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Changes in motion *vs.* bonding in positively *vs.* negatively cooperative interactions

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From a consideration of the interactions between non-covalent bonds, it is concluded that positively cooperative binding will occur with a benefit in enthalpy and a cost in entropy, and that negatively cooperative binding will occur with a cost in enthalpy and a benefit in entropy; experimental data support these conclusions.

In this communication, we illustrate the relationship between changes in intermolecular motion in non-covalent complexes and cooperative binding. We define an interaction as occurring with positive cooperativity with respect to a second interaction when the binding affinity associated with the first interaction is increased upon addition of the second interaction. Conversely, an interaction occurs with negative cooperativity with respect to a second interaction when the binding affinity associated with the first interaction is decreased upon the addition of the second interaction.

Consider a structural model for positive cooperativity. Two sets of amide–amide hydrogen bonds that can be made simultaneously are shown in Fig. 1. When made separately, they would consist of (i) the rightmost chain hydrogen bonded to the central chain (set R) and (ii) the leftmost chain hydrogen bonded to the central chain (set L). Consider set R made in isolation; the formation of the hydrogen bonds is opposed by the relative motions of the two chains. These include the relative translational motions of the chains, and the internal wagging of the chains. The internal wagging of the central chain can be reduced by forming hydrogen bonds from it to the leftmost chain, *i.e.*, by making the two sets of interactions simultaneously. Put in another way, the central chain forms a better template to form the hydrogen bonds of the R set if the central chain is simultaneously hydrogen bonded to form the L set. Thus, the two sets of hydrogen bonds can be strengthened by their simultaneous formation, and the system forms a model to illustrate positive cooperativity.

In the above model, the positive cooperativity is due to one set of hydrogen bonds reducing the motions associated with the other set. This reduction in motion is usefully compared to the effects of a reduction in temperature. On reducing the temperature of a pure substance, whether liquid or solid, it is universally observed that the change is favourable in enthalpy[1] (the bonding within the substance is improved). It is also adverse in entropy (the order within the substance is increased). It is also almost universally observed that a temperature reduction leads to a volume reduction of a substance (the water to ice transition is a rare exception). In summary, the internal 'tightening' of the structure upon the temperature reduction is universally associated with an enthalpic benefit and an entropic cost, and typically associated with a volume reduction, and these changes should correspond to those that are characteristic of positive cooperativity.

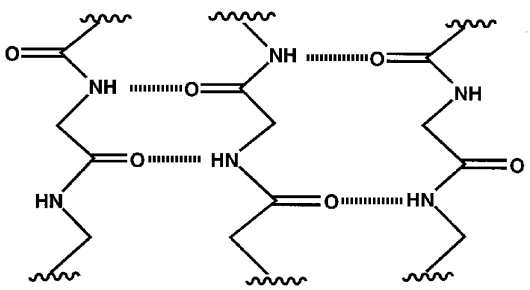

Fig. 1 Structural model for positive cooperativity (see text for details).

This construction of positive cooperativity, involving the interaction of two non-covalently bonded interfaces, permits an understanding of the consequences of positive cooperativity as observed in numerous studies. Thus, NOES indicate reductions in non-covalent bond lengths (*cf.* volume reduction) within a β-hairpin when this structure is extended to a β-sheet.[2] In the dimerisation of glycopeptide antibiotics, positive cooperativity due to concomitant ligand binding is associated with structural tightening.[3] In the case of vancomycin, this phenomenon has been investigated by isothermal calorimetry in the physiologically relevant pH range 3–7.[4] In 9 cases in this pH range, the positive cooperativity is always accompanied by an increase in exothermicity. In 8 of these 9 cases, the positively cooperative binding is accompanied by an increase in the adverse entropy of binding (the exception is the ligand di–Ac–Lys–D–Ala–D–Ala and where dimerisation is studied at pH 3). Additionally, the thermodynamics of unfolding of polypeptides corresponding to different segments of the ankyrin repeats of Drosophila Notch have been investigated.[5] The data show that inclusion of a putative seventh, C-terminal ankyrin sequence doubles the stability of the Notch ankyrin domain (a 1000-fold increase in the folding equilibrium constant). The positively cooperative binding of the seventh sequence is associated with a *ca.* 50% increase in enthalpy of unfolding.

Even where positively cooperative binding occurs at a *single* non-covalently bonded interface, structural tightening is observed, and is correlated with increased exothermicity and increased adverse entropy of binding.[6]

Similar effects are found when cooperative binding is expressed in the process of crystallisation. For a series of hydrocarbons, the greater the degree of positive cooperativity that is exercised in a crystallisation process, the greater is the exothermicity, the adverse entropy, and volume contraction upon crystallization.[7]

The arguments above for cooling a substance as a model for positive cooperativity can be extended to propose warming a substance as a model for negative cooperativity. A schematic structural model to represent negative cooperativity is given in Fig. 2. The negative cooperativity arises through an inability to make simultaneously two non-covalent interfaces where each interface takes up the geometry that gives the optimal free energy of binding when each is made in isolation. In contrast to Fig. 1 where two interfaces can be made to mutual benefit, in Fig. 2 one interface is made at the cost of a distortion of the geometry preferred by the other. In contrast to the case of positive cooperativity, the impossibility of simultaneously making the two interfaces with each in the geometry that is optimal for the free energy of binding means that they are now overall less well bonded and mutually disordering, rather than mutually constraining. As a result, one or both of the interfaces of Fig. 2 are less well bonded and are more dynamic than when

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made in isolation (*i.e.*, one or both will be 'loosened'). Thus, the exercise of negative cooperativity should cause bonding to be more favourable in entropy, and less favourable in enthalpy.

Negative cooperativity caused by the introduction of mismatches within dodecamer DNA duplexes can be studied by observing the melting behaviour of the duplexes.[8] The introduction of only two mismatches into such duplexes causes, relative to the fully matched duplex, the loss of only two hydrogen bonds and a loss of duplex stability ($\Delta\Delta G$) of 46–47 kJ mol^{-1}. However, the introduction of the two mismatches causes large reductions in bonding within the dodecamers, and large increases in their internal motions. It is because these changes have opposing effects upon the changes in dodecamer stability that $\Delta\Delta G$ is smaller. Specifically, the introduction of the two mismatches reduces the experimentally determined enthalpy of duplex formation from −438 kJ mol^{-1} to −222 kJ mol^{-1} ($\Delta\Delta H$ = +216 kJ mol^{-1}), and reduces the entropy of duplex formation from −1159 J mol^{-1} K^{-1} to −587 J mol^{-1} K^{-1} ($\Delta\Delta S$ = +572 J mol^{-1} K^{-1}). Since the negative cooperativity reduces the melting temperature of the duplex, a correction (not previously considered[7]) must be made for the heat capacity change upon melting of the DNA. Several papers have recently determined the heat capacity change occurring upon melting DNA duplexes.[9–13] Values lie in the range 0.19–0.39 kJ K^{-1} mol^{-1} per base pair. We can therefore make a correction for the heat capacity effect upon $\Delta\Delta H$ which, since the matched and mis-matched duplexes melt at 345 and 310 K, respectively, lies in the range 80–164 kJ mol^{-1}. Therefore, the introduction of only two mis-matches reduces the favorable enthalpy of the dodecamer duplex formation by a value in the range 52–136 kJ mol^{-1}. This is a large effect that suggests that the introduction of the *mis-matches* loosens the non-covalent bonding between the *matched* bases in the dodecamer duplex.

We note also the negative cooperativity involved in the binding of O_2 to haemoglobin. The binding of O_2 to the tense (T) form of the haemoglobin tetramer is negatively cooperative with respect to O_2 binding to the relaxed (R) form.[14] In the terminology of the present paper, the T form is loosened towards the R form as negatively cooperative binding of O_2 occurs. Thus, as O_2 binding promotes the T to R transition, there should be an uptake of heat by, and increase in disorder within, the haemoglobin tetramer. This is exactly the situation found in the case of trout haemoglobin.[15] Starting from the T state, O_2 binding occurs with an exothermicity very near to zero, and a favourable $T\Delta S$ term of +21 kJ mol^{-1}. In contrast, O_2 binding to the R state is exothermic (ΔH = −32 kJ mol^{-1}) and slightly unfavourable in entropy ($T\Delta S$ = −3 kJ mol^{-1}). The difference in the two sets of thermodynamic data reflects the way in which O_2 binding promotes the disordering of the T state tetramer towards the R state tetramer.

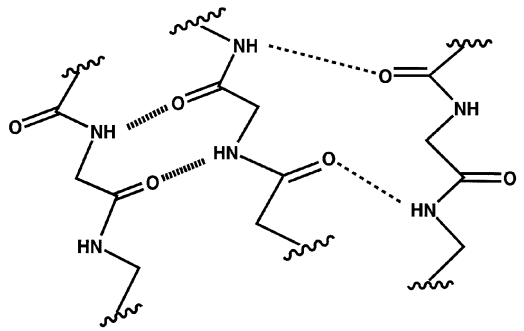

Fig. 2 Structural model for negative cooperativity (see text for details).

The model developed in this paper gives a plausible physical basis for the understanding of the enormous variations in enthalpies and entropies of binding found for a large selection (136) of drug-receptor interactions.[16] Among these, *ca.* 10% are remarkably *endothermic* (83–125 kJ mol^{-1}) and therefore highly entropy-driven. It is difficult to place a plausible physical interpretation upon these data unless this 10% binds such that there is negative cooperativity between the drug–receptor interface and the pre-existing interactions within the receptor. In this interpretation, the endothermicity is largely due to a reduction of bonding within the receptor upon drug binding and the favourable entropy term is due to the associated decrease of order within the receptor.

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