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Crystal structure of quinone reductase 2 in complex with cancer prodrug CB1954

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Abstract

CB1954 is a cancer pro-drug that can be activated through reduction by *Escherichia coli* nitro-reductases and quinone reductases. Human quinone reductase 2 is very efficient in the activation of CB1954, approximately 3000 times more efficient than human QR1 in terms of k_{cat}/K_m. We have solved the three-dimensional structure of QR2 in complex with CB1954 to a nominal resolution of 1.5 Å. The complex structure indicates the essentiality of the two nitro groups: one nitro group forms hydrogen bonds with the side-chain of Asn161 of QR2 to hold the other nitro group in position for the reduction. We further conclude that residue 161, an Asn in QR2 and a His in QR1, is critical in differentiating the substrate specificities of these two enzymes. Mutation of Asn161 to His161 in QR2 resulted in the total loss of the enzymatic activity towards activation of CB1954, whereas the rates of reduction towards menadione are not altered.

Keywords: CB1954; Quinone reductase; QR1; QR2; Substrate specificity; Menadione

Quinone reductase 2 (QR2, also called NQO2: NRH:quinone oxireductase 2) was first described in 1961 [1], and has been ignored for many years until recently [2]. Cloning of mouse and human forms of QR2 as well as its structural solution [2,3] renewed interest in this protein. Recent studies have also indicated that the genetic polymorphisms of QR2 are associated with a number of neurological diseases, such as Parkinson's disease, Schizophrenia, and others [4–7]. However, the other isoform, quinone reductase 1 (QR1, also termed DT-diaphorase, NQO1), has been very well studied [8]. In particular, more and more evidence points to QR1 having a function to protect cells against the toxicity of electrophiles and reactive forms of oxygen, and its induction protects cells against carcinogenesis. Therefore, QR1 is accepted as belonging to the group of enzymes classified as phase II detoxification enzymes. While the biological functions of QR2 have yet to be determined, it should be noted that, like the QR1 quinone reductase, it catalyzes the obligatory two-electron reduction of quinones to hydroquinones without the accumulation of a dissociated semiquinone [9], and consequently without the formation of free radicals that could potentially damage cells.

Structurally, QR1 and QR2 are very similar [10]. Both proteins form dimeric complexes featuring two independent, equivalent active sites, which are located at the opposite ends of the dimer interface. Residues from both monomers line the active sites, which are deep cavities extending from the protein surface to the isoalloxazine rings of the bound FAD cofactors. QR1 monomer is composed of two domains: a major catalytic domain (residue 1–220) and a small C-terminal domain (residue 221–273). The overall topology of QR2 structure is very similar to the catalytic domain of QR1, with r.m.s. for Cα carbon of the catalytic domain less than 1 Å. The C-terminal domain of QR1 (220–273) is entirely absent in QR2, and the C-terminal residues of QR2 (221–230) have no sequence homology with the corresponding residues in QR1. The conformation and locations of QR1 and QR2 bound co-factor FADs are almost identical when the Cα carbons are aligned between the two catalytic domains.

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One of the differences between the two structures is the presence of metal ions in QR2, reported to be zinc ions [2]. The metal site is tetracoordinate, with two histidine ligands, a main-chain carbonyl oxygen, and one cysteine ligand. The cysteine ligand is covalent in nature. We have observed that QR2 retains its zinc ions, even after extensive purification in the presence of high concentrations of EDTA.

Although QR2 and QR1 have high sequence and structural similarities, they possess some significantly different catalytic properties [9]. While both QR1 and QR2 can catalyze the two-electron reduction of quinones such as menadione (see Fig. 1 for structure), QR1 uses NADH or NADPH as electron donors, whereas QR2 cannot use NADH or NADPH as electron donors efficiently. Instead, QR2 can use NRH (ribosyl dihydronicotinamide) as the electron donor. QR2 can also use a variety of NRH analogs (see Fig. 1 for structure) as electron donors in the reduction of quinones in vitro [11]. While NADH and NADPH are electron donors for a variety of enzymes in a variety of reactions, and their biological metabolism and concentration are very well characterized, very little is known about NRH and its related analogs. At present, it is still not clear whether NRH is the biological electron donor for QR2. As a consequence, the true biological function of QR2 is still yet to be revealed.

In addition to the differences in the preference of electron donors for catalysis, QR1 and QR2 have very different inhibitors. The very well-characterized inhibitors for QR1, such as dicumarol and cibacron blue, do not significantly inhibit QR2 activity [12], whereas a number of natural polyphenols, such as resveratrol and quercetin, are strong inhibitors of QR2 [13]. Another striking difference between the functions of QR1 and QR2 is that even though animals with gene disruption of either protein exhibit similar phenotypes, they exhibit opposite properties toward menadione toxicity. Animals with QR2 gene disruption exhibited increased resistance to quinone toxicity, whereas animals with QR1 gene disruption showed increased sensitivity to quinone toxicity [14,15]. This is a very intriguing paradox, as both proteins share similarities in structure, catalytic mechanism, and activity towards menadione reduction.

CB1954 (5-(aziridin-1-yl)-2,4-dinitrobenzamide, see Fig. 1 for structure) has been of intense interest as a prodrug in the treatment of cancer in antibody-directed enzyme prodrug therapy (ADEPT) and gene-directed enzyme prodrug therapy (GDEPT) [16,17], and it is currently in phase I clinical trials for prostate cancer treatment in Britain. As a prodrug, CB1954 exhibits a minimal toxic effect on the hematopoietic system [18]. It is not a quinone derivative and therefore, it cannot be reduced by typical quinone reductases, such as NADPH:cytochrome P450 reductase or xanthine oxidase. These enzymes can only transfer a single electron at a time and are incapable of reducing nitro groups [17]. However, rat QR1 can serve as a nitroreductase in reducing CB1954, and the reaction product, 5-(aziridine-1-yl)-4-hydroxylamino-2-nitrobenzamide (Fig. 1), is a highly toxic compound that reacts bifunctionally in cells to induce DNA–DNA interstrand cross-linking [19], which consequently kills the cancer cells. However, the reduction of CB1954 by human QR1 is very inefficient [11]. Recently, it was discovered that QR2 is a much more efficient enzyme in the reduction of CB1954 [11]. It is 3000 times more efficient than human QR1 in the reduction of CB1954 when assayed in vitro. This led to the development of a novel co-substrate-mediated antitumor prodrug therapy, in which CB1954 is given simultaneously with QR2 electron donors. This in turn decreases IC_{50} of CB1954 from a concentration in the range of hundreds of micromolar to less than 1 μM, and suggests that QR2 may be the cellular enzyme responsible for the activation of CB1954. QR2 is highly expressed in tissues of certain cancers, such as prostate cancer [20]. Therefore, this approach could prove to be very intriguing in the treatment of these types of cancers and definitely warrants more research.

In view of the great importance of CB1954 in cancer treatment, it is of fundamental importance to understand the mechanism and specificity of activation of CB1954 by QR2. We have determined the three-dimensional structure of QR2–CB1954 complex at 1.5 Å resolution, which reveals insight into the mechanism of CB1954 activation and specificity. This structure and its analysis will aid in the design of more specific cancer prodrugs towards QR2 or QR1.

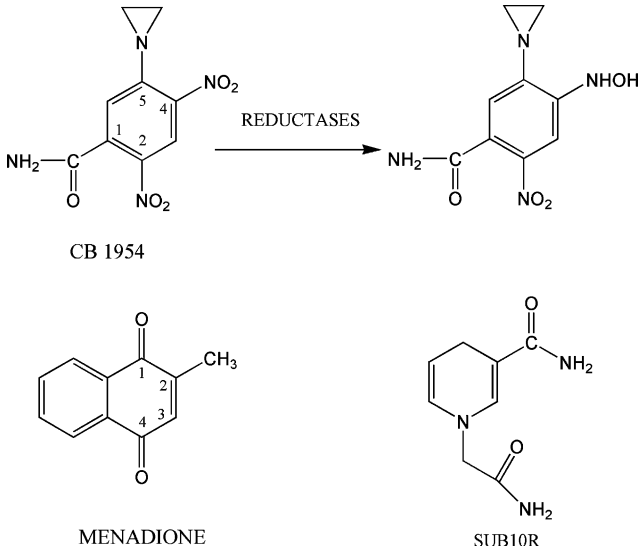

Fig. 1. Structures of small molecules involved in this study.

Materials and methods

Chemicals and reagents. CB1954, nicotinamide, and menadione were purchased from Sigma. All other chemicals were of the highest purity commercially available. 1-(Carbamoylmethyl)-3-carbamoylpyridinium iodide was prepared by stirring and heating a mixture of nicotinamide and 2-iodoactamide in DMF at 60 °C for 3 h as reported [11]. Subsequent reduction to produce the dihydronicotinamide derivative (SUB10R, Fig. 1) was carried out as reported [11].

Protein methods. To produce recombinant QR2, the coding region of QR2 cDNA fragments was amplified by PCR and cloned into pET23d vector (Novagen) between *Nco*I and *Xho*I sites with the first Met as part of the *Nco*I sequence and the native termination codon before the *Xho*I site. The insert was verified by DNA sequencing. The plasmid of the full-length protein of QR2 without any change in amino acids is transformed into B834 *Escherichia coli* strain (Novagen). The recombinant protein was purified by passing through DEAE Sepharose, Superdex 75 (Pharmacia), and Mono-Q columns (Pharmacia), sequentially to obtain the homogeneous protein samples. Protein concentration was determined by the Bio-Rad protein assay kit according to manufacturer's protocol. Crystals of the native protein as well as the complex with CB1954 were all grown in conditions containing 0.1 M Hepes (pH 7.0), 10 μM FAD, and 1.2–2.0 M ammonium sulfate. The mutant QR2 (Asn161His) was generated by site-directed mutagenesis method. The mutant protein was expressed and purified similarly to the wild-type QR2.

Crystallographic analysis. All diffraction data were collected at 100 K using a CCD detector at beamline 19BM of Advanced Photon Source (APS), Argonne National Laboratories. Raw data were processed using the HKL2000 software [21]. Crystals of the native QR2, QR2–CB1954 complex all belong to the $P2_12_12_1$ space group. The structure of QR2–CB1954 complex was solved by the molecular replacement method with native QR2 structure [2] (1qr2, Protein Data Bank) and subsequently refined with CNS [22]. The density of CB1954 was clearly shown after Fourier-difference transformation. Figures were prepared using PDB-viewer [23] and rendered with POV-ray [24] software packages. The atomic coordinates of QR2–CB1954 complex have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, with access code 1XI2.

Enzymatic assays. QR2 activity was assayed according to Zhao et al. [12]. The enzyme activity was determined spectrophotometrically at either 37 or 25 °C in pH 8.5 buffer (50 mM Tris, 140 mM NaCl, and 0.1% Tween 20) with menadione and SUB10R as co-substrates. The reaction was initiated by addition of 2–8 ng QR2 and the catalysis was monitored at wavelength of 350–370 nm with a Unicam UV–vis spectrophotometer. The reduction of CB1954 was conducted with SUB10R as co-substrate as follows: in a reaction mixture of 200 μl (100 mM Tris, pH 8.5, 100 mM NaCl, 0.1% Tween 20, 1 μM FAD, and 100 μM SUB10R), varying concentrations of CB1954 were added to the reaction buffer. The catalysis was initiated by addition of 8 ng of wild-type or mutant QR2 proteins. After incubating at room temperature for 10 min, the absorbance of the sample at 370 nm was measured. The reduction of absorbance at 370 nm is the result of both the consumption of SUB10R ($\varepsilon = 6400\ M^{-1}\ cm^{-1}$) and reduction of CB1954 ($\varepsilon = 5600\ M^{-1}\ cm^{-1}$). The conversion of CB1954 was calculated accordingly.

Results and discussion

CB1954–QR2 complex structure

CB1954 is chemically a mono-functional alkylation agent by virtue of its aziridine functional group. It exhibited a dramatic and highly selective activity against the rat Walker 256 tumor and could actually cure this tumor [17]. This is due to the activation of CB1954 by rat QR1, which reduces the 4-nitro group to a 4-hydroxylamine (Fig. 1). The resulting 4-hydroxylamine derivative is a bifunctional agent that can form DNA–DNA interstrand cross-links [25]. However, the human form of QR1 is very inefficient in catalyzing this reaction. On the other hand, human QR2 is 3000 times more efficient in the activation of CB1954 than human QR1 when analyzed in vitro [26]. The crystal of QR2–CB1954 complex was obtained by co-crystallization of QR2 in the presence of 1 mM CB1954 with ammonium sulfate as precipitants. The complex crystal belongs to the same space group as the apo-protein crystal ($P2_12_12_1$) with near identical cell dimensions (Table 1, crystallographic and refinement statistics). The structure was solved by molecular replacement with the apo-protein structure (1QR2 [2], Protein Data Bank). The CB1954 molecules were identified after Fourier difference transformation (Fig. 2A). The overall structure of QR2 in the complex is indistinguishable with the apo-protein structure, indicating that the CB1954 binding site is preformed. CB1954 is clearly shown to bind to the active site (Fig. 2A), occupying very similar positions as menadione in the QR2–menadione complex [2] and resveratrol in the QR2–resveratrol complex we solved earlier [13]. The CB1954 molecule occupies two-thirds of the active-site cavity, whereas resveratrol occupies the entire cavity (Fig. 2B). The benzene ring of CB1954 sits near parallel to the plane of the isoalloxazine ring, stacked on top of ring C of the isoalloxazine moiety of FAD. Three phenylalanine side-chains (F131′, F178′, and F126′) sitting on top of the benzene ring of CB1954. Those hydrophobic interactions hold the benzene ring of CB1954 parallel to the plane of isoalloxazine ring of co-factor FAD. The orientation of CB1954 molecule is unmistakable, with the aziridine functional group and the amide group pointing towards the solvent. The 4-nitro group of CB1954 is close in proximity to the N5 atom of FAD, and the distance between the nitrogen (4-nitro group) and N5 of FAD is 3.3 Å, positioning the 4-nitro group to accept a hydride from the reduced FAD. This explains that only the 4-nitro group of CB1954 is reduced by QR2. The positioning of the 4-nitro group is only possible as a result of the two hydrogen bonds formed between the 2-nitro group and the amino acid side-chains of QR2 (Fig. 3). One of the two oxygens in the 2-nitro group forms one hydrogen bond with the

Table 1
Summary of crystallographic analysis

QR2–CB1954	
Data collection statistics	
Space group	$P2_12_12_1$
Unit cell	$a = 83.48$ Å, $b = 106.46$ Å, $c = 56.69$ Å
Resolution (Å)	50.0–1.5
Completeness (%; $I/\sigma \geqslant 0$)[a]	99.5 (99.1)
Average I/σ	30.4
R_{merge} (%)[b]	5.0 (31.3)
Refinement statistics	
Resolution range (Å)	50–1.5
R factor[c]/R_{free} (%)	21.6/23.2
rmsd bond length	0.014
Rmsd angles	1.8
Mean B factor value	17.1

[a] Values for the highest resolution shell are given in parentheses.

[b] $R_{merge} = \sum|I - \langle I\rangle|/\sum\langle I\rangle$, where I and $\langle I\rangle$ are the measured and averaged intensities of multiple measurements of the same reflection. The summation is over all the observed reflections.

[c] R factor $= \sum||F_o| - |F_c||/\sum|F_o|$, where F_o denotes the observed structure factor amplitude and F_c denotes the structure factor calculated from the model.

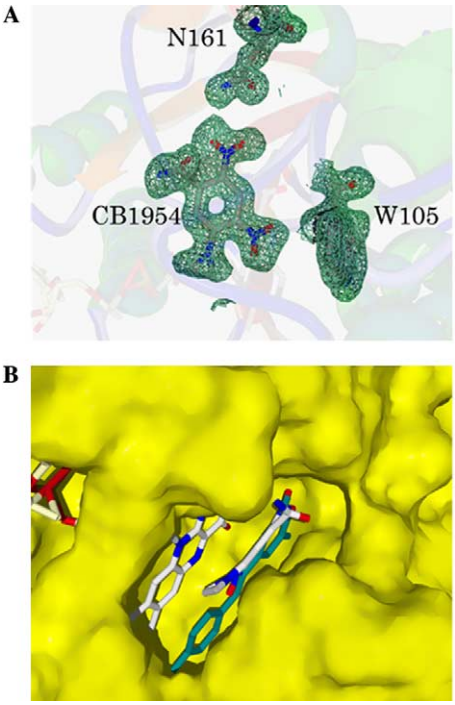

Fig. 2. CB1954 occupies the active site of QR2. (A) Electron density ($2F_o - F_c$, 1δ cutoff) of CB1954 and two interacting residues. (B) The CB1954 binding cavity is shown in a surface representation. In comparison with the QR2–resveratrol structure, CB1954 occupies about 2/3 of the active site, whereas resveratrol (blue) occupies the entire QR2 active site cavity.

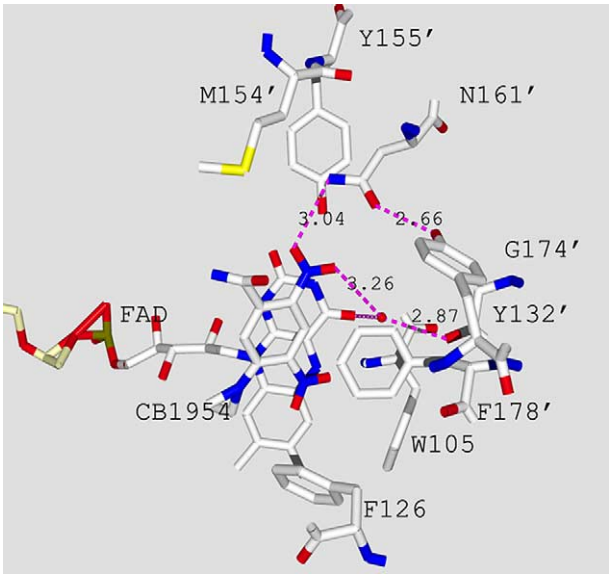

Fig. 3. CB1954–QR2 interactions. The benzene ring of CB1954 is sitting on top of the isoalloxazine ring of FAD. The 2-nitro group of CB1954 forms a hydrogen bond with the side-chain of N161 and another hydrogen bond with the carbonyl oxygen of G174 through a water molecule.

amide group of N161, while the other oxygen forms a hydrogen bond through a water molecule with carbonyl oxygen of G174 and atom O4 of the bound FAD. The orientation of the side-chain of N161 was further held in position by a strong hydrogen bond with the hydroxyl group of Tyr132. All these hydrogen bonds between CB1954 and QR2 are relatively weak, conferring the relatively low affinity of CB1954 towards QR2 (K_m of 61 μM) in comparison with the affinity of resveratrol towards QR2 (K_d of 35 nM) [13]. The amide group of CB1954 exhibited minimal interaction with QR2, forming a pair of weak hydrogen bonds with the main-chain amide of Gly150 (3.33 Å) and the carbonyl of Gly149 (3.23 Å). Both the amide group and the aziridine group point towards the solvent, leaving ample space for the design of additional derivatives possessing additional functionalities to be substrates of QR2.

Difference between QR1 and QR2 in the activation of CB1954

Even though CB1954 can be activated by both human QR1 and QR2, the efficacy of catalysis is very different, as QR2 is 3000 times more efficient in the reduction of CB1954 than QR1 when assayed in vitro [26]. This is reflected in both the K_m values and K_{cat} values [26]. CB1954 possesses higher affinity towards QR2 than QR1, with K_m values of 0.26 and 1.4 mM, respectively, reported in the literature [26] and we have obtained a much smaller K_m (61 μM) for CB1954 towards QR2. This could be due to the fact that CB1954 is not very soluble and studies in the literature have used exceedingly long procedures to quantify the reduced CB1954 product (HPLC separation and subsequent integration of the chromatogram) or could be due to the fact that we used a different co-substrate. However, the difference in catalysis efficacy is more reflected on the k_{cat} values. For QR2, the k_{cat} value for the reduction of CB1954 is $3.6 \times 10^2\ min^{-1}$ with NRH as co-substrate, whereas the k_{cat} for QR1 is only $0.6\ min^{-1}$ with NADH as co-substrate when assayed under the same assay conditions [26].

The active sites of QR1 and QR2 are very similar, with the majority of the residues conserved between the two proteins. When the two structures are aligned, the r.m.s. of the main chain Cα is about 1 Å, with the active site residues aligned very well and the two co-factor FAD molecules virtually overlap each other (Fig. 4). W105 and F106 in both proteins form the bottom of the active sites, and residues F178, M154, Y132, Y155, and F126 (Y126 in QR1) are conserved and similarly positioned. The isoalloxazine rings of the bound cofactor FAD in both proteins are basically superimposed upon the three-dimensional structural alignment. As a matter of fact, the active sites from both proteins are so similar that they not only share similar catalytic mechanism but also share some similar substrates. Both proteins can catalyze the reduction of menadione, DCIP (2,6-dichlorophenolindophenol), and other 1,4-quinones at similar rates [26]. Both proteins can use NRH as electron donors with similar efficiency. However, one significant difference at the active sites between QR1 and QR2 is residue 161, which is an asparagine residue in QR2 and a histidine in QR1. H161 was suggested to be

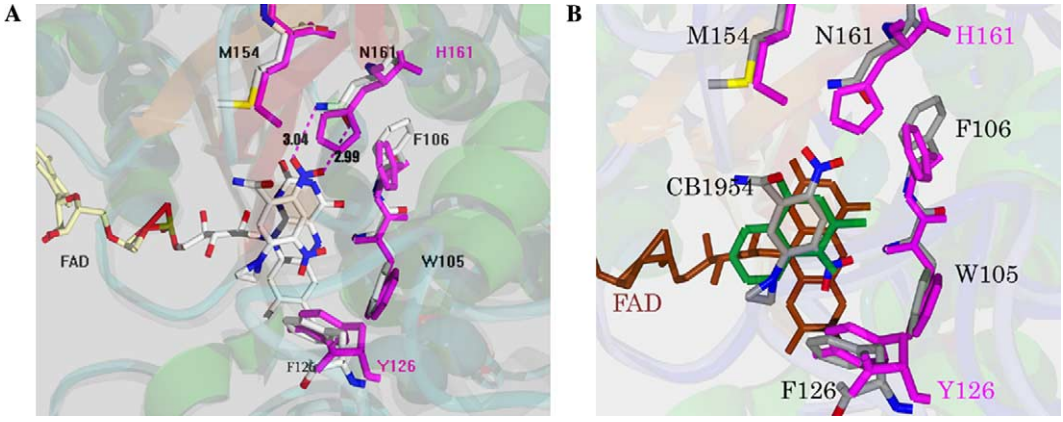

Fig. 4. Active-site comparison of QR2–CB1954 complex with QR1. (A) The active sites of QR2 and QR1 are very similar. One significant difference is residue 161, Asn in QR2 and His in QR1. When the structures of QR2 and QR2 are aligned, most of the active site residues are positioned similarly (QR1 in pink), except residue 161. Due to its larger side-chain, histidine 161 would clash with CB1954 if CB1954 would bind to QR1 in a similar manner as it binds QR2. (B) Comparison of the positions of CB1954 and menadione (green). The mutation of N161 to H161 would not affect the binding and orientation of menadione to QR2, as there is no possible clash between His161 with any atoms in menadione.

involved in the catalysis in stabilizing the developing negative charge on Y155 during the hydride transfer in QR1 catalysis [27]. The role of N161 in QR2 is very different, as it cannot be protonated to possess a positive charge. The comparable catalytic activities in the reduction of menadione between QR1 and QR2 might also indicate that H161 might not play a very important role in the catalysis as suggested. On the other hand, in QR2, N161 plays critical roles in the interaction with resveratrol and CB1954. In the QR2–resveratrol complex, the side-chain of N161 forms a critical hydrogen bond with the 5-hydroxy group of resveratrol that seals off the hydrophobic cavity from the solvent [13], whereas the side-chain of N161 in QR2–CB1954 complex forms a critical hydrogen bond with the 2-nitro group of CB1954 to hold it in position for the reduction of the 4-nitro group. The active site of QR2 is a 17 Å in length and 7 Å wide cavity that is a perfect fit for a trans-stilbene structure [13], which is shared among a number of classes of nature polyphenols, including trans-stilbene derivatives, flavonoids, chalcones, and 3-phenylcoumarins. The replacement of N161 by H161 will shorten the active site cavity by about 1.9 Å (Fig. 4A). In the QR2–CB1954 complex, the side-chain of N161 is at optimum distance to interact with the 2-nitro group of CB1954 and further places the 4-nitro group in position for the reduction. However, the change of N161 to H161 would hinder the binding of CB1954, as the side-chain of His161 extends about 1.9 Å into the active site cavity. Even bound, the CB1954 molecule would be pushed out of optimal position for the reduction of the 4-nitro group, which gives rise to the catalytic activity difference between QR1 and QR2 in the reduction of CB1954. This should have great implications in the design of CB1954 analogs specific for either human QR1 or QR2, as both proteins are overexpressed in certain type of cancers, respectively. Upon alignment of the structures of QR2-menadione and QR2–CB1954 complexes, CB1954 and menadione occupy the same position at the QR2 active sites, with the benzene ring overlapping with the 1,4-quinone ring. The 4-nitro group in CB1954 overlaps with the 1-carbonyl group in menadione and the amide group in CB1954 overlap with the 4-carbonyl of menadione. Since there is no substitution group at the 3 position in menadione, the mutation of Asn161 to His161 would not hinder the binding and positioning of menadione at the active site (Fig. 4B), and hence both QR1 and QR2 are active in the reduction of menadione.

Mutation of Asn161 to histidine abolished the enzymatic activity of QR2 in the activation of CB1954

In order to confirm the importance of residue 161 in the differentiation of substrate specificities between QR1 and QR2, we generated a QR2 mutant in which residue Asn161 was mutated to histidine by site-directed mutagenesis. The mutant protein was expressed and purified similarly to the wild-type QR2, with no reduction of expression levels or alteration in the behavior on ion-exchange and size-exclusion chromatography. We tested the activity of the mutant protein in the activation of CB1954 using SUB10R as co-substrate, which was proved to be effective in the activation of CB1954 both in vitro and in vivo in a cellular toxicity assay [11]. In comparison with the wild-type QR2, the mutant QR2 (N161H) exhibited no detectable activity in the reduction of CB1954 up to concentrations of 150 μM CB1954 (Fig. 5A). The activity was monitored as the reduction of absorbance of the reaction mixture at a wavelength of 370 nm, which is due to the reduction of CB1954 as well as the oxidation of SUB10R. This observation reaffirms our structural observation that Asn161 is critical in the positioning of CB1954 at the QR2 active site. We further analyzed the catalytic activities of the mutant QR2 in the reduction of menadione. We used constant concentrations of menadione (100 μM) and varying concentrations of co-substrate SUB10R to compare the

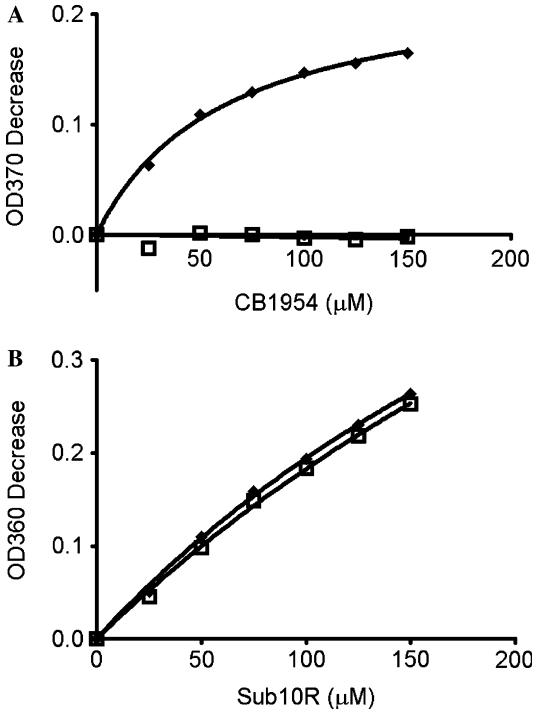

Fig. 5. Comparison of mutant QR2 (Asn161 to His) with wild-type QR2 in the reduction of menadione and the activation of CB1954. (A) The mutant protein is inactive in the activation of CB1954. The activation of CB1954 was monitored by the reduction of the 4-nitro group with SUB10R as the electron-donor, which resulted in the reduction of absorbance at 370 nm from both CB1954 reduction and SUB10R oxidation. The absorbance decrease due to the reduction of CB1954 is plotted against its concentration. The K_m for CB1954 obtained is 61 ± 5 μM in the presence of 100 μM SUB10R. The mutant protein is indicated by symbol, □; whereas the wild-type QR2 is indicated by (♦). No activity was detected for the mutant QR2. (B) Reduction of menadione by wild-type QR2 (♦) and mutant QR2 (□) was at the same rate when substrate SUB10R was used as the electron donor. Constant concentrations of menadione (100 μM) were reduced with varying concentrations of SUB10R. The reduction rate was monitored by the consumption of SUB10R.

activities of wild-type QR2 and mutant QR2. At all co-substrate (SUB10R) concentrations, the two proteins exhibited indistinguishable activities toward reduction of menadione. This is also consistent with our structural analysis, as mutation of Asn161 to His would not interfere with the binding and orientation of menadione at the active site.

The high conservation of the primary and tertiary structure between QR1 and QR2 gives an initial indication that these two enzymes may have similar properties and biological functions [10]. However, the difference in the utilization of electron-donors (co-substrates) indicates otherwise, as QR1 uses the universal reductase co-substrate, NAD(P)H, whereas QR2 uses an unidentified reduced nicotinamide homolog, suggested to be ribosyl-dihydronicotinamide. What may it be, the electron-donor for QR2 exists in minimal amount under normal cell growth conditions, as indicated by the activation of CB1954 only occur significantly with the co-application of QR2 co-substrates [11]. Certainly, this is advantageous in some respect in the use of cancer pro-drugs such as CB1954, since the administration would have minimal toxicity to tissues even with high QR2 levels and made it possible for the gene therapy by tissue-specific introduction of CB1954 reductases that can use NAD(P)H as co-substrate such as nitro-reductases [28]. Furthermore, QR1 belongs to the class of detoxification enzymes (phase 2), which can be induced by certain oxidants and antioxidants [29]. QR2 does not belong to this class of enzymes as it is not induced under conditions that induce QR1. How evolution differentiated the biological functions of these two very homologous enzymes remains to be elucidated; however, the difference at residue 161 is a critical determinant in the substrate specificity differences between these two enzymes.

Implication in the design of additional CB1954 analogs

There is an intense effort in the development of next generation of CB1954-related analogs which are specific for QR1, QR2 or other nitro-reductases [30–32]. The structural analysis of the QR2–CB1954 complex indicates that the 2-nitro group prevents the effective reduction of CB1954 by QR1, and consequently, the repositioning of this group to 3- or 5-position should facilitate the effective reduction by QR1. On the other hand, the amide group and aziridine group exhibit minimal interactions with the protein, and further derivatization of these groups to enhance their pharmacological properties should be feasible without disturbance to the affinities and activities towards QR1 or QR2. Overall, the structure of the QR2–CB1954 complex should pave the way for the design of the next generation of cancer prodrugs that can be activated by QR1 or QR2.

Acknowledgments

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