BINDING ENERGY AND ENZYMIC CATALYSIS

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Summary

Substrate-solid interactions may provide a better estimate of substrate-enzyme interactions than do substrate-solvent interactions. The free-energy of transfer of a methylene group from water to a non-polar solid is -2.0 kcal mol^{-1} which is more favourable than previous estimates of the binding energy of this group.

The intrinsic binding energy resulting from the non-covalent interaction of a substrate with an enzyme appears to be responsible for a large fraction of the difference in rates of enzyme and non-enzyme catalysed reactions.[1] These binding forces compensate for "entropic strain"[1 2 3] and destabilisation of the substrate brought about by geometric, electrostatic and solvation effects.[3]

The addition of the small molecule A as a substituent to the larger molecule B to form AB often results in a much more favourable free-energy of binding for AB compared with the sum of the binding energies of A and B, when compared at the same standard states. This is due to the loss of entropy upon binding being very similar for the two molecules B and AB and hence the more favourable free-energy of binding AB reflects mainly the intrinsic binding energy between A and the enzyme - the anchor principle.[1] However, this principle does not explain why these approximated

intrinsic binding energies are often much more favourable than those indicated by "hydrophobic interactions" estimated from the observed free-energies of transfer of the substituent A from water to non-aqueous solvents.[3] For example, the free-energy of transfer of a methylene group from water to amino acid - tRNA synthetases, ca. -3.3 kcal. mol^{-1}, equation 1, is more than three times as favourable as that for transfer of a methylene group from water to a non-polar liquid, ca. -1.0 kcal.mol^{-1},[5] equation 2.

$$\underline{\overline{-CH_2-}}_{\text{water}} \xrightarrow{\Delta G = -3.3 \text{ kcal.mol}^{-1}} \underline{\overline{-CH_2-}}_{\text{enzyme}} \qquad (1)$$

$$\underline{\overline{-CH_2-}}_{\text{water}} \xrightarrow{\Delta G = -1.0 \text{ kcal.mol}^{-1}} \underline{\overline{-CH_2-}}_{\text{non-polar liquid}} \qquad (2)$$

If, as is often suggested, the interior of a protein is analogous to an organic liquid these two processes should show similar free-energy changes. There are many other examples which show that these estimations of the interactions between a substituent and a non-polar liquid underestimate the forces between the same substituent and an enzyme.[3] A better estimate of these forces may be obtained from the free-energy of transfer of the substituent from water to the solid phase. The maximum favourable free-energy of transfer of a methylene group from water to a non-polar solid is about -2.0 kcal.mol^{-1}. This value is obtained using the enthalpies of fusion of hydrocarbons in addition to equation (2). This procedure thus accounts for a significant part of the observed binding energy that cannot be explained by previous estimations. However, substrate-enzyme interactions appear to be greater than substrate-solvent or substrate-solid interactions presumably because the number, and possibly the magnitude, of the dispersion forces are greater within the enzyme-substrate complex.

There are several reasons for believing that the number of the

favourable dispersion or van der Waals interactions of a substituent with an enzyme are greater than with a liquid solvent.

The atoms in an enzyme molecule are very closely packed since they are linked together by covalent bonds and, in some cases, the polypeptide chains are cross-linked by covalent disulphide bonds. The fraction of space occupied by the atoms in a molecule of protein is 0.76[6] which may be compared with 0.74, the value for the most efficient known way of packing spheres, cubic close-packing or hexagonal close-packing. In a liquid the fraction of space occupied is very much less, for example upper limits for water, cyclohexane and carbon tetrachloride are 0.36, 0.44 and 0.44, respectively.[6] The inside of a protein thus contains little space and is more analogous to a solid than to a liquid. It may be more appropriate, therefore, to compare equation (1) with the free energy of transfer of a substituent from water to a solid, equation (3). An indication of this energy change may be estimated from the fusion of hydrocarbons. The

$$\underline{\overline{-CH_2-}}_{\text{water}} \longrightarrow \underline{\overline{-CH_2-}}_{\text{pure liquid}} \longrightarrow \underline{\overline{-CH_2-}}_{\text{solid}} \qquad (3)$$

enthalpy of melting per additional methylene group in n-alkanes, up to $C_{20}H_{42}$, containing an even number of carbon atoms is 0.99 ± 0.03 kcal.mol^{-1}.[7] There is a phase transition just below the melting point of n-alkanes containing an odd number of carbon atoms and the enthalpy of melting per additional methylene group in this series is 0.89 ± 0.03 kcal.mol^{-1}, including the heat of transition.[7] The heat capacity changes per additional methylene group are also approximately constant, about 7 and 3.5 cal.deg^{-1} mol^{-} for the liquid and solid respectively.[7] The hypothetical enthalpy of melting per additional methylene group at, say, 25°C will therefore not deviate much from the above values. There will, of course, be an unfavourable entropy change on transfer from the liquid to the solid but it can now be estimated from equations (2) and (3) that the maximum favourable

free-energy of transfer of a methylene group from water to a non-polar solid is about -2.0 kcal.mol^{-1}.

The atoms in an enzyme surrounding the substrate are very compact and many are separated only by their covalent radii. In a liquid a solute is surrounded by molecules separated by their van der Waals radii. Hence the effective coordination number is much greater in the enzyme than it is in a liquid. For example, the van der Waals radii for carbon, nitrogen and oxygen are about 2.2 times greater than their respective covalent radii and a very simple treatment of the packing of spheres shows that the coordination number of a sphere is about 2.5 times greater when surrounded by other spheres separated by such covalent radii than when they are separated by van der Waals radii. In a liquid a large fraction of the surface of a solute molecule is surrounded by empty space between the solvent molecules.[8] Even in a non-polar solid the closest approach of neighbouring molecules is governed by their van der Waals radii therefore the energy of transfer of a group from water to a solid may underestimate the interaction energy between the same group and an enzyme.

The common procedure of estimating binding energies from the partitioning ratios of compounds between water and a non-polar solvent, equations (1) and (2), involves an examination of the following changes in interaction energies. Firstly, since the initial state is the same in the two systems, changes in energy when the solute is removed from water will be the same in each case. When a solute is transferred to a non-polar liquid, equation 2, there is a gain of solute-solvent interactions and a loss of solvent-solvent interactions, which is equivalent to the free-energy of formation of a cavity in the solvent of a suitable size to accommodate the solute molecule.[9] When a solute is transferred to an enzyme, equation 1, there is a gain of solute-enzyme interactions if water is initially absent from the binding site. If water is initially bound to the site of binding and is displaced by the substituent then there is also

a gain of water-water interactions and a loss of water-enzyme interactions. However, the sum of the free-energy changes in these two processes would be thermodynamically unfavourable, otherwise water would not initially be bound to the enzyme, and hence, in this situation, the observed free-energy of transfer of a substituent from water to an enzyme provides a lower limit to the magnitude of enzyme-substrate interactions.

Approximately 16 localised water molecules have been detected within the chymotrypsin molecule[10] but much larger numbers in general for proteins are probably unlikely.[11] If water is not initially bound to the enzyme then the transfer in equation (1) could be thermodynamically more favourable than that in (2) because the latter process involves a loss of solvent-solvent interactions, i.e., the free-energy of cavity formation, and thus the free-energy change for (2) provides an underestimation of solute-solvent interactions. According to the scaled particle theory,[12] and other calculations,[13] the free-energy of cavity formation in non-polar liquids is of similar magnitude, but, of course, of opposite sign, to the free-energy of interaction of the solute with the solvent. This, therefore, may explain, in some cases, part of the discrepancy between equations (1) and (2) since the enzyme may have a ready-made "cavity" and the binding of a substituent to this site would not result in the unfavourable changes which accompany cavity formation in liquids.

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