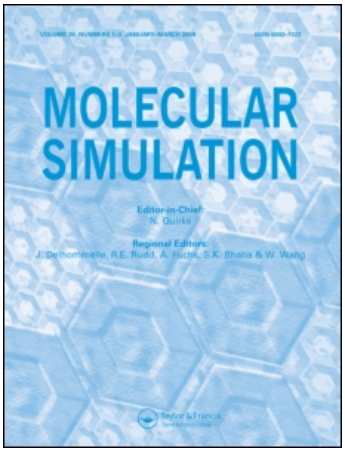

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MOLECULAR MODELLING OF HUMAN DT-DIAPHORASE FOR ENZYME-DIRECTED BIOREDUCTIVE DRUG DESIGN

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The enzyme DT-Diaphorase (NAD(P)H:quinone acceptor oxidoreductase, EC 1.6.99.2.; DTD) has been recognised as a good target for enzyme-directed bioreductive drug development. This is due to elevated levels of enzyme activity in several human tumour types and its role in the bioreductive activation of several quinone-based anti-cancer drugs.

Bioreductive drugs are designed to exploit one of the features of solid tumours, namely tumour hypoxia. However, selectivity of bioreductive drugs is not only governed by oxygen levels, but also by the levels of the enzymes catalysing bioreductive activation, leading to the concept of "enzyme-directed bioreductive drug development" introduced by Workman and Walton in 1990. This concept requires the identification of tumours within a patient that have elevated levels of enzyme activity (enzyme profiling) and treating the patient with drugs activated by such enzymes. DTD has been singled out as a particularly good candidate for such targeting. In order to rationalise the design of drugs to target DTD, molecular modelling techniques have been employed.

The human DTD three-dimensional structure has been modelled with homology to the known rat DTD structure (about 85% identity) and the model refined using energy minimisation. Drug-binding orientations have been determined and molecular dynamics simulations performed. Using data from a series of quinone based compounds with a broad range of substrate specificity we examine drug-enzyme interactions and suggest how DTD substrate specificity might be further optimised.

Keywords: DT-Diaphorase; flavoprotein; bioreduction; molecular model

INTRODUCTION

DTD is a cytosolic flavoprotein that catalyses the two-electron reduction of different compounds including quinones [1]. Its physiological function is

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believed to be the detoxification of quinones, although in certain cases the hydroquinone generated may undergo further reactions leading to DNA-damaging species [2]. This ability to activate quinone-based compounds in conjunction with the fact that elevated levels of DTD activity have been noted in several types of human tumour, particularly NSCLC, has led to interest in this enzyme as a target for anti-cancer drug design. The notion of 'enzyme-directed bioreductive drug development' [3] requires the identification of patients with elevated levels of enzyme activity in the tumour and treatment with drugs specifically activated by such an enzyme. DTD is an enzyme where this approach can be used and hence is a potential target for rational drug design. Whilst the three-dimensional structural co-ordinates are only available for the rat structure [4], both rat [5] and human [6] sequences are known and share high homology.

Relatively minor structural changes to substrates result in major changes in both substrate specificity and cytotoxic potency. Some quinone based substrates are shown in Figure 1. Replacement of the aziridine moiety in

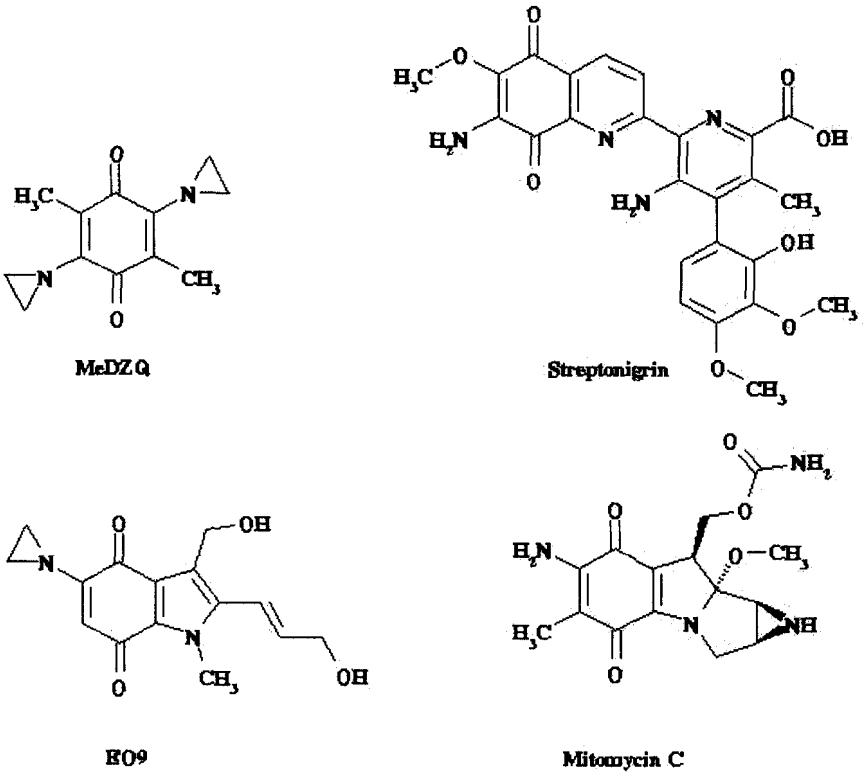

FIGURE 1 Quinone-based DTD substrates.

EO9 has dramatic results. Conversion to —OMe results in a loss of a third in rate of binding, and addition of $-CH_3$ to the aziridine or opening of the ring results in almost complete loss of binding [7]. Additionally, substituting the OH groups for larger groups could also affect binding [8]. By using molecular modelling we hope to explain these observations and to use the models to improve substrate specificity.

METHODS

Modelling Human DTD

Human DTD was modelled with homology to the available rat DTD structure including included tetramethyl-1,4-benzoquinone (duroquinone), Cibacron blue (a potent inhibitor) and FAD (PDB entry 1QRD; [4]). The amino-acid sequences had 85% identity and 91% similarity with a single amino-acid insertion at the N-terminus. The physiological dimer was created from the PDB coordinates using appropriate transformations.

The model was minimised with the substrate, inhibitor and co-factor constrained to their spatial positions. Partial charges for the inhibitor, co-factor and substrate atoms were determined from *ab-initio* calculations at the STO-3G level. The complex was minimised using the CHARMM force field [9]. The resulting structure was used as a starting model for docking simulations.

Docking Substrates

Optimal drug binding orientations were identified using GRID [10] calculations on the minimised human DTD model after the duroquinone (DQN) substrate and cibacron blue (CBD) inhibitor were removed. GRID contours for the aromatic carbon probe indicated that the aromatic moeities of the substrates were likely to protrude in only one direction within the binding site. This direction led to the space previously occupied by the inhibitor. Other probes were used in determining binding orientations. Using these GRID contours as a guide, substrates were docked into the enzyme at one of the active sites.

Each enzyme-substrate complex then underwent a minimisation protocol of 500 steps Steepest Descent on hydrogens alone, 5000 steps Steepest Descent on sidechains, FAD and substrate and finally 15000 steps ABNR on sidechains, FAD and substrate. The final structures were observed in order to identify any interactions which could explain the experimental results regarding sensitivity to substrate modifications.

RESULTS AND DISCUSSION

All the quinone-based substrates moved with respect to DQN in order to allow for steric interactions on one side of the molecule (*e.g.*, the aziridine of EO9). Analysis of this region showed that Trp 106 provides a 'wall' against which the two CH_2 groups of the aziridine produce a hydrophobic contact. The distance is enough to allow the aziridine or an —OMe to interact favourably. The addition of a CH_3 group to the aziridine, or ring opening, evidently provides a steric interaction with the Trp which forces the double-bonded oxygen atoms to be unfavourably positioned for reduction and hence the rate is significantly or totally reduced. Figure 2 shows EO9 in its position in the final model with the aziridine group next to Trp 106.

The substitution of the OH group marked (a) in Figure 2 with a bulky group (*e.g.*, aromatic) also significantly reduces activity. The model shows

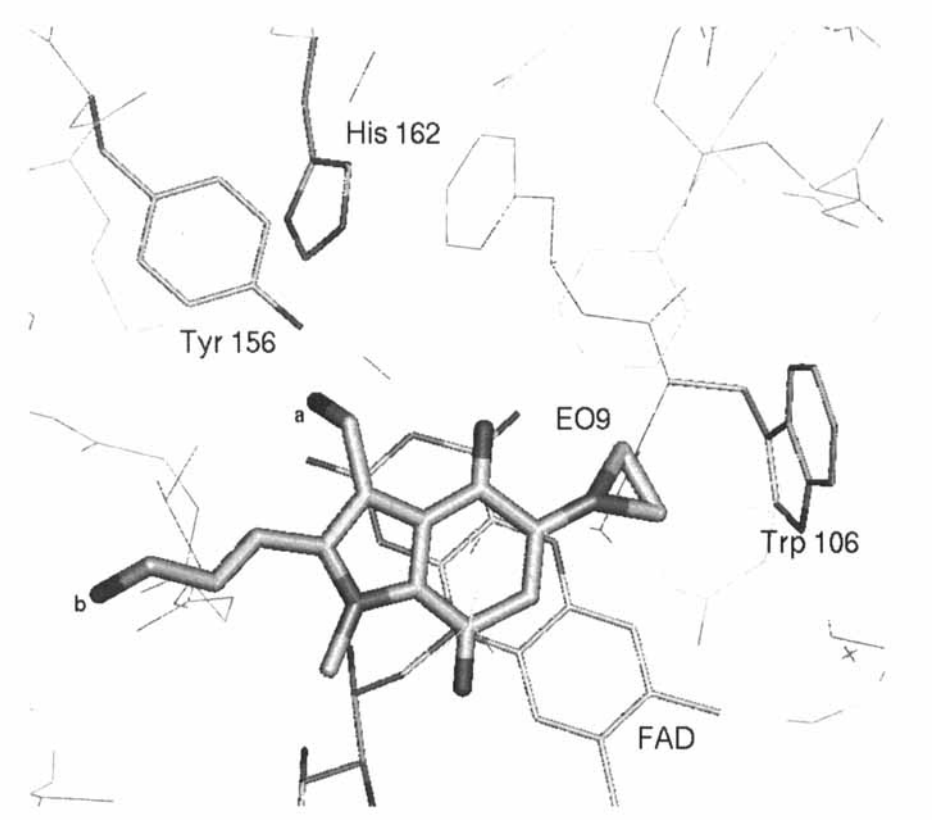

FIGURE 2 EO9 docked in active site near Trp 106. (See Color Plate I).

that this group will be within close proximity to the residues Tyr 156 and His 162 which have been postulated to be essential components in the reduction mechanism [4]. In contrast, alterations at the OH group (b), located in the binding site entrance, tend to be tolerated.

The MeDZQ binding model placed the aziridines in the same position as those of EO9. Streptonigrin filled the binding pocket very well, the final structure overlaying regions previously occupied by the inhibitor CBD in the original structure. Mitomycin C bound slightly out-of-plane with respect to DQN and the other substrates.

Further work using MM/QM calculations is needed to study the mechanistic implications of these models, although they do suggest explanations for some experimental data and should also significantly assist rational drug design of novel substrates for this enzyme.

CONCLUSIONS

A model of the human DTD structure was constructed with homology to the known rat structure. Models of substrates bound to the enzyme were produced. The models suggest that the small modifications that result in significant differences in the ability of quinone-based compounds to be reduced, do so due to steric interactions in the active site. In particular Trp 106 provides a 'wall' at the rear of the active site and certain substitutions can disrupt mechanistically essential amino-acids. These models therefore indicate structural restraints on potential bioreductive quinone-based anti-tumour compounds which are metabolised by DTD, and hence can assist in the rational design of such drugs.

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