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How can enzymes be so efficient?

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Dudley Williams and his colleagues discuss how ligands can gain binding energy to their receptors, and substrate transition states to their enzymes, by tightening the protein structures, with a decrease in their dynamic behaviour.

Introduction

FREQUENTLY, the binding constants for the association of substrate transition states to enzymes are amazingly high, lying in the range $10^{16\pm4}$ M^{-1}.[1–5] Additionally, in the cases of many substrate/receptor interactions, the high strength of the binding is difficult to understand. For example, the small molecule biotin binds to the protein receptor streptavidin with K = $10^{13.4}$ M^{-1}.[6,7] A common approach to the problem considers simply the non-covalent bonding (*e.g.*, hydrogen bonds) at the interface between the small molecule and the receptor system. But does Nature have other tricks up its sleeve to promote strong binding?

Origin of ligand binding energy

The vancomycin group of antibiotics form dimers that are in equilibrium with monomers. When the natural ligands that recognise the antibiotics bind to them, the ligand binding promotes dimerisation.[8(a)] Ligand binding can stabilise the dimer (the receptor system) by a factor of up to 100

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M^{-1}. This finding necessitates that ligand binding is stronger to the antibiotic dimer than to the antibiotic monomer.[8(b)] That is, ligand binding and dimerisation are positively cooperative. A structural model[9] to understand positively cooperative binding is given in Fig. 1. In Fig. 1, a portion of a protein receptor (blue) is shown prior to (above), and after (below), binding to a ligand (purple). The formation of the two hydrogen bonds depicted within the receptor is opposed by the motions of the two peptide backbones of the receptor. The internal motions of the chain that is presented to the ligand can be reduced by the formation of hydrogen bonds from it to the ligand (Fig. 1, lower panel). Since motion opposes bonding, the restriction of the internal motions of this chain upon ligand binding results in strengthening of the hydrogen bonds within the receptor.

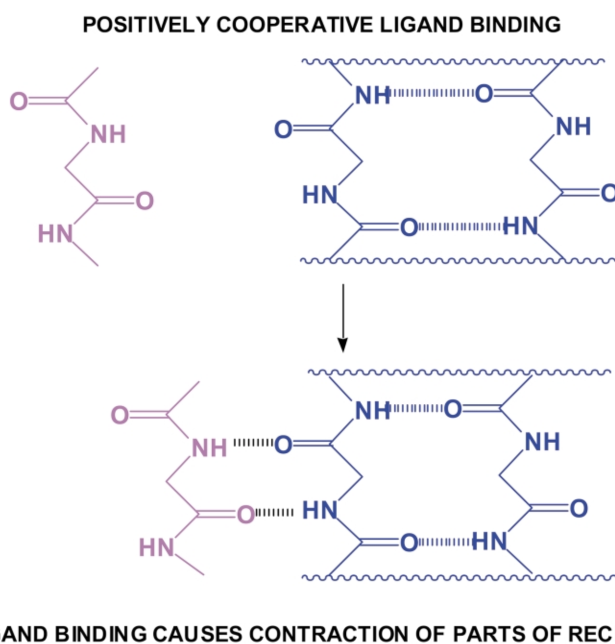

Fig. 1 Structural model for positively cooperative binding.

The 'damping down' of the motions of the depicted receptor residues (Fig. 1) upon ligand binding can, in turn, improve non-covalent bonding more deeply inside a receptor. Such transmission will result in an improvement in non-covalent bonding at *all* sites within the receptor that are coupled with positive cooperativity to ligand binding. Ligand binding to the receptor is improved because the receptor system is stabilised by ligand binding.

The restriction of internal dynamics of the receptor caused by positively cooperative ligand binding is usefully compared to cooling parts of the receptor. It occurs with an improvement in internal bonding (a benefit in enthalpy, ΔH) which outweighs the cost of the motional restriction (a cost in entropy, expressed in terms of TΔS). Also as in the case of cooling, it occurs with better packing within the receptor. A consequence of this better receptor packing will be a reduced degree of exchange of the affected receptor amide NHs following positively cooperative ligand binding.[10]

To test whether ligand binding energy is more generally derived in this manner, we determined the degree of deuterium exchange (from D_2O, as determined by electrospray mass spectrometry) of the amide NHs of streptavidin both in the absence and the presence of biotin.[10] Four molecules of biotin bind to a streptavidin tetramer, and it seemed plausible that the above model might apply since the binding is remarkably exothermic ($\Delta H = -134$ kJ mol^{-1}) and adverse in entropy ($T\Delta S = -57$ kJ mol^{-1}),[11] plausibly corresponding to extensive simultaneous tightening of the streptavidin tetramer. Through the binding of biotin, ~24 exchangeable amide hydrogens per sub-unit were protected from solvent exchange, and the protection was widely spread through the streptavidin structure. The extensive reduction in the dynamic behaviour of the receptor upon ligand binding is not evident from a comparison of the X-ray structure of streptavidin when free and when bound to biotin.[12–13] This is presumably because changes in receptor dynamics may be masked by crystal packing forces. Additionally, the associated changes in distances may be relatively small.

Binding of transition states by enzymes

Does the above origin of ligand binding energy through improved receptor packing find analogy in the binding of transition states by enzymes? If so, enzymes should improve their packing efficiency in the transition state for reaction. Thus, catalysis would be improved because the *enzyme* is further stabilised in the transition state/enzyme system. Two lines of evidence support this conclusion:

Enthalpy benefit

First, the benefit in entropy of enzyme catalysis (originating in the ordering of the catalytic groups on the enzyme structure) should be offset by the cost in entropy of reducing the dynamic behaviour of the enzyme in the transition state. Moreover, this proposed reduction in dynamic behaviour of the enzyme should provide a benefit in improved non-covalent bonding (benefit in enthalpy) within the enzyme. Strikingly, where data are available, enzyme-catalysed reactions are, relative to the corresponding reaction in solution, greatly favoured in enthalpy (Table 1).[2,14]

Table 1 Benefit in enthalpy ($\Delta\Delta H^{\#}$) of some enzyme-catalysed reactions relative to the reactions in free solution[2,14]

Enzyme	$\Delta\Delta H^{\#}$, kJ mol^{-1}	Rate Accel (s^{-1}) due to $\Delta\Delta H^{\#}$
Chorismate dismutase	−33	10^{6}
Chymotrypsin	−66	10^{12}
Staphylococcal nuclease	−63	10^{11}
Bacterial α-glucosidase	−80	10^{14}
Urease	−93	10^{16}
Yeast OMP decarboxylase	−143	10^{25}

In the case of cytidine deaminase, both the enthalpic and entropic contributions have been derived. Enzyme catalysis increases the reaction rate by 10^{16} M^{-1}, due to a benefit in enthalpy ($\Delta\Delta H^{\#}$) of −84 kJ mol^{-1}, and a benefit in entropy ($T\Delta\Delta S^{\#}$) of only 7 kJ mol^{-1}.[15] Almost all of the rate enhancement comes from overall improvements in bonding rather than from overall benefits in entropy. The data are consistent with extensive improvements in *bonding within the enzyme* in the transition state.

Reduction in backbone amide NH exchange

Second, enzymes should undergo less amide NH to ND exchange when bound to

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transition-state analogues. Two recent sets of data give convincing support to this idea. Hydrogen/deuterium (H/D) exchange into backbone amide bonds in hypoxanthine–guanine phosphoribosyl transferase (HGPRT) was used to study the dynamic properties of human HGPRT. The extent of exchange was measured for enzyme alone, for enzyme bound to reactant/product, and for enzyme bound to a transition-state analogue.[16] There are 207 backbone amide NHs in the enzyme. Of these, after 1 h in D_2O, the isolated enzyme exchanged 160, an equilibrium reactant/product complex exchanged 139, and the transition-state analogue complex exchanged 126. Thus, the enzyme structure becomes better packed to provide binding energy for the reactant/product, and then the packing is improved further to provide even greater binding energy for the transition-state analogue. Equally striking results are found for the binding of a transition-state analogue to a purine nucleoside phosphorylase, which is a trimer.[17] The transition-state analogue (immucillin–H) binds to the enzyme extremely strongly ($K = 23 \times 10^{-12}\ M^{-1}$) when only one of the three catalytic sites is occupied. Deuterium exchange occurred at 167 slow-exchange sites in 2 h when no catalytic site ligands are present. A substrate analogue and product prevented H/D exchange at 10 of these sites. When only one of the three sites of the homo-trimer was filled with the transition-state analogue immucillin–H, 27 of the slow exchange sites were protected from exchange in *all three* sub-units. The hallmark of the positively cooperative binding of the transition-state analogue is to reduce the dynamic behaviour of the receptor (trimer) system to such a degree that a further 81 backbone NHs are protected from exchange. The reduction in dynamic behaviour occurs almost throughout the trimer, and binding energy of the transition-state analogue can therefore be derived in a highly delocalised manner.

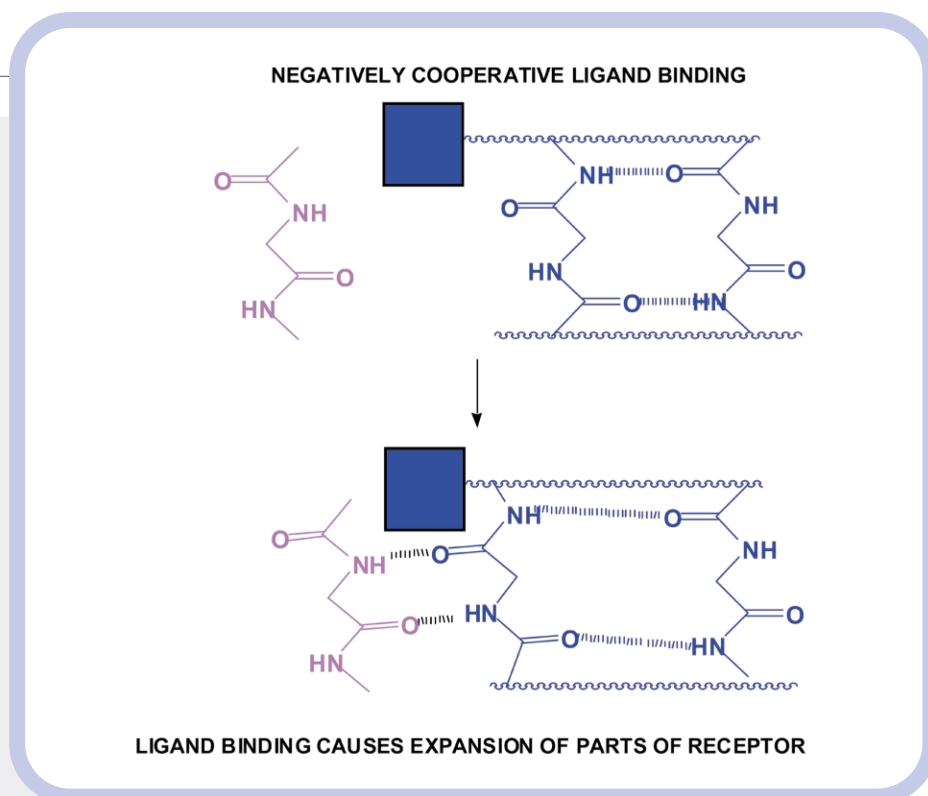

Fig. 2 Structural model for negatively cooperative binding.

Negative cooperativity

A structural model for negative cooperativity[9] is shown in Fig. 2. Here, the surface chain of the protein receptor must incorporate a structural feature (*e.g.*, steric inhibition by the blue square) that inhibits ligand binding to the structure of the isolated receptor. Thus, when ligand binding occurs, the ground state structure of the receptor must be distorted from its preferred geometry (Fig. 2, upper panel) to a state (Fig. 2, lower panel) in which its internal non-covalent bonding is weakened. Thus, ligand binding that is negative cooperativity should cause receptors to become more dynamic, and the extent of amide NH exchange of the receptor peptide backbone should increase upon ligand binding. Equally, the reduction in bonding efficiency within the receptor upon negatively cooperative ligand binding will make ligand binding less favourable in enthalpy, and more favourable in entropy than would otherwise be the case.

Using the above definition of negative cooperativity, the binding of O_2 to haemoglobin is seen to be negatively cooperative. Why this is so is evident from the classical model of Monod, Wyman and Changeux (MWC model, Fig. 3). The

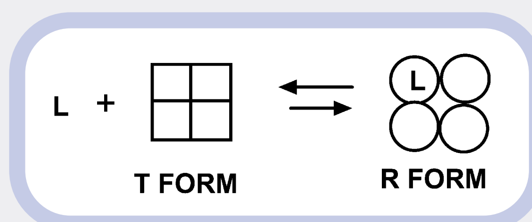

Fig. 3 Illustration of the MWC model for the binding of O_2 (in the general case, of a ligand, L) to the haemoglobin tetramer (showing binding of only the first ligand molecule).

available form of the free haemoglobin tetramer is the tense (T) form. The optimal binding of the ligand ($L = O_2$) is incompatible with the geometry existing in the T state. The receptor therefore adopts a modified geometry when O_2 is bound, and this modified geometry is that existing in the relaxed (R) state. The negatively cooperative binding does indeed force a loosening of the T state of the tetramer, through the breaking of inter-subunit salt bridges (as established by Perutz and co-workers[18]) to give the R state. However, *all* non-covalent interactions within a receptor system that are *coupled* with negative cooperativity to ligand binding should loosen. To test this conclusion, we determined the change in dynamic behaviour of the haemoglobin tetramer polypeptide backbone when it binds O_2 with negative cooperativity.

Upon the binding of oxygen to haemoglobin, a further 7–8 backbone amide NHs per α-chain, and a further 16 per β-chain, undergo H/D exchange upon exposure to D_2O.[10] These large effects are in accord with the predictions regarding the changes associated with negatively cooperative binding. Interestingly, they were not evident from prior X-ray studies. This is again presumably because the dynamic changes that can be picked up by experiments carried out in solution are masked by crystal packing forces, and/or the changes in distances of the affected regions of the protein were too small to be picked up.

We note that the binding of O_2 to haemoglobin is, using a different definition of cooperativity, defined to be *positively* cooperative. This is because the work required to populate the R form of the tetramer is, through communication between the sub-units, largely achieved upon the binding of the first molecule of

the O_2 ligand (Fig. 3). Therefore, *subsequently* binding O_2 ligands bind *more strongly* (to the now available R form), and this justifies a description of positively cooperative binding. However, this definition of positively cooperative binding is applicable only where there are multiple ligand binding sites, and the definitions adopted in Figs. 1 and 2 are of wider applicability. They also have the advantage of accounting naturally for occurrence of tense (T) and relaxed (R) form of receptors in the MWC model, because it is negatively cooperative ligand binding that drives the relaxation (= loosening).

Summing up

In summary, ligands can gain binding energy to their receptors, and substrate transition states to their enzymes, by tightening the protein structures with a decrease in their dynamic behaviour. These benefits add to our existing knowledge of the origins of non-covalent binding energies. Large benefits require large proteins, and hence we can see a reason why enzymes are large. The decreases in dynamic behaviour are accompanied by benefits in enthalpy and costs in entropy, and by a reduction in the extent of backbone amide NH exchange. Negative cooperativity is associated with the opposite effects. The findings have implications for protein/protein recognition, which is crucial in studies of the proteome. The binding between two proteins is not simply a function of the properties of the surface patches of the proteins that come together. It is also a function of how these interactions internally modify the structures of the proteins.

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