MOLECULAR MODELLING CALCULATIONS ON THE BINDING OF D- AND L-XYLOSE TO WILD-TYPE ALDOSE REDUCTASE AND ITS H110Q AND H110A MUTANTS

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INTRODUCTION

Human aldose reductase (hAR), an NADPH-dependent enzyme, catalyzes the reversible reduction of a wide variety of carbonyl-containing compounds to their respective alcohol counterparts (Flynn and Green, 1993). Although a physiological role for this enzyme has not yet been established, inhibitors of hAR appear to be effective in the treatment of diabetic neuropathy (Masson and Boulton, 1990).

The active site pocket of hAR is lined by seven aromatic and four apolar residues, and is very hydrophobic. The only polar side chains within the pocket are Gln49, Cys298, Tyr48 and His110. The nicotinamide ring of the NADPH cofactor is located at the bottom of the pocket, with the hydroxyl group of Tyr48 and the imidazole ring of His110 positioned above it.

The catalytic mechanism of hAR appears to be relatively simple, involving the protonation of the substrate by an acid-base catalyst and a stereospecific transfer of the 4-pro-R hydrogen, presumably in the form of a hydride ion, from the C4 of the nicotinamide ring to the carbonyl carbon of the substrate. An important step in the mechanism is the transfer of a proton from a proton donor group in the enzyme to the carbonyl oxygen of the substrate. Cys298, Tyr48 and His110 are all proton donor candidates, however because Cys298 is not conserved in the aldo-keto superfamily, its role as a putative proton donor group in hAR is considered less likely (Bohren et al., 1989). In order for His110 to act as a proton donor, it is necessary that its Ne atom is protonated when the carbonyl substrate is bound. Unfortunately, X-ray diffraction cannot locate hydrogen atoms as a result of their weak electron density. Thus, any experimental information concerning the protonation state of His110 in hAR is not available. Additionally, the preferred binding conformation of substrate in the active site pocket of hAR has not yet become available, presumably because...
of the difficulties in either crystallising a suitable hAR-substrate complex or soaking substrate into crystals. Therefore, molecular modelling calculations on the binding of two substrates, D- and L-xylose, in the active site of wild-type hAR and two of its site-directed mutants (H110A and H110Q) have been performed in order to elucidate a picture of the protonation state of His110 with these substrates bound (De Winter and von Itzstein, 1994). In this study we have built the eight different enzyme-substrate (ES) complexes for which the interaction enthalpies between enzyme (E) and substrate (S) were calculated in the following manner. Firstly, the H110A and H110Q mutants of the wild-type enzyme were constructed by replacing His110 with the appropriate residue. In addition, two wild-type configurations were considered, one in which His110 was configured with Nε protonated (hereafter referred to as the HisI configuration), and one in which Nδ was protonated (HisII configuration). Thus, in total, four different E systems were investigated. Secondly, either D- or L-xylose was manually docked into the active site of each enzyme, yielding eight different ES complexes. Finally, conformational space of the ES complexes within the active site was explored using a slightly adapted procedure of Taylor and von Itzstein (1994), which gave eleven interaction enthalpies ($\Delta H_{\text{inter}}(E-S)$) for each complex. These interaction enthalpies have then been used to deduce an estimation of the corresponding $K_M$ constants, and the differences between the predicted and experimental $K_M$'s have been used to obtain a picture of the protonation state of His110.

Figure 1. (a) A plot of the average interaction enthalpies against the experimental $\log(K_M)$ values for the eight enzyme-substrate complexes. The six points which were used for the calculation of the linear regression line are shown by black squares. The equation of the fitted line through these points is $\log(K_M) = 11.2 + 0.33\Delta H_{\text{inter}}$. The abbreviations used are: HI-D (HisI/D-xylose); HII-D (HisII/D-xylose); A-D (Ala/D-xylose); Q-D (Gln/D-xylose); HI-L (HisI/L-xylose); HII-L (HisII/L-xylose); A-L (Ala/L-xylose); Q-L (Gln/L-xylose). Dotted squares show the corresponding values of the HisII configuration complexes with either D-xylose (HII-D) or L-xylose (HII-L). (b) As for (a) except that the HisII values instead of HisI values were used for the calculation of the regression line. Dotted squares show the values of the HisI configurations with either D-xylose (HI-D) or L-xylose (HI-L).