sites [7, 8].

The data of present communication show that certain quinones, including the naturally occurring ones may act as potent inhibitors of glutathione reductase. This may be an additional factor of their toxicity apart from their redox cycling and reaction with cellular nucleophiles [9].

Abbreviations: GSSG, oxidized glutathione; GSH, reduced glutathione; DTT, dithiothreitol; TN, turnover number; TN/K_m, bimolecular rate constant.

Reprint requests to N. K. Čėnas.

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Materials and Methods

Yeast glutathione reductase (Sigma) was additionally purified up to $A_{280}/A_{460} = 7.7–7.9$ [1]. The enzyme concentration was determined using $\varepsilon_{460} = 11\ mM^{-1}cm^{-1}$. NADPH, $NADP^+$ and DTT (Serva), GSSG and GSH (Chemapol, Czechoslovakia), rhein (4,5-dihydroxy-9,10-anthraquinone-2-carbonic acid, Aldrich), duroquinone (Sigma) were used as received. Alizarin (2,3-dihydroxy-9,10-anthraquinone), 9,10-anthraquinone-2-sulphonate, 1,5-dioxy-9,10-anthraquinone and 9,10-phenanthrenequinone (Reakhim, USSR) were recrystallized from ethanol or benzene. Chrisophanol (2-methyl-4,5-dihydroxy-9,10-anthraquinone) was extracted from the cultural fluid of *Verticillium dahliae,* javanicin (2-methoxymethylene-3-methyl-5,8-dioxy-7-methoxy-1,4-naphtoquinone) – from *Fusarium decemcellulare,* and pigment G-2N (2-methyl-4,7,9,12-tetraoxy-5,6-dihydrobenz[a]-naphtacene-8,13-dione) – from *Frankia* sp. ANP 190107 as described earlier [10, 11]. The structural formulae of some uncommonly used quinones are presented in Fig. 1. The reaction rate was determined using a Hitachi-557 spectrophotometer according to the NADPH absorption change ($\Delta\varepsilon_{340} = 6.2\ mM^{-1}cm^{-1}$) at 25 °C in 0.1 M K-phosphate-citrate, pH 7.0, containing 1 mM EDTA. The enzymatic GSH-linked reduction of $NADP^+$ was done in the presence of 10 mM DTT [12]. TN of the enzyme is a number of NADP(H) consumed by FAD per 1 s.

Results and Discussion

By analogy to previous data [1, 2], the GSSG-linked oxidation of NADPH by glutathione reductase follows a "ping-pong" mechanism. TN_{max} of the enzyme (at saturating concentration of both substrates) at pH 7.0 is 240 s^{-1}.

Since the partially substituted quinones may react with GSH formed during the reaction of glutathione reductase [9], only the fully substituted compounds were tested. It was found that rhein was potent inhibitor of glutathione reductase, acting as the mixed-type inhibitor for NADPH (Fig. 2). The inhibition is not linear according to the in-

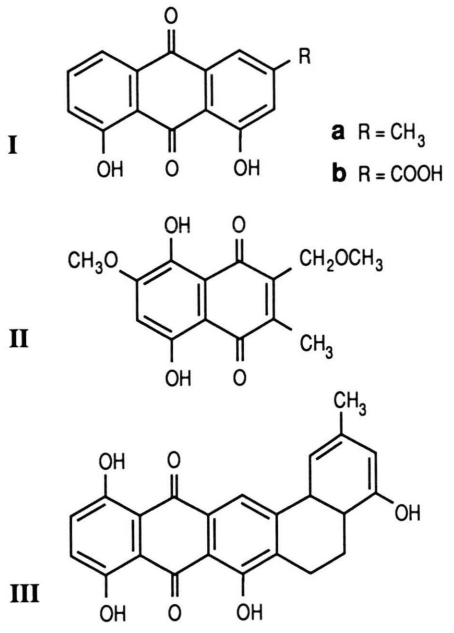

Fig. 1. Structural formulae of quinones used: chrisophanol (**Ia**), rhein (**Ib**), javanicin (**II**), G-2 N (**III**).

tercepts (reciprocal maximal rates of reaction). The double reciprocal plot of inhibition degree *vs.* inhibitor concentration [6] gives K_i = 1.1 μM and the maximal degree of inhibition, 93%. The inhibition of rhein is linear according to the slopes (reciprocal TN/K_m of NADPH) giving K_i = 8 μM. Javanicin was another mixed-type inhibitor possessing K_i of 70 μM and 100 μM (according to intercepts and slopes). Other quinones were either linear competitive inhibitors (alizarin, K_i = 20 μM; chrisophanol, K_i = 34 μM; G-2 N, K_i = 40 μM; 1,5-dioxyanthraquinone, K_i = 70 μM), either uncompetitive inhibitors for NADPH (duroquinone, K_i = 200 μM; anthraquinone sulfonate, K_i = 50 μM; phenanthrene quinone, K_i = 1.8 μm, maximal inhibition degree, 73%). Since phenanthrene quinone was the oxidant of glutathione reductase (TN_{max} = 5 s^{-1}, TN/K_m = 10^4 $M^{-1}s^{-1}$) the inhibition patterns were obtained after the substraction of rates of this reaction. All quinones tested were the uncompetitive inhibitors for GSSG.

The reverse reaction of glutathione reductase – GSH-linked reduction of $NADP^+$ is characterized by a transition from "ping-pong" to "sequential" mechanism at high concentrations of $NADP^+$ [12]. This is indicated by the transition of a series of parallel lines to the converging ones in the Lineweaver-Burk plots (Fig. 3). In this case rhein de-

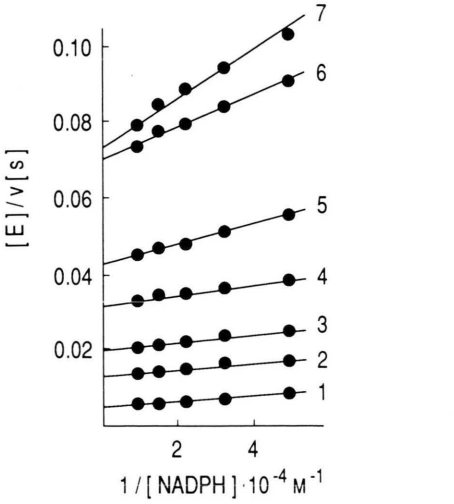

Fig. 2. The influence of rhein on the forward reaction of glutathione reductase. Concentration of rhein: 0 (1), 2 μM (2), 5 μM (3), 10 μM (4), 20 μM (5), 35 μM (6) and 55 μM (7); concentration of GSSG: 300 μM.

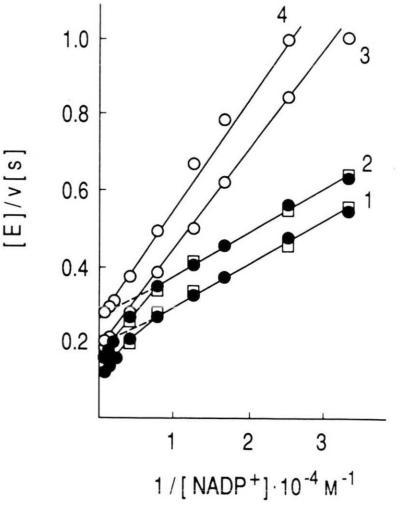

Fig. 3. The influence of rhein on the reverse reaction of glutathione reductase. Concentration of rhein: 0 (1, 2), 20 μM (3, 4); concentration of GSH: 30 mM (1, 3), 10 mM (2, 4). The data in the absence and in the presence of 50 μM of anthraquinone sulfonate are marked by symbols (●) and (□), respectively, on curves 1 and 2.

creases TN/K_m of $NADP^+$ and does not affect TN_{max}, while anthraquinone sulfonate almost does not influence the reaction kinetics.

The data presented indicate that certain quinones (rhein, phenanthrene quinone) are potent inhibitors of glutathione reductase, similar to certain flavin derivatives, used as antimalarial agents [7]. Thus the additional factor of influence of quinones on the system of glutathione metabolism is demonstrated, which may attribute to their toxicity to some extent. This is especially interesting for naturally occurring oxyquinones which activity is accociated with the inhibition of mitochondrial electron transport chain (rhein, G-2N) [11, 13] or redox cycling (javanicin) [10]. It seems that quinones being uncompetitive inhibitors for NADPH bind to the interface domain of enzyme which is distinct from NADP(H) and GSSG-binding sites [8]. Such a character of binding is proposed for flavins and nitrofurans [6, 7], which inhibit glutathione reductase in a similar way. Evidently, oxyquinones being competitive inhibitors for NADPH bind at its binding site in oxidized glutathione reductase. It is known that certain oxyquinones bind to NAD(P)H-binding centers of flavoproteins [13, 14]. The mixed character of inhibition of rhein (Fig. 2) and javanicin may be explained by their binding both at the NADP(H)-binding site and at the interface domain. The binding of rhein to the NADP(H)-binding site is evident from data of Fig. 3. The reasons for different specificity of rhein comparing to other oxyquinones remain unclear. It is possible that 2-carboxyl group of rhein takes a definite part in the complexation, since an analoguous 2-methyl-substituted compound, chrisophanol (Fig. 1) inhibits glutathione reductase in a different way.

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