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One- and two-electron reduction of quinones by glutathione reductase

Narimantas K. Čénas, Gelmé A. Rakauskiené and Juozas J. Kulys

Institute of Biochemistry, Lithuanian Academy of Sciences, Vilnius (U.S.S.R.)

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Yeast glutathione reductase (E.C. 1.6.4.2) catalyzes the oxidation of NADPH by *p*-quinones and ferricyanide with a maximal turnover number (TN_{max}) of 4–5 s^{-1}. $NADP^+$ stimulates the reaction and the TN_{max}/K_m value of acceptors is reached at $NADP^+/NADPH \geqslant 100$. TN_{max} is increased up to 30–33 s^{-1}. The stimulatory effect of $NADP^+$ may be associated with its complexation with the NADPH-binding site in the reduced enzyme ($K_d = 40$–60 μM). It is suggested that $NADP^+$ shifts the electron density towards FAD in the two-electron-reduced enzyme and, evidently, changes its one-electron-reduction potentials, while quinones oxidize an equilibrium form of glutathione reductase containing reduced FAD. In the absence of $NADP^+$ the reduction of quinones by glutathione reductase proceeds mainly in a two-electron manner. At $NADP^+/NADPH = 100$ a one-electron reduction makes up 44% of the total process. At pH 6.0–7.0 the reduced forms of naphthoquinones undergo cyclic redox conversions. A hyperbolic dependence exists of the log TN/K_m of quinones on their one-electron-reduction potentials.

Introduction

Glutathione reductase (E.C. 1.6.4.2) catalyzes the oxidation of NADPH by glutathione and contains FAD and redox-active disulfide in the active centre [1–6]. During the reaction, NADPH reduces FAD which transfers the electrons to disulfide. A two-electron-reduced enzyme, containing the charge-transfer complex of FAD and reduced disulfide is further oxidized by glutathione [1–6]. The steady-state kinetics of glutathione reductase is described by a 'hybrid ping-pong' scheme [3] and the reductive half-reaction is a rate-limiting step [5]. According to the X-ray data, the NADPH and glutathione binding sites are separated by the isoalloxazine ring of FAD [7]. It was found that the two-electron-reduced enzyme, oxidized by glutathione, contains tightly bound NADPH repeatedly attached to the active centre [3].

According to the classification of Hemmerich and Massey [8] glutathione reductase is characterized as flavin dehydrogenase-transhydrogenase and catalyzes a one-step transfer of two redox equivalents to its physiological oxidizer. However, one-electron reactions are also characteristic of glutathione reductase. The enzyme is efficiently by the cation radical of methyl viologen [9] and oxidized by ferricyanide [10,11]. Recently, it has been found that glutathione reductase reduces 2,4,6-trinitrobenzenesulfonate (TNBS) in a one-electron manner [12]. Contrary to glutathione reduction, the reactions involving artificial oxidizers are stimulated by $NADP^+$ [12].

Quinones are widely used as substrates in the study of flavoprotein redox reactions. With the use of quinones Yamazaki et al. [13–15] estimated a quantitative relationship between one- and two-electron transfers in the reactions of NADPH oxidizing flavoenzymes. Among the advantages of quinones, the well-characterized thermodynamics of their one- and two-electron reduction [16,17] may be mentioned, as well as the applicability of the outer spherical electron transfer model to their redox reactions [16,18,19]. Another major consideration when studying the reactions of quinones with flavoproteins is the toxicity of quinoidal xenobiotics – the products of pollution [20] and various drugs [21,22], caused by their one-electron reduction with low-potential dehydrogenases. Semiquinones, formed in this reaction, undergo cyclic redox conversion leading to some 'oxidative stress' – the formation of superoxide and hydroxyl radicals.

Abbreviations: TNBS, 2,4,6-trinitrobenzenesulfonate; TN, turnover number.

Correspondence: N.K. Čénas, Institute of Biochemistry, Lithuanian Academy of Sciences, Vilnius, U.S.S.R.

The aim of the present work, was to study the relationship between one- and two-electron transfers in the oxidation of glutathione reductase by *p*-quinones and to elucidate the role of $NADP^+$ as effector in these reactions.

Materials and Methods

Yeast glutathione reductase (Sigma, grade III) was additionally purified according to the method of Massey and Williams [2]. The enzyme had the ratio $A_{280}/A_{460} = 7.7–7.9$. The concentration of the enzyme was determined spectrophotometrically with the use of $\epsilon_{460} = 11.3\ mM^{-1} \cdot cm^{-1}$. Cytochrome *c* from horse heart (Serva), NADPH, $NADP^+$ and NADH (Boehringer), tetramethyl-1,4-benzoquinone (Sigma), 2,5-dimethyl-1,4-benzoquinone (Aldrich), 5,8-dioxy-1,4-naphthoquinone (Fluka AG) and glutathione (Chemapol, Czechoslovakia) were used as received. 1,4-Benzoquinone, 2-methyl-1,4-benzoquinone, 1,4-naphthoquinone and 2-methyl-1,4-naphthoquinone (Reachim, U.S.S.R.) were purified by sublimation in vacuum or were recrystallized twice from benzol or ethanol. Potassium ferricyanide (Reachim) was recrystallized from water.

The reaction rate was determined by the decrease in the NADPH absorption at 340 nm. NADPH concentration was 10–150 μM and that of glutathione was 20–500 μM, of quinone and ferricyanide was 20–150 μM, and of enzyme was 1–500 nM. The benzosemiquinone-mediated cytochrome *c* reduction rate during the enzymatic oxidation of NADPH by benzoquinone was monitored at 550 nm ($\Delta\epsilon_{550} = 20\ mM^{-1} \cdot cm^{-1}$). The concentration of cytochrome *c* was 35–40 μM, the rate of NADPH oxidation did not exceed 0.3 $\mu M \cdot s^{-1}$. An Hitachi-557 spectrophotometer was used. Measurements were made under aerobic conditions at $25 \pm 0.1°C$ in 0.1 M potassium-phosphate-citrate buffer solutions (pH 5.0–8.0) prepared from a 0.1 M solution of K_2HPO_4 and citric acid containing 1 mM EDTA.

The steady-state kinetic parameters of the reactions (K_m, turnover number (TN) and bimolecular rate constants (TN/K_m), were determined using Lineweaver-Burk coordinates at constant concentrations of one substrate and changing another one. The maximal turnover number (TN_{max}) was estimated from the dependence of TN on fixed substrate concentrations following the method of Cornish-Bowden [23]. TN corresponds to the number of NADPH oxidized by FAD per 1 s.

Results

$NADP^+$ as effector of diaphorase reactions

At pH 7.0 the oxidation of NADPH by glutathione, catalyzed by glutathione reductase, follows a 'ping-pong' mechanism, as judged from the series of parallel lines in Lineweaver-Burk coordinates. The TN_{max} value makes up 240 s^{-1} and TN/K_m of NADPH is $2 \cdot 10^7\ M^{-1} \cdot s^{-1}$. $NADP^+$ acts as a competitive inhibitor for NADPH ($K_i = 60\ \mu M$) and is uncompetitive for glutathione.

A study of diaphorase reactions of glutathione reductase revealed that at fixed NADPH concentrations and variable concentrations of a nonphysiological oxidizer the data on the initial rates linearize well in Lineweaver-Burk coordinates (Fig. 1). The TN value of the reaction does not depend on the NADPH concentration (10–150 μM) and is equal to 4–5 s^{-1} for the

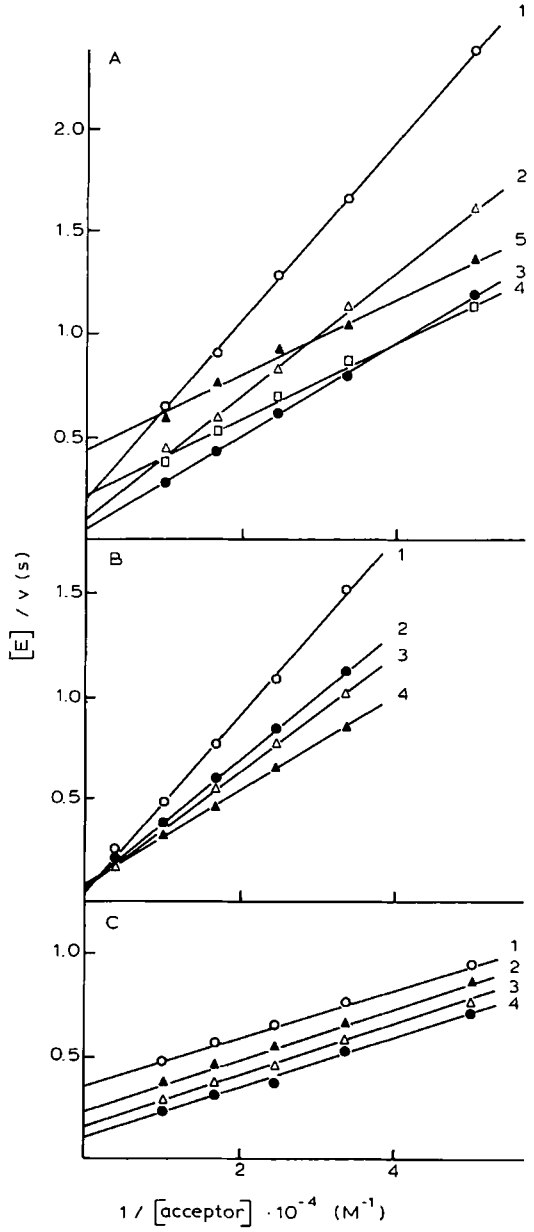


Fig. 1. The effect of $NADP^+$ on the diaphorase activity of glutathione reductase at pH 7.0. (A) NADPH concentration, 10 μM, $NADP^+$, 0 (1), 0.2 (2), 0.3 (3), 2.0 (4) and 4.0 mM (5); electron acceptor, 1,4-benzoquinone. (B) $NADP^+$ concentration, 100 μM; NADPH, 31 (1), 19 (2), 14 (3) and 10 μM (4); electron acceptor, ferricyanide. (C) $NADP^+$ concentration, 4.0 mM; NADPH, 10 (1), 15 (2), 24 (3) and 35 μM (4); electron acceptor, ferricyanide.

TABLE I

The relation between TN/K_m of glutathione reductase nonphysiological electron acceptors and their one- (E_7^1) and two-electron (E_7^0) reduction potentials (standard) and 'hydride-transfer potentials' ($E_7^{H^-}$) at pH 7.0

Acceptor	TN/K_m ($M^{-1} \cdot s^{-1}$)		E_7^1, V [16,17]	E_7^0, V [16,30]	$E_7^{H^-}$, V [c] [31–33]
	$NADP^+ = 0$	$NADP^+/NADPH = 100$			
1,4-Benzoquinone	$2.4 \cdot 10^4$	$5.8 \cdot 10^4$	0.09	0.29	0.19
2-Methyl-1,4-benzoquinone	$4.0 \cdot 10^3$	$1.6 \cdot 10^4$	0.01	0.21	0.13
5,8-Dioxy-1,4-naphthoquinone	– [a]	$2.7 \cdot 10^4$	−0.11	−0.06	−0.12
1,4-Naphthoquinone	$1.05 \cdot 10^3$	$2.8 \cdot 10^3$	−0.15	0.04	−0.03
2,5-Dimethyl-1,4-benzoquinone	$1.5 \cdot 10^3$	$3.0 \cdot 10^3$	−0.08	0.16	0.07
2-Methyl-1,4-naphthoquinone	$3.6 \cdot 10^2$	$6.1 \cdot 10^2$	−0.20	−0.03	−0.13
Tetramethyl-1,4-benzoquinone	– [b]	$8.5 \cdot 10$	−0.26	0.04	−0.08
Ferricyanide	$1.1 \cdot 10^4$	$7.8 \cdot 10^4$	0.41	–	–

[a] Not determined due to high activation by $NADP^+$ formed.
[b] Not determined due to low reaction rate.
[c] Determined according to $E_7^{H^-} = E_7^0 - 0.03$ ($pK_{QH_2/QH^-} - 7$), when $pK_{QH_2/QH^-} > 7.0$ [31].

most active compounds oxidizing glutathione reductase – 1,4-benzoquinone, ferricyanide, 2-methyl-1,4-benzoquinone. TN/K_m of oxidizers presented in Table I do not depend on the NADPH concentration. The TN/K_m values for 1,4-benzoquinone and 2-methyl-1,4-benzoquinone do not change when NADH is used as substrate. In this case, TN of the reaction is 0.3 s^{-1} when 50 μM of NADH is used.

$NADP^+$ increases the rate of the diaphorase reaction, but the reaction parameters show a complex dependence on the $NADP^+$ and NADPH concentrations. With fixed NADPH concentrations, an increase in the $NADP^+$ concentration up to 0.5–0.8 mM increases the TN and TN/K_m values. However, a further increase in the $NADP^+$ concentration decreases TN (Fig. 1A). An increase in the NADPH concentration at low fixed concentrations of $NADP^+$ (20–500 μM) leads to a negligible increase of TN and decrease of TN/K_m (Fig. 1B). However, at millimolar concentrations of $NADP^+$, the NADPH concentration increase shows almost no effect on the TN/K_m value, and the data are presented as a series of parallel lines in Lineweaver-Burk coordinates (Fig. 1C). Thus, TN/K_m for electron acceptors acquires a constant value both in the absence of $NADP^+$ and at a high $NADP^+$/NADPH ratio. NADPH inhibition, with respect to an acceptor, takes place at intermediate concentrations of $NADP^+$. As follows from Fig. 2, the maximal TN/K_m value for ferricyanide and benzoquinone are observed when $NADP^+/NADPH = 100$ and are further determined merely by this ratio ($NADP^+$ concentration 10 μM to 4 mM, NADPH concentration 10–150 μM). TN_{max} of the diaphorase reaction at pH 7.0, determined in the presence of 4 mM

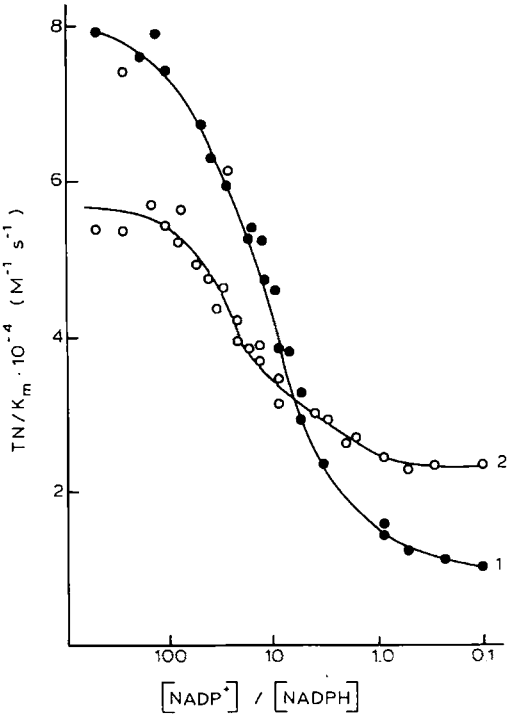


Fig. 2. The dependence of TN/K_m of ferricyanide (1) and 1,4-benzoquinone (2) on the $NADP^+$/NADPH ratio at pH 7.0.

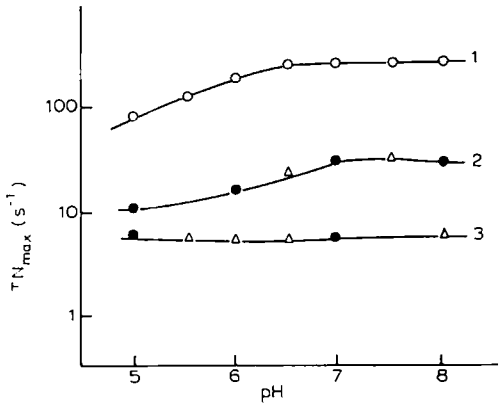


Fig. 3. The pH profile of glutathione reductase (1) and diaphorase reactions (2, 3). $NADP^+$ is absent in curves 1 and 3. The $NADP^+$ concentration was 4 mM (2). The electron acceptors were ferricyanide (△) and 1,4-benzoquinone (●).

$NADP^+$ and 10–40 μM NADPH is 30–33 s^{-1} and coincides for ferricyanide, 1,4-benzoquinone, 2-methyl-1,4-benzoquinone. The TN/K_m values for electron acceptors, determined at $NADP^+/NADPH = 100$, are presented in Table I. In the presence of 4 mM $NADP^+$, the TN/K_m value of NADPH in the diaphorase reaction is $2.8 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ that is close to the values of the same parameter of the NADPH: glutathione reductase reaction determined at the same $NADP^+$ concentration. A decrease in pH decreases the stimulatory effect of $NADP^+$ in the diaphorase reaction (Fig. 3).

Relationship between one- and two-electron transfer in the reduction of quinones

When the $NADP^+$/NADPH ratio exceeds 10, naphthoquinones, reduced by glutathione reductase, undergo cyclic redox conversions in an aerobic medium at pH 6.0–7.0. This is evident from the amount of oxidized NADPH which greatly exceeds the initial amount of naphthoquinone. This shows that napthoquinones are reduced in a one-electron way, since at pH 6.0 semiquinones formed in this reaction are reoxidized rapidly by oxigen and futher take part in cyclic conversions [24]. In contrast to this, the oxidation of naphthohydroquinones under the same conditions is rather slow [24].

A quantitative relation between one- and two-electron transfers has been estimated by the method of Yamazaki et al. [13–15]. It is based on the fact that at pH < 7.2 the rate of cytochrome *c* reduction by benzohydroquinone is negligible and benzosemiquinone formed in a one-electron process reduces cytochrome *c* at a high rate ($k = 1.5 \cdot 10^6$ $M^{-1} \cdot s^{-1}$). The reaction is not related to the formation of superoxide due to a high redox potential of benzosemiquinone [17]. As is judged from the experiments with DT-diaphorase, reducing quinones in a two-electron way [14], the reaction between benzoquinone and benzohydroquinone formed is not responsible for cytochrome *c* reduction, since the equilibrium constant of semiquinone formation is too low. So, at high concentrations of cytochrome *c* and low rates of NADPH oxidation, the percentage of one-electron flux is expressed as the ratio between the rate of cytochrome redution and the double rate of NADPH oxidation in the presence of benzoquinone.

At pH 6.5 in the absence of $NADP^+$, a one-electron transfer comprises up merely 3.6% of an overall reduction rate of benzoquinone (Fig. 4A). The efficiency of the process does not depend on the benzoquinone concentration and does not change when NADH is used as substrate. However, when $NADP^+/NADPH = 100$ a one-electron process comprises 44% of the total rate (Fig. 4A). The reaction proceeds without any lag period. It was found that under the conditions mentioned in the absence of benzoquinone, glutathione reductase does not reduce cytochrome *c*. The general pattern of change

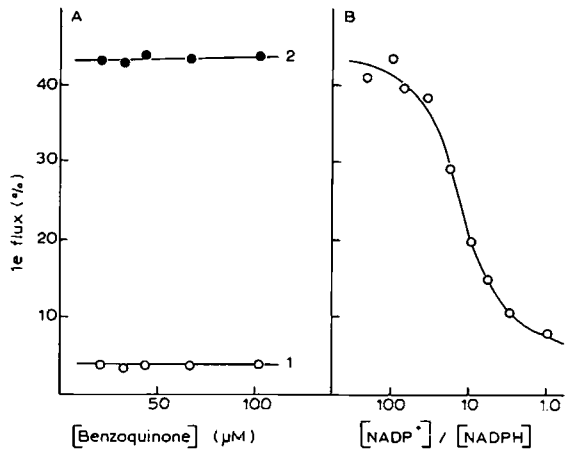


Fig. 4. One- and two-electron reduction of 1,4-benzoquinone by glutathione reductase at pH 6.5. (A) The dependence of a one-electron flux on the benzoquinone concentration in the absence of $NADP^+$ (1) and at $NADP^+/NADPH = 100$ (2). (B) The dependence of the efficiency of one-electron transfer on the $NADP^+$/NADPH ratio. The NADPH concentration was 30 μM.

in the efficiency of the process with respect to the $NADP^+$/NADPH ratio (Fig. 4B) is similar to the data of Fig. 2.

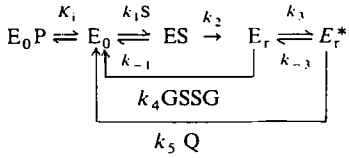
Discussion

The data presented here show that the reduction of quinones and ferricyanide by yeast glutathione reductase proceeds in a similar way as the TNBS reduction by the enzyme from erythrocytes [12]. The reaction is stimulated by $NADP^+$, and the efficiency of activation increases with an increase in pH (Figs. 1–3). It is possible that some existing differences may be determined by structural features of TNBS , which binds specifically to glutathione reductase [26]. In our case, the reaction rate did not depend sigmoidally on the oxidizer concentration and was not inhibited by NADPH in the absence of $NADP^+$ which is characteristic of the TNBS reduction [12]. This enables the proposition of a less sophisticated mechanism of the reduction of nonphysiological electron acceptors by glutathione reductase.

Diaphorase reactions of glutathione reductase are associated with its flavin cofactor, since no change or an increase in diaphorase activity is observed when the glutathione reduction activity is lost after modification of catalytic -SH groups [11,25]. Sometimes, an increase in diaphorase activity correlates with a disappearance of the FAD and thiolate charge transfer complex and a complete reduction of FAD by NADPH [2]. However, the participation of a fully reduced native enzyme in the diaphorase reaction is unlikely, since the super reduction is slow and is efficiently prevented by $NADP^+$ [2]. For this reason it is necessary to study the properties of various forms of the two-electron-reduced enzyme.

The reduction of glutathione reductase by an excess of NADPH produces a complex of the reduced enzyme and repeatedly bound NADPH ($K_d = 2.1\ \mu M$ [3]). Since the NADPH concentrations used markedly exceed this K_d value, one can conclude that TN/K_m of an oxidizer in the absence of $NADP^+$ corresponds to the oxidation of the enzyme-NADPH complex. The data from Figs. 1 and 2 indicate that $NADP^+$ converts glutathione reductase into a more reactive form competing for a NADPH binding site. It is known that $NADP^+$ at high concentrations can replace NADPH in the complex with the reduced enzyme [3]. The complex formed possesses a lower absorption coefficient at 460 nm which indicates increased FAD reduction [1,3]. Thus, $NADP^+$ activation may be determined by a shift of the electron density towards FAD in the two-electron-reduced enzyme. Since half the maximal activation of the acceptor is observed at $NADP^+/NADPH = 20–30$ (Fig. 2), the K_d value of the complex formed must be 40–60 μM. When $NADP^+/NADPH \geqslant 100$, TN/K_m of the acceptor must correspond to the oxidation of the enzyme-$NADP^+$ complex. A decrease in TN at high $NADP^+$ concentrations is due to $NADP^+$ competition with respect to the NADPH binding site in the oxidized enzyme (Fig. 1) [3].

The data given can be expressed approximately by a simple 'ping-pong' scheme where glutathione (GSSG) and a nonphysiological oxidizer (Q) oxidize various equilibrium forms of the reduced glutathione reductase (E_r and E_r^*), whereas $NADP^+$ and NADPH do not compete for the binding sites of oxidizers, but affect the equilibrium constant between the two forms of the reduced enzyme (k_3/k_{-3})



(1)

where E_0, E_0P and ES are the oxidized enzyme, tis complex with $NADP^+$ and the enzyme-substrate complex, S and P are NADPH and $NADP^+$ and K_i is the $NADP^+$ binding constant to the oxidized enzyme. The glutathione reduction rate (V_1) is expressed in the following way

$$\frac{[E]}{V_1} = \frac{1}{k_2} + \frac{k_{-1}+k_2}{k_1 k_2 [S]}\left(1+\frac{[P]}{K_i}\right) + \frac{1}{k_4[GSSG]}\left(1+\frac{k_3}{k_{-3}}\right) \quad (2)$$

and the reduction rate of the acceptor (V_2)

$$\frac{[E]}{V_2} = \frac{1}{k_2} + \frac{1}{k_3} + \frac{k_{-1}+k_2}{k_1 k_2 [S]}\left(1+\frac{[P]}{K_i}\right) + \frac{1}{k_5[Q]}\left(1+\frac{k_{-3}}{k_3}\right) \quad (3)$$

As follows from Eqns. 2 and 3, the TN/K_m value for NADPH is identical both for glutathione reductase and diaphorase activities (experimentally observed at high $NADP^+$ concentrations) and the diaphorase reaction proceeds according to a formal 'ping-pong' scheme (Fig. 1C) with a limiting step, k_3. This seems reasonable, since TN_{max} of the diaphorase reaction does not depend on the nature of the electron acceptor (Fig. 3). An increase in the TN_{max} and TN/K_m values of the electron acceptors caused by $NADP^+$ should be attributed to the ratio k_3/k_{-3} increase (Eqn. 3). Evidently, the distribution of electrons in the active centre with bound NADPH differs insignificantly from that of a free state, since the use of NADH, having a low affinity for reduced enzyme [3], does not change the TN/K_m value of quinones. A decrease in TN/K_m of glutathione with increasing k_3 (Eqn. 2) must be negligible, since $k_3/k_{-3} \ll 1$ (the content of reduced FAD in the two-electron-reduced enzyme is low [27]). Therefore, the model agrees satisfactorily with the data that $NADP^+$ does not influence the rate constant of glutathione reductase oxidation with glutathione [1].

The data presented here show that glutathione reductase reduces quinones according to a mixed mechanism. In the absence of $NADP^+$, the efficiency of one-electron reduction is low and it is by the order lower than the efficiency of one-electron transfer of lipoamide dehydrogenase, possessing an analogous catalytic mechanism (Fig. 4) [15]. Therefore, the efficiency of the two-electron reduction of quinones by glutathione reductase is close to that of DT-diaphorase [14], but the observed rates are too slow to be physiologically important.

The data of the Table I indicate that at $NADP^+/NADPH = 100$ the the log TN/K_m of quinones exhibits a hyperbolic dependence on their one-electron-reduction potentials (E_7^1) (Fig. 5A) and no correlations exist between reactivity and two-electron (standard) potential (E_7^0) or 'hydride-transfer potential' (E_7^{H-}) [31], reflecting the energetics of a Q/QH^- couple. Since when $NADP^+/NADPH = 100$, a one-electron process makes up a significant part of the total reaction rate, it is possible to conclude that the data indicate the applicability of the outer-spherical electron-transfer model to the reactions of quinones [18,28,29]. The limiting value of TN/K_m reaching $10^5\ M^{-1} \cdot s^{-1}$ (Fig. 5A) does not reveal such a low level of diffusion-controlled rate, since the quinone TN/K_m values determined experimentally are $(1 + k_{-3}/k_3)$-fold lower (Eqn. 3) than that of actual flavin oxidation. The relation between reactivity and E_7^1 is observed also in the absence of $NADP^+$ when the reaction follows mainly a two-electron mechanism (Fig. 5B). However, according to these data one cannot propose an unequivocal mechanism for two-electron (hydride) reduction of quinones, since the data employing E_7^0 or E_7^{H-} are less scattered than in Fig. 5A. A one-electron step in the two-electron reduction of quinones should not be excluded, since the relation

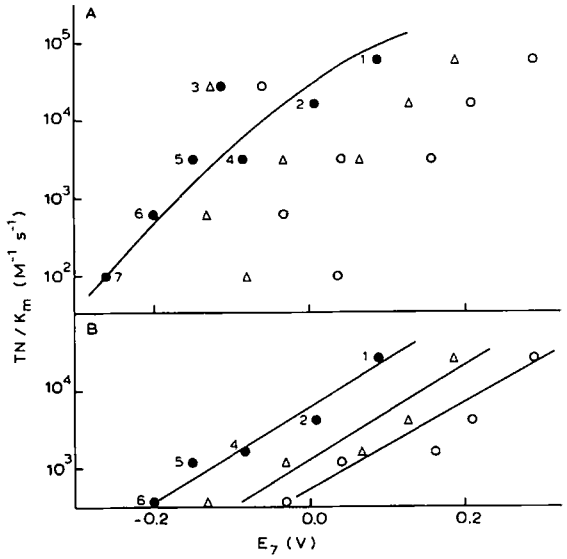


Fig. 5. The relation between log TN/K_m of quinones and their redox potentials E_7^1 (●), E_7^0 (○) and $E_7^{H^-}$ (△) at $NADP^+/NADPH = 100$ (A) and in the absence of $NADP^+$ (B). 1,4-Benzoquinone (1), 2-methyl-1,4-benzoquinone (2), 5,8-dioxy-1,4-naphthoquinone (3), 2,5-dimethyl-1,4-benzoquinone (4), 1,4-naphthoquinone (5), 2-methyl-1,4-naphthoquinone (6) and tetramethyl-1,4-benzoquinone (7).

oxidation of NADH analogues by quinones [30], where biradical intermediates of reaction are detected and a three-step hydride transfer (electron-proton-electron) is most probable. Thus, one can suppose that the quinone reduction by glutathione reductase in the absence and in the presence of $NADP^+$ may posses a common electron-transfer step. Evidently, $NADP^+$ bound to a reduced enzyme may change the potentials of the one-electron reduction of FAD and affect the rates of the subsequent processes – the transfer of a proton and second electron. The effect of the reaction product on the one-electron reduction potentials of flavins is well known for NADH: cytochrome b_5 reductase [34] and flavocytochrome b_2 [35]. Studies of glutathione reductase show that the reduced enzyme-$NADP^+$ complex undergoes a gradual conversion to a product, characterized as the complex of $NADP^+$ and anionic flavinsemiquinone possessing the 410 nm absorption band [1]. It would be interesting to determine whether cytochrome *c* accepts electrons from bound or free benzosemiquinone. However, at present it seems hardly possible in this case.

References

1 Williams, C.H., Jr. (1976) in The Enzymes (Boyer, P.D., ed.) 3rd Edn., Vol. 13, pp. 89–173, Academic Press, New York.
2 Massey, V. and Williams, C.H., Jr. (1965) J. Biol. Chem. 240, 4470–4480.
3 Bulger, J.E. and Brandt, K.G. (1971) J. Biol. Chem. 246, 5578–5587.
4 Colman, R.F. and Black, S. (1965) J. Biol. Chem. 240, 1796–1803.
5 Huber, P.W. and Brandt, K.G. (1980) Biochemistry 19, 4568–4575.
6 Arscott, L.D., Thorpe, C. and Williams, C.H., Jr. (1981) Biochemistry 20, 1513–1520.
7 Pai, E.F. and Schulz, G.E. (1983) J. Biol. Chem. 258, 1752–1757.
8 Hemmerich, P. and Massey, V. (1979) in Proceedings of the Third International Symposium on Oxidases and Related Redox Systems (King, T.E., ed.), pp. 1–13, Albany, New York.
9 Llobel, A., Fernandez, V.M. and Lopez-Barea, A. (1986) Arch. Biochem. Biophys. 250, 378–381.
10 Dubler, R.E. and Anderson, B.M. (1981) Biochim. Biophys. Acta 659, 70–85.
11 Tsai, C.S., Godin, J.R.P. and Tsai, Y.H. (1984) in Flavins and Flavoproteins (Bray, R.C., Engel, P.C. and Mayhew, S.G., eds.), pp. 505–508, Walter de Gruyter, Berlin.
12 Carlberg, I. and Mannervik, B. (1986) J. Biol. Chem. 261, 1629–1635.
13 Lyanagi, T. and Yamazaki, Y. (1969) Biochim. Biophys. Acta 172, 370–381.
14 Iyanagi, T. and Yamazaki. Y. (1970) Biochim. Biophys. Acta 216, 284–294.
15 Nakamura, M. and Yamazaki, Y. (1972) Biochim. Biophys. Acta 267, 249–257.
16 Rich, P.R. and Bendall, D.S. (1980) Biochim. Biophys. Acta 592, 506–518.
17 Swallow, A.J. (1982) in Functions of Quinones in Energy Conserving Systems (Trumpower, B.L., ed.), pp. 59–72, Academic Press, New York, San Francisco, London.
18 Marcus, R.A. and Sutin, N. (1985) Biochim. Biophys. Acta 811, 265–322.
19 Grampp, G. and Jaenicke, W. (1987) J. Electroanal. Chem. 229, 297–303.
20 Chesis, P.L., Levin, D.E., Smith, M.T., Ernster, L. and Ames, B.N. (1984) Proc. Natl. Acad. Sci USA 81, 1696–1700.
21 Thayer, W.S. (1977) Chem. Biol. Interact. 19, 265–278.
22 Davies, K.J. and Doroshow, J.H. (1986) J. Biol. Chem. 261, 3060–3067.
23 Eisenthal, R. and Cornish-Bowden, A. (1974) Biochem. J. 139, 715–720.
24 Lind, C., Hochstein, P. and Ernster, L. (1982) Arch. Biochem. Biophys. 216, 178–185.
25 Pinto, M.C., Mata, A.M. and Lopez-Barea, J. (1985) Eur. J. Biochem. 151, 275–281.
26 Carlberg, I. and Mannervik, B. (1979) FEBS Lett. 98, 263–266.
27 Lively, C.R. and McFarland, J.T. (1986) Biochem. Biophys. Res. Commu. 136, 22–29.
28 Meyer, T.E., Watkins, J.A., Przysiecki, C.T., Tollin, G. and Cusanovich, M.A. (1984) Biochemistry 23, 4761–4767.
29 Čénas, N.K., Pocius, A.K., Butkus, A.A., Kulys, J.J. and Antanavičius, V.S. (1986) Biokhimyia 51, 285–292.
30 Fukuzumi, S., Koumitsu, S., Hironaka, K. and Tanaka, T. (1987) J. Am. Chem. Soc. 109, 305–316.
31 Carlson, B.W. and Miller, L.L. (1985) J. Am. Chem. Soc. 107, 479–485.
32 Nagaoka, T., Sakai, T., Ogura, K. and Yoshino, Y. (1987) J. Chem. Soc. Faraday Trans. I 83, 1823–1833.
33 Bailey, S.I. and Ritchie, I.M. (1985) Electrochim. Acta 30, 3–12.
34 Iyanagi, T., Watanabe, S. and Anan, K.F. (1984) Biochemistry 23, 1418–1425.
35 Tegonni, M., Janot, J.M. and Labeyrie, F. (1984) Biochimie 66, 127–134.