Rational Drug Design: Binding Free Energy Differences of Carbonic Anhydrase Inhibitors

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The free energy perturbation method has been applied to calculate the binding energy of sulphonamide inhibitors to carbonic anhydrase; the agreement with experiment gives further evidence for the reliability of this method even for anionic inhibitors and supports its use in drug design.

A prerequisite for rational drug design is the prediction of binding free energy differences between modified compounds acting at the same receptor. To be totally convincing, such calculations must include dynamic effects in both macromolecule and ligand, they must include solvation and entropic effects, and they must yield differences in free energy of binding rather than enthalpies, so as to conform with experiment. The free energy perturbation approach seems capable of fulfilling these requirements and recent studies[1—3] have suggested that the difference in binding free energies of a ligand to an enzyme can be calculated to an accuracy of better than 1 kcal mol^{-1} (cal = 4.184 J). Here, we extend that type of study to a more clinical example, the inhibition of carbonic anhydrase. The inhibitors considered are negatively charged and it is particularly encouraging that the optimistic hopes for the method are fully justified, even for anionic ligands which are normally difficult to treat theoretically. The zinc metalloenzyme carbonic anhydrase [E.C.4.2.1.1] catalyses the reversible hydration of carbon dioxide. Its sulphonamide inhibitors have found wide application as diuretics, antiepileptic agents, and as a possible treatment of glaucoma.[4] Their action is mainly exerted *via* the unsubstituted sulphonamido group which co-ordinates with the zinc ion in the enzyme active site as SO_2NH^-, mimicking the transition state. The wide range of affinity constants spanned by these inhibitors depends significantly on the electronic structure of their common fragment modulated by the substituents.[5,6]

In this work, our aim is to obtain quantitatively correct values of the binding free energy differences on making a chemical modification of the inhibitor, just as the experimentalist has traditionally done. For this purpose we have chosen to study the binding of *p*-chlorobenzenesulphonamide and benzenesulphonamide to carbonic anhydrase using the free energy perturbation method, within the framework of a molecular dynamics simulation, as incorporated in the AMBER suite of programs.[7]

The perturbation consists of a hypothetical transformation of the anionic form (H^-) of benzenesulphonamide (H) into the anionic form (Cl^-) of *p*-chlorobenzenesulphonamide (Cl) as shown in the thermodynamic cycle in Scheme 1, where CA

represents the enzyme. The difference in binding energy, $\Delta G_6 - \Delta G_5$, can be related to $\Delta G_7 - \Delta G_4$ at high pH where the sulphonamide is in its anionic form in solution; at pH 6.5, for which an experimental binding energy is available it is also necessary to consider the free energy of ionisation and the difference in free energy of hydration of the neutral forms of H and Cl ($\Delta G_3 = \Delta G_4 - \Delta G_2 + \Delta G_1$). Fortunately, H and Cl have very similar pK_a values (9.88 and 9.95 respectively[8]) and this correction can be ignored, though it is given in Table 1. In this cycle, the solvation free energy difference between the two inhibitors (ΔG_4) and the binding free energy difference for the two inhibitors in the enzyme–inhibitor complex (ΔG_7) can be obtained by considering a set of intermediate states coupled to the dimensionless parameter λ in the range of 0—1; $\lambda = 0$ represents the Hamiltonian for Cl^- and $\lambda = 1$ represents the Hamiltonian for H^-. The free energy change between each state is calculated in a number of windows from equation (1),[9] where ΔH is obtained by changing the Hamiltonian from that of the reference state by the amount corresponding to $\Delta\lambda$; this change may occur in both the (+) and (−) directions. $<>_\lambda$ indicates that the average is to be taken over the state characterized by the coupling parameter λ.

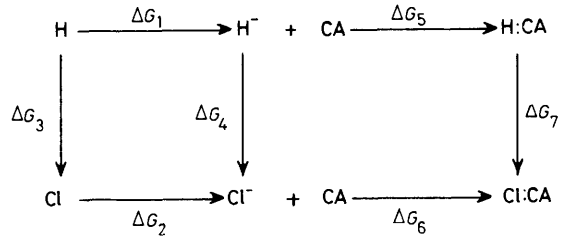
$$\Delta G = -RT \ln <\exp(-\Delta H/RT)>_\lambda \quad (1)$$

The co-ordinates for the native human erythrocite carbonic anhydrase C enzyme were taken from the 2.0 Å resolution *X*-ray structure.[10,11] The starting geometry of the enzyme–inhibitor complex was generated with the aid of the computer graphics program HYDRA.[12] The drug atoms were assigned parameters consistent with the AMBER force field,[13—15] and further details are given in ref. 16. For the solvent, the Jorgensen TIP3P water potentials[17] were used throughout.

For the enzyme–inhibitor system, a bath of water molecules was centred on the atomic co-ordinates of the inhibitor sulphur atom. Water molecules which were in a short contact (<2.4 Å) with any atom on the solute and which were beyond 18 Å of the inhibitor were eliminated. All the water molecules and the amino acid residues lying within 15 Å of the benzenesulphonamide were allowed to move in the minimization and molecular dynamics simulations; other residues were included in the determination of the energy and forces, but were fixed at their starting positions. The structure was minimized as follows: initially only the water molecules were minimized, so as to relax the sphere of water without disrupting the enzyme, then the whole structure within the 15 Å sphere was minimized. Harmonic forces were used to restrain any water molecules from leaving the 15—18 Å boundary; this was achieved by constraining the oxygen atoms of these water molecules to their initial position using a force constant of 0.6 kcal $Å^{-1}$; the constraint co-ordinates were updated every few hundred steps. The minimized structure was equilibrated for 5.25 ps (10 500 steps with a time step of 0.0005 ps) at 298 K, using SHAKE[18] and a 10 Å non-bonded cutoff. A slightly longer cutoff was used for the enzyme simulation as electrostatic effects are more important in enzymes owing to the charged side chains. However, it must be noted that because the configurations are generated according to the Hamiltonian for state λ, both the perturbed and unperturbed structures are treated equally. It should also be noted in this context that both perturbed and unperturbed inhibitors have the same charge.

In order to calculate the difference in free energy of hydration of the two inhibitors, the benzenesulphonamide anion was placed in a box of 411 water molecules which had been generated by a Monte Carlo simulation. After minimization the system was equilibrated for 14 ps (8000 steps with time step of 0.0005 ps followed by 5000 steps with a time step of 0.002 ps) at constant temperature and pressure (298 K, 1 atm) under periodic boundary conditions[19] and an 8 Å non-bonded cutoff. SHAKE was used to constrain bond lengths to their equilibrium values. A constant dielectric constant of 1.0 was used throughout. Because changing a hydrogen atom to a chlorine atom does not represent a large volume change, the differing boundary conditions used in the two parts of the simulation are unlikely to introduce major errors; periodic boundary conditions were not used in the enzyme simulation as this would have involved many more water molecules and would have been prohibitively expensive.

Perturbation calculations were carried out, in both cases, by a series of 21 successive simulations, starting with the values of the coupling parameter $\lambda = 1$ and incrementing by −0.05. To ensure that the average was evaluated over the most important configurations, 500 steps of equilibration and 500 steps of data collection were applied to each window. Owing to the serious computational demands of the calculation (8 h Cray X-MP/48



Scheme 1

Table 1. Relative free energies of hydration and binding in kJ mol^{-1}.

	ΔG_4 Inhibitor	ΔG_7 Enzyme–inhibitor	$\Delta\Delta G$ theory	$\Delta\Delta G$ expt.[a]
$(H^- \rightarrow Cl^-)$				
(+)	−30.79	−33.15		
(−)	−31.25	−33.10		
Average $(H^- \rightarrow Cl^-)$	−31.02	−33.12		
$(Cl^- \rightarrow H^-)$				
(+)	27.36			
(−)	27.92			
Average $(Cl^- \rightarrow H^-)$	27.64			
Average	−29.33 (+1.69)[b]	−33.12	−3.80 (−4.20)[c]	−6.31

[a] From ref. 20. [b] The standard deviation provides an estimation of the statistical errors in the simulation.[21] [c] Corrected for differences in pK_a.

time), the determination of ΔG_7 for the simulation running in the direction ($Cl^- \rightarrow H^-$) has not been carried out.

The results of the calculations are reported in Table 1, together with the experimental relative binding energy.[20] The calculated $\Delta\Delta G$ value of -3.80 kJ mol^{-1} was obtained by subtracting the average of the ΔG_4 values for the $H^- \rightarrow Cl^-$ and $Cl^- \rightarrow H^-$ simulations from ΔG_7. The theoretical and experimental values differ by only 2.5 kJ mol^{-1} (0.6 kcal mol^{-1}); the level of agreement is within the statistical errors inherent in the methodology.

The results justify the optimism building around the free energy perturbation method. Its reliability has now been tested on anionic ligands, for which the relative free energies of solvation and desolvation often play a major role in determining the relative binding affinities for a common receptor site. This provides a convincing argument that the method will be very useful in aiding the design of molecules to fit a specific enzyme binding site.

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