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Catalytic Properties of NAD(P)H:Quinone Acceptor Oxidoreductase: Study Involving Mouse, Rat, Human, and Mouse-Rat Chimeric Enzymes

SHIUAN CHEN, RICHARD KNOX, ALEXANDER D. LEWIS, FRANK FRIEDLOS, PAUL WORKMAN,[1] PAULIS S. K. DENG, MAISIE FUNG,[2] DONNA EBENSTEIN, KEBIN WU, and TA-MING TSAI[2]

Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, California 91010 (S.C., P.S.K.D., M.F., D.E., K.W., T.-M.T.), CRC Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK (R.K., F.F.), and CRC Department of Medical Oncology, University of Glasgow, Glasgow G61 1BD, UK (A.D.L., P.W.)

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SUMMARY

NAD(P)H:quinone acceptor oxidoreductase (quinone reductase) (DT-diaphorase, EC 1.6.99.2) is involved in the process of reductive activation of cytotoxic antitumor quinones and nitrobenzenes. In this study, we initially examined the relative abilities of mouse, rat, and human quinone reductases to reduce two prodrugs, CB 1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide] and EO9 [5-(1-aziridinyl)-3-(hydroxymethyl)-2-(3-hydroxy-1-propenyl)-1-methyl-1H-indole-4,7-dione]. By using *Escherichia coli*-expressed quinone reductases and evaluating them under identical conditions, we confirmed previous findings showing that the human enzyme is not as effective as the rat enzyme in reducing CB 1954 and EO9, although the two enzymes have similar NAD(P)H-menadione reductase activities. Interestingly, although the amino acid sequence of mouse quinone reductase is more homologous to that of the rat enzyme, we found that the mouse enzyme behaves similarly to the human enzyme in its ability to reduce these compounds and to generate drug-induced DNA damage. To determine the region of quinone reductase that is responsible for the catalytic differences, two mouse-rat chimeric enzymes were generated. MR-P, a chimeric enzyme that has mouse amino-terminal and rat carboxyl-terminal segments of quinone reductase, was shown to have catalytic properties resembling those of rat quinone reductase, and RM-P, a chimeric enzyme that has rat amino-terminal and mouse carboxyl-terminal segments of quinone reductase, was shown to have catalytic properties resembling those of mouse quinone reductase. In addition, MR-P and RM-P were found to be inhibited by flavones with K_i values similar to those for rat and mouse quinone reductases, respectively. Based on these results, we propose that the carboxyl-terminal portion of the enzyme plays an important role in the reduction of cytotoxic drugs and the binding of flavones.

Quinone reductase (EC 1.6.99.2), also referred to as DT-diaphorase, is a flavoprotein that catalyzes the obligatory two-electron reduction of quinones (such as menadione; see Fig. 1 for structure) and quinonoid compounds to hydroquinones, using either NADH or NADPH as the electron donor (1, 2). In the past few years, evidence has been accumulated showing that quinone reductase is involved in the process of reductive activation of several cytotoxic antitumor quinones, such as mitomycins, anthracyclines, and aziridinylbenzoquinones, in cells (3–5). The enzyme can also act as a nitroreductase, in that it reduces nitrobenzenes such as CB 1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide] (6) (see Fig. 1 for structure). The enzymatic reduction of these compounds gives rise to reactive intermediates that can then undergo nucleophilic additions to DNA and other macromolecules, suggesting a possible mechanism for their cytotoxicity (7). Thus, these compounds are prodrugs and therefore important drugs for cancer chemotherapy.

Although experimental evidence that quinone reductase is responsible for the reductive activation of antitumor quinones and related compounds has been provided, the molecular basis for the activation of these compounds by this enzyme is not yet clearly understood. Particularly, it has been recognized that a major difference exists in the abilities of the human and rat quinone reductases to activate cytotoxic antitumor compounds. Human quinone reductase is not as active as rat quinone reductase in activating most, if not all, of

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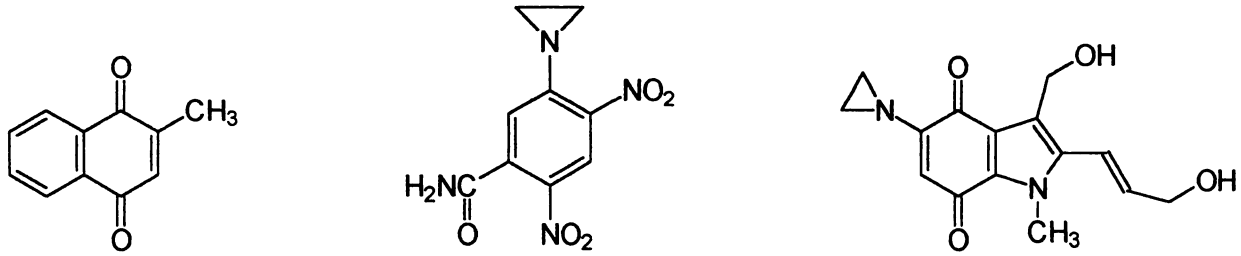
[1] Current address: Cancer Research Department, Zeneca Pharmaceuticals, Macclesfield, Cheshire SK10 4TG, UK.

[2] Supported by the Summer Student Research Program, American Heart Association, California Affiliate.

ABBREVIATIONS: quinone reductase, NAD(P)H:quinone acceptor oxidoreductase; NRH, dihydronicotinamide riboside; NARH, dihydronicotinic acid riboside; SDS, sodium dodecyl sulfate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; kb, kilobase(s); PCR, polymerase chain reaction; HPLC, high performance liquid chromatography.



1. MENADIONE 2. CB 1954 3. EO9

Fig. 1. Structures of the compounds used in this study.

the available antitumor quinones and nitrobenzenes. For example, the rate of the reductive activation of the indoloquinone antitumor agent EO9 [5-(1-aziridinyl)-3-(hydroxymethyl)-2-(3-hydroxy-1-propenyl)-1-methyl-1H-indole-4,7-dione] (see Fig. 1 for structure) by the rat enzyme was found to be significantly lower than that by the human enzyme (5). Similarly, the rat enzyme reduces CB 1954 at a faster rate than does the human enzyme (K_{cat} ratio = 6.4) (6), resulting in a large difference in the effectiveness of this prodrug. Therefore, CB 1954 is a very potent antitumor agent in rats but is not effective against human tumors *in vivo*. It has also been shown that the rat enzyme is more effective than the human enzyme in reducing diaziquone and mitomycin C, two clinically used anticancer agents (3, 4), as well as 2,5-dimethyl-3,6-diaziridinyl-1,4-benzoquinone, steptonigrin, and mitomycin A (8). These results indicate that the available cytotoxic drugs are better substrates for the rat enzyme and are not ideal prodrugs for activation by quinone reductase in human tumors.

The mouse quinone reductase cDNA was recently cloned and the mouse enzyme was successfully expressed in *Escherichia coli* (9). As expected, the amino acid sequence of the mouse quinone reductase was found to be more homologous to the rat enzyme than the human enzyme. There are 17 and 37 differences between the mouse sequence and the rat and human sequences, respectively (9). Thus, it was thought that the mouse enzyme would be similar to the rat enzyme in reducing the aforementioned prodrugs in a more efficient manner, compared with the human enzyme. Unexpectedly, in the present study the mouse enzyme was found to reduce two prodrugs, CB 1954 and EO9, at rates similar to those of the human enzyme and not the rat enzyme.

In addition to cytotoxic prodrugs, quinone reductase can also reduce vitamin K (e.g., menadione). In this regard, it can function physiologically as one of several vitamin K reductases in the vitamin K cycling involved in the hepatic post-translational modification of vitamin K hydroquinone-dependent blood coagulation factors (10). This enzyme is potently inhibited by anticoagulants, such as dicoumarol and warfarin (11, 12). Flavones have been found to be potent inhibitors of rat quinone reductase and have been suggested to be potentially useful oral anticoagulants (13). In a recent study (9), mouse quinone reductase was found to be significantly less sensitive to flavones than was rat quinone reductase.

Recognizing the catalytic differences for prodrugs and binding affinity differences for flavones between the mouse and rat quinone reductases, two mouse-rat chimeric enzymes were generated and evaluated. In this paper, we report the results obtained from studies with *E. coli*-expressed mouse, rat, human, and mouse-rat chimeric quinone reductases, and we discuss the implications of our current findings for further characterization of the molecular basis of the drug activation mechanism of this enzyme, as well as its flavone binding site.

Experimental Procedures

Materials. CB 1954 was synthesized at the CRC Centre for Cancer Therapeutics at the Institute of Cancer Research. EO9 was obtained from the New Drug Development Office (EORTC, Amsterdam, Holland). NRH was prepared from dihydronicotinamide ribotide (Sigma) by treatment with alkaline phosphatase, as described previously (14). NARH was prepared from nicotinic acid mononucleotide (Sigma), followed by chemical reduction (14).

Generation of mouse-rat chimeric quinone reductase cDNAs. For this study, mouse-rat chimeric quinone reductases were generated to determine which portion of the enzyme is more important in the reduction of prodrugs. By examining the nucleotide sequences of rat and mouse quinone reductases, we identified a conserved *Pst*I restriction site in the region encoding amino acid residues 93–95 (Fig. 2). Although there is only one *Pst*I site in the mouse quinone reductase cDNA, an additional *Pst*I site is present in the rat quinone reductase cDNA, in the region encoding amino acid residues 157 and 158. Because there is no *Pst*I site in the expression vector pKK233.2, a chimeric enzyme containing the amino-terminal portion of rat quinone reductase and the carboxyl-terminal portion of mouse quinone reductase was generated as follows. Both the mouse and rat quinone reductase cDNAs were ligated into the expression vector pKK233.2 through *Nco*I and *Hind*III sites (9, 15). Therefore, both expression plasmids were digested with *Nco*I and *Pst*I. The 0.3-kb fragment encoding the amino-terminal region of the rat enzyme and the 5.2-kb fragment encoding the carboxyl-terminal region of the mouse enzyme as well as the expression vector were gel purified and ligated together to generate the expression plasmid RM-P. The presence of an additional *Pst*I site in the rat quinone reductase cDNA complicated the construction of MR-P, the expression plasmid for the counterpart of the aforementioned chimeric enzyme. The second *Pst*I site in the rat quinone reductase cDNA was mutated by a primer-directed amplification method before the construction of MR-P. Two oligonucleotide primers (A and B) were synthesized. Oligonucleotide A contained the sequence around the second *Pst*I site, with sequence changes to mutate the *Pst*I site (5′-ATGTACTCTCTACAGGGTGTC-3′). Oligonucleotide B was a reverse primer and contained the 3′-end sequence plus an additional 25 nucleotides with an unrelated sequence (5′-AGCAGAGAGCATAAGAACAGAAAGCTTATTTTCTAGCTTTGATCTG-3′). PCR was performed with these two primers using rat quinone reductase cDNA as the template. The PCR product was gel purified and extended using the full length cDNA as the template. The second PCR

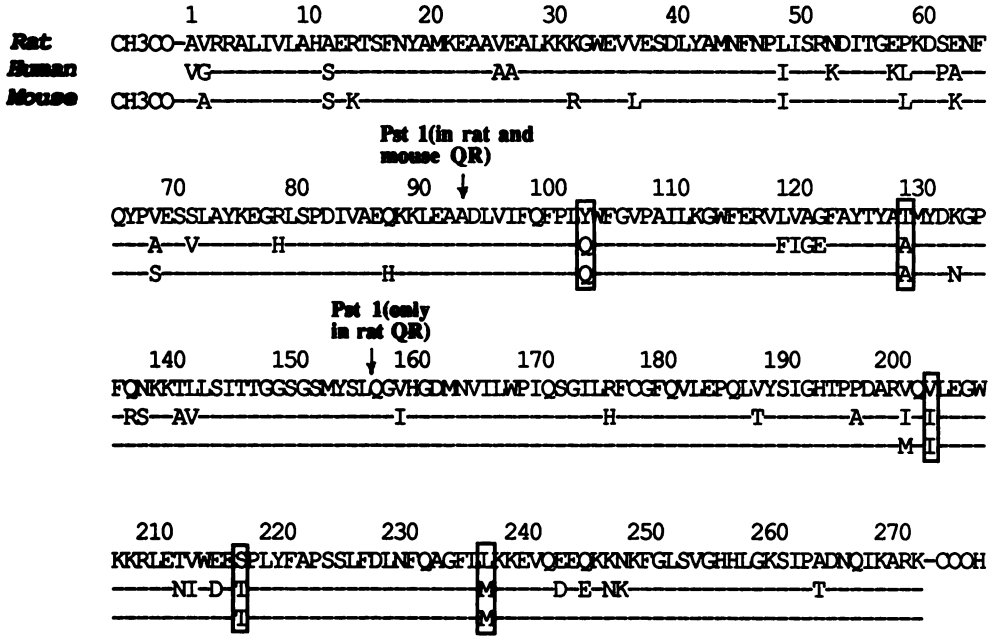


Fig. 2. Amino acid sequences of quinone reductases (*QR*) from rat, human, and mouse liver. For the amino acid sequences of the human and mouse enzyme, only the residues that are different from those of the rat enzyme are shown. The positions of *Pst*I restriction sites are indicated. In addition, the residues (after the first *Pst*I site) that are identical between the human and mouse enzymes, but different from those of the rat enzyme, are *boxed*.

was performed with two new oligonucleotide primers (C and D). Oligonucleotide C contained the sequence around the first *Pst*I site (5′-CTGGAAGCTGCAGACCTG-3′), and oligonucleotide D contained the sequence complementary to the unrelated sequence in oligonucleotide B (5′-AGCAGAGAGCATAAGAACAGA-3′). Using these two primers, only mutant cDNA was amplified. The mutant cDNA was subcloned into the PCR1000 vector from the TA cloning kit (Invitrogen Co.) and sequenced to ensure that no sequence errors were introduced during the PCR amplification. The mutated rat quinone reductase cDNA *Pst*I-*Hind*III fragment (0.54 kb) was then ligated to the 5-kb *Pst*I-*Hind*III fragment containing nucleotides coding for the amino-terminal region of the mouse quinone reductase and the expression plasmid pKK233.2, to generate the expression plasmid MR-P.

Enzyme preparations. The study was carried out with *E. coli*-expressed rat, human, mouse, and mouse-rat chimeric quinone reductases. The *E. coli* expression and purification of the rat quinone reductase have been published (15). The mouse quinone reductase cDNA has recently been cloned and the enzyme has been expressed in *E. coli* (9). The human quinone reductase was purified from *E. coli* transformed by a human quinone reductase expression plasmid, which was kindly provided by Dr. N. Gibson and colleagues (University of Southern California, Los Angeles, CA). Two chimeric enzymes, MR-P and RM-P, were expressed and purified from *E. coli* using procedures identical to those used for the rat and mouse enzymes (9, 15).

Purified MR-P and RM-P were checked for their purity by SDS-polyacrylamide gel electrophoresis (Fig. 3). We previously reported that mouse and human quinone reductases migrated faster than rat quinone reductase on SDS gels (6, 9). It is noteworthy that MR-P and RM-P migrate in fashions similar to those of mouse and rat quinone reductases, respectively, suggesting that the differences in the amino portions of the quinone reductases of the two species are responsible for their different migration rates on SDS-polyacrylamide gels. The most interesting difference within this region is residue 63. This is glutamic acid in the rat quinone reductase and lysine in the mouse quinone reductase (Fig. 2). The results suggest that a negatively charged residue (glutamic acid) at this position provides a conformation for quinone reductase that migrates more slowly than the enzyme with a positively charged residue (lysine) at this position. In agreement with this explanation, human quinone reductase has a neutral amino acid, alanine, at position 63 and migrates with a rate

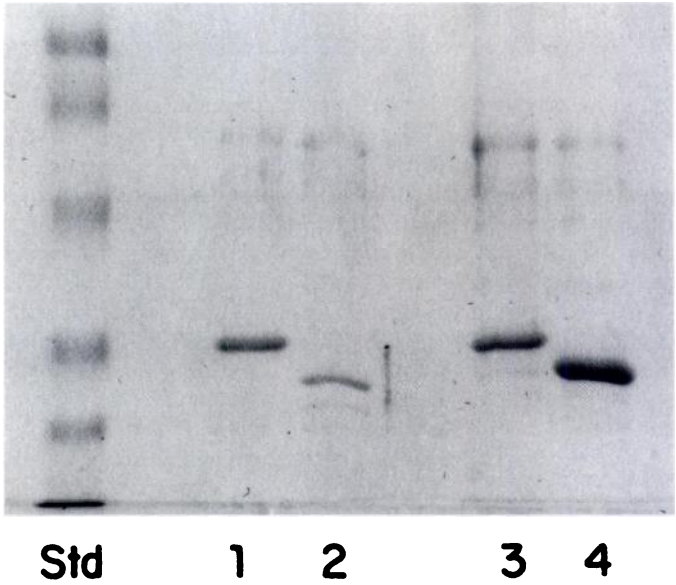


Fig. 3. SDS-polyacrylamide gel electrophoresis of *E. coli*-expressed rat, mouse, and chimeric (MR-P and RM-P) quinone reductases. The molecular weights of the molecular weight markers (*Std*) are 97,400, 66,200, 45,000, 31,000, 21,500, and 14,000. *Lane 1*, rat quinone reductase; *lane 2*, mouse quinone reductase; *lane 3*, RM-P; *lane 4*, MR-P.

intermediate between those of the rat and mouse quinone reductases (16).

Enzyme assays and kinetic analyses. The NADH-menadione reductase activity of the enzyme was determined spectrophotometrically by measuring the reduction of MTT at 610 nm [ϵ(610 nm) = 11.3×10^3 M^{-1} cm^{-1}] at 25° (17). The assay mixture (1 ml) contained NADH (200 μM), menadione (1.5–12.5 μM), MTT (0.3 mg/ml), and potassium phosphate (50 mM, pH. 7.5). In this assay, menadione is the electron acceptor and MTT is included to continuously reoxidize the menadiol formed.

The reduction of CB 1954 by quinone reductase was analyzed by HPLC. Quinone reductase was incubated with NADH (500 μM) and CB 1954 at different concentrations (0.1–2 mM), in sodium phosphate buffer (10 mM, pH 7) at 37°. At various times, aliquots (10 μl) were

injected onto a Partisphere SCX (250- × 4.7-mm) HPLC column and eluted isocratically (1.5 ml/min) with 50 mM sodium phosphate containing 1% methanol. The eluate was continuously monitored for absorbance at 340 and 260 nm, and the spectra of the eluting components were recorded using a diode array detector (ABI 1000S). This separation system could resolve all of the expected reduction products (6), and the reduction of CB 1954 was monitored by either the decrease in its peak area or the increase in the area of the peak corresponding to the reduction product 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide.

The reduction of EO9 by quinone reductase was analyzed spectrophotometrically by measuring the reduction of cytochrome *c* at 550 nm [ϵ(550 nm) = 21.1 × 10^3 M^{-1} cm^{-1}]. The assay mixture (1 ml) contained NADH (2 mM), EO9 (1–200 μM), cytochrome *c* (77 μM), bovine serum albumin (0.14%), and Tris buffer (50 mM, pH 7.5). In this assay, EO9 is the electron acceptor and cytochrome *c* is included to continuously reoxidize the product formed. All reactions were carried out at 37°.

DNA damage assay. DNA damage induced by the reduction of EO9 by quinone reductase was evaluated by investigating the conversion of supercoiled (form I) plasmid pBR322 DNA to the relaxed (form II) and linearized (form III) configurations. Incubations contained quinone reductase (2.5 μg), EO9 (100 μM), DNA (1.5 μg), NADH (2 mM), and the quinone reductase inhibitor dicoumarol (100 μM, when added), in a final volume of 100 μl of 0.1 mM sodium phosphate buffer, pH 7.4. Dicoumarol was added in an excess concentration to ensure complete inhibition of the purified enzyme. After incubation for 30 min at 37°, the reaction was stopped by the addition of 15 μl of stop buffer (5 mM EDTA, 0.5% SDS, 60%, v/v, glycerol, 0.1%, v/v, bromphenol blue).

Aliquots (25 μl) of stopped reaction mixtures were added to a horizontal 1% (w/v) agarose gel (20 × 24 cm) and electrophoresed at 40 V for 15–20 hr at room temperature. DNA was stained with ethidum bromide (0.5 μg/ml) and photographed under UV transillumination. Densitometric analysis of gel negatives was performed using a Molecular Dynamics laser densitometer with Image Analysis software (Protein Databases, New York, NY). Correction factors were used to account for differential staining of DNA forms by ethidum bromide.

Results and Discussion

The *E. coli*-expressed mouse quinone reductase has a higher NAD(P)H-menadione reductase activity than does either *E. coli*-expressed rat or human quinone reductase. As shown in a previous paper (9), the *E. coli*-expressed mouse quinone reductase was twice as active as the *E. coli*-expressed rat and human quinone reductases. The K_m value of menadione for the mouse enzyme was found not to be significantly different from those for the rat and human enzymes. Furthermore, the K_m values of NADPH and NADH for the mouse enzyme were found to be similar to those for the human enzyme but slightly higher than the values for the rat enzyme (9).

Mouse, rat, and human quinone reductases all converted the nitrobenzene prodrug CB 1954 to a single product that was identified as 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide, in agreement with previously published data (6). This hydroxylamine is the cytotoxic form of CB 1954. No other CB 1954 metabolites were found. In contrast to its ability to reduce menadione, as indicated in Fig. 4, mouse quinone reductase, resembling the human enzyme, reduced CB 1954 less efficiently than did the rat enzyme. Under the conditions used in this experiment, the rate of reduction of CB 1954 by the human and mouse forms of the enzyme was approximately 5.5-fold lower, compared with the rat enzyme. The mouse and human enzymes had similar kinetic parameters with respect to CB 1954, but both of these enzymes had a higher K_m and a much lower V_{max} than did the rat enzyme (Table 1).

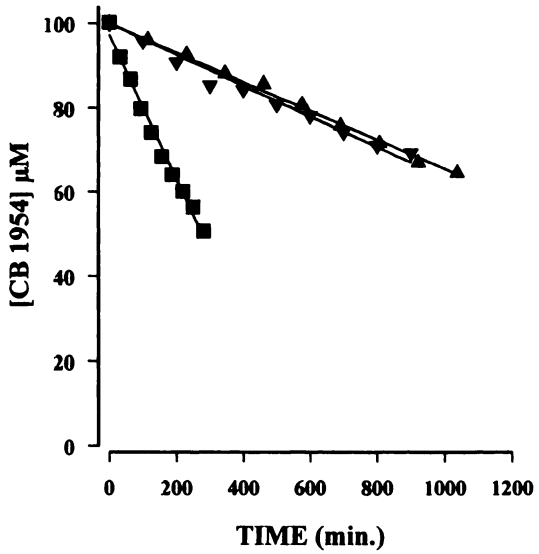


Fig. 4. Reduction of the prodrug CB 1954 by the mouse (▼), human (▲), and rat (■) forms of quinone reductase. Reduction was monitored by HPLC, and NADH, at an initial concentration of 500 μM, was used as a cofactor. The enzyme concentration was normalized to 10 μg/ml. In all cases the product of the reduction is 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide. This experiment was repeated three times, and similar results were obtained from three analyses.

For the reduction of CB 1954, NADH could be replaced by NRH without loss of activity of any of the forms of the enzyme. NARH could not be used as an electron donor (data not shown). It has been shown previously that NRH (and other simple reduced pyridinium compounds) but not NARH can act as a cofactor substitute for the rat form of the enzyme (14). It therefore appears that both the mouse and human enzymes share these lax structural requirements for a cofactor, without any requirement for the adenine nucleotide portion of NADH. A previous photoaffinity labeling study using four different photoaffinity labeling analogues of NADH generated results suggesting that, upon binding to the active site of quinone reductase, the adenine nucleotide portion of NADH is in a unrestricted environment (18).

The indoloquinone prodrug EO9 (which is currently in phase I clinical trials under the auspices of the European Organization for Research and Treatment of Cancer) is also less efficiently reduced by the mouse quinone reductase, with a K_m value approximately 2-fold higher than those for the rat

TABLE 1

Kinetic constants for the reduction of CB 1954 and EO9 by rat, mouse, human, and mouse-rat chimeric quinone reductases

	CB 1954		EO9	
	$K_{m_{app}}$	V_{max}	$K_{m_{app}}$	V_{max}
	μM	nmol/min/mg	μM	μmol/min/mg
Rat	840 ± 40	140 ± 8	27 ± 1	1620 ± 60
Mouse	1280 ± 80	20 ± 2	40 ± 3	180 ± 10
Human	1370 ± 70	20 ± 2	25 ± 6	210 ± 30
RM-P	1530 ± 170	20 ± 3	39 ± 3	150 ± 10
MR-P	840 ± 50	150 ± 9	20 ± 1	1690 ± 110

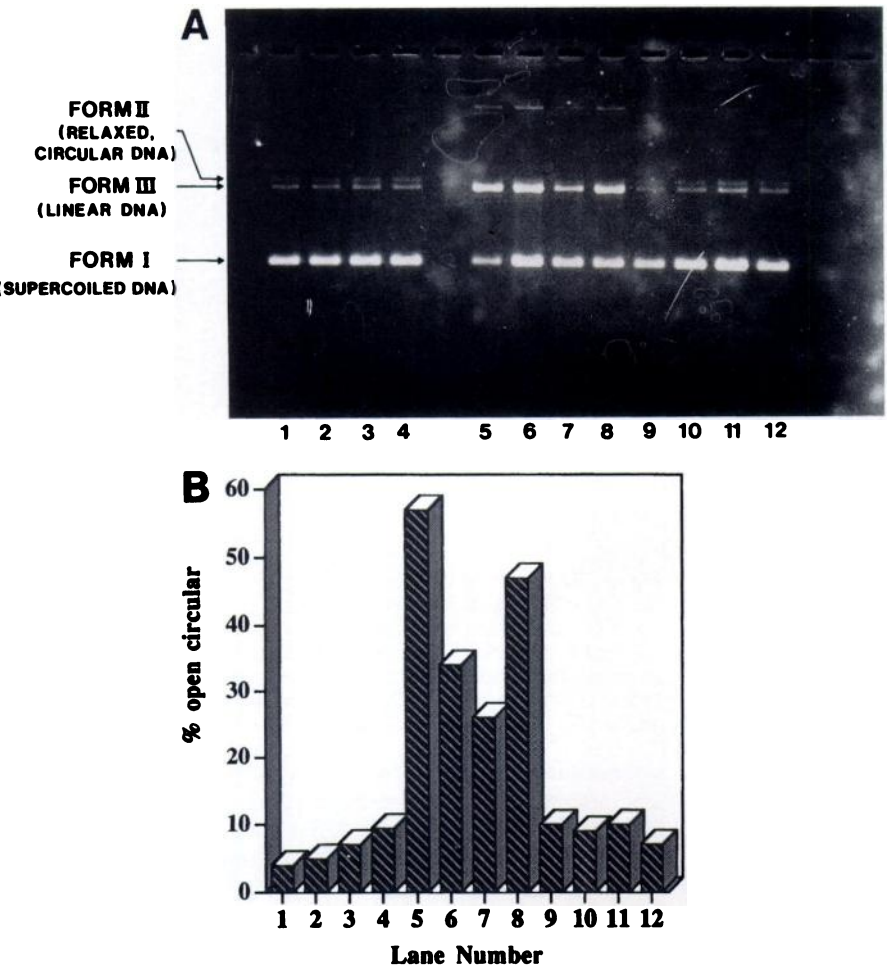


Fig. 5. Analysis of DNA damage by EO9 incubated with purified quinone reductases. A, Ethidium bromide-stained gel; B, histogram of the percentage of open circular DNA (form II) formed. EO9 was incubated with plasmid DNA, cofactor, and purified enzymes as described in Experimental Procedures. The gel was stained with ethidium bromide and the percentage of open circular DNA (form II) formed was quantified by densitometric scanning. *Lanes 1-4*, controls in which drug, enzyme, or cofactor was omitted or plasmid DNA alone was added, respectively; *lanes 5-8*, rat, human, mouse, and rat mutant (Lys-113 to His-113) quinone reductases, respectively; *lanes 9-12*, as in *lanes 5-8* but containing 100 μM dicoumarol. Although the mutant K113H is included in this experiment, its preparation and catalytic properties will be described in another paper. Lys-113 is important for the binding of the FAD prosthetic group. However, the mutation did not significantly affect the ability to reduce CB 1954 and EO9 or to cause EO9-induced DNA damage. This experiment was repeated twice, and similar results were obtained from two analyses.

and human enzymes. In addition, in comparison with the rat quinone reductase both the human and mouse enzymes had a lower (approximately 8-fold) V_{max} value for EO9 reduction.

As indicated in the introduction, the reduction of the prodrugs by quinone reductase gives rise to reactive intermediates that can undergo nucleophilic additions to DNA and cause DNA structural changes. A DNA damage assay was performed to determine whether the mouse enzyme would produce less EO9-induced DNA damage than the rat enzyme, because the mouse enzyme is less effective than the rat enzyme in reducing EO9. The results shown in Fig. 5 revealed that incubation of quinone reductase with the cofactor NADH and EO9 caused DNA single-strand breaks. As predicted, based on the kinetic data, the amount of DNA damage was greater with the rat enzyme than with the mouse or human enzyme. The DNA damage was inhibited in the presence of dicoumarol, an inhibitor of quinone reductase, indicating that the damage was enzyme dependent.

To summarize the results presented so far, by using purified recombinant quinone reductases and evaluating them under identical conditions we have confirmed previous findings that the human enzyme is not as effective as the rat enzyme in reducing cytotoxic prodrugs such as CB 1954 and EO9, although these enzymes have similar NAD(P)H-menadione reductase activities. Furthermore, we have also found that the mouse enzyme behaves similarly to the human enzyme in its ability to reduce these compounds and to generate drug-induced DNA damage. To determine the region of quinone reductase that is important for the reductive activation of the drugs, two mouse-rat chimeric enzymes were generated. As shown in Table 1, MR-P and RM-P have catalytic properties very similar to those of the rat and mouse quinone reductases, respectively. These results clearly indicate that the carboxyl-terminal region (after the conserved *Pst*I restriction site) of quinone reductase plays a critical role in the catalytic differences between the rat and mouse (and presumbly human) quinone reductases. Based on these results, we propose that the amino acid residues that are within the carboxyl-terminal region and identical between the human and mouse enzymes (because they have similar catalytic

TABLE 2

K_m values of NADH and inhibitory constants (K_i) of chrysin for rat, mouse, human, and mouse-rat chimeric quinone reductases

The enzyme activity was measured using NADH as the electron donor and menadione as the electron acceptor. Chrysin is a competitive inhibitor of the enzyme with respect to NADH, and the K_i values were determined using the method described by Liu *et al.* (13). The specific activities of rat, mouse, and human quinone reductase preparations have been reported (9). The specific activities of RM-P and MR-P were determined to be 4310 ± 1770 and 4420 ± 310 μmol of MTT reduced/mg/min, respectively.

	$K_{m_{app}}$ (NADH)	K_i (chrysin)
	μM	nM
Rat	110 ± 50	80 ± 20
Mouse	120 ± 10	680 ± 240
Human	100 ± 40	70 ± 30
RM-P	120 ± 30	580 ± 70
MR-P	90 ± 20	80 ± 10

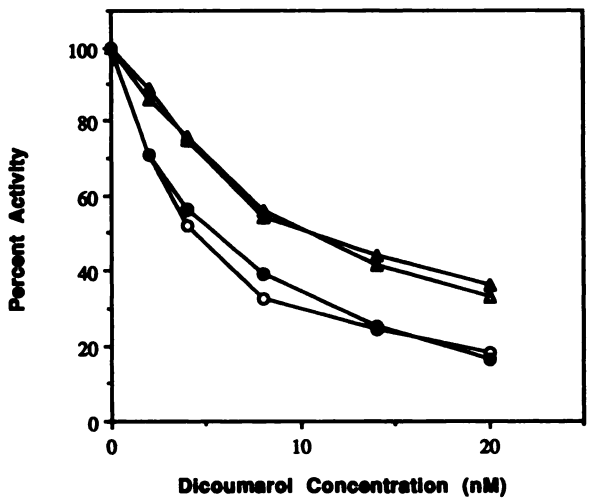


Fig. 6. Inhibition of chimeric quinone reductases by dicoumarol. The inhibition study was performed with rat, mouse, and chimeric quinone reductases at amounts providing identical levels of NADH-menadione reductase activity. The assay conditions are described in Experimental Procedures. ○, Rat quinone reductase; △, mouse quinone reductase; ●, MR-P; ▲, RM-P.

properties) but different from those of the rat enzyme are responsible for the difference in their reactivities toward the aforementioned cytotoxic drugs. There are only five such differences (Fig. 2). We are performing site-directed mutagenesis at these positions to evaluate our hypothesis and to further study the molecular basis of drug activation by quinone reductase.

Because we already know that there is a major difference between the rat and mouse quinone reductases regarding their profiles of inhibition by flavones, such as chrysin (5,7-dihydroxyflavone) (9), we also examined the ability of chrysin to inhibit the two chimeric enzymes. It has been shown that flavones are competitive inhibitors, with respect to NAD(P)H, of quinone reductase (13). As expected, the K_m values of NADH for rat, human, mouse, and mouse-rat chimeric quinone reductase preparations were found to be similar (Table 2). On the other hand, these preparations were inhibited to different degrees by chrysin. MR-P and RM-P were inhibited by chrysin with relative potencies similar to those for rat and mouse quinone reductase, respectively. The K_i values were also determined (Table 2). These results indicate that the carboxyl-terminal region of quinone reductase also plays a critical role in the binding differences for flavones between the rat and mouse quinone reductases. Further mutagenesis at this region will be useful to determine the critical amino acid residues involved in flavone binding. Finally, MR-P and RM-P were inhibited by dicoumarol with relative potencies similar to those for the rat and mouse quinone reductases, respectively (Fig. 6).

Activation of antitumor prodrugs by quinone reductase is particularly attractive with respect to therapeutic selectivity, because the enzyme is frequently expressed at higher levels in tumors, compared with corresponding normal tissues (19, 20). Information on the molecular basis of catalytic activation will be very important for the design of improved prodrugs for clinical use. Such structure-function studies of quinone reductase will also contribute to the understanding of the binding mechanism of flavones, which are potentially useful oral anticoagulants.

Acknowledgments

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Send reprint requests to: Shiuan Chen, Division of Immunology, Beckman Research Institute of the City of Hope, 1450 E. Duarte Rd., Duarte, CA 91010.