Pure & Appl. Chem., Vol. 66, Nos 10/11, pp. 1975–1982, 1994.
Printed in Great Britain.

Functional roles of natural products: The involvement of extended arrays of weak interactions in cooperative binding phenomena

Dudley H. Williams,* Mark S. Searle, Patrick Groves, Joel P. Mackay, Martin S. Westwell, Daniel A. Beauregard & Michael F. Cristofaro.

Cambridge Centre for Molecular Recognition, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

Abstract: A factorisation of the free energy of binding into various "costs" and "benefits" has provided the basis for a semi-quantitation of some weak interactions in solution. It has become clear that the entropic cost of motional restriction on binding (**A** + **B** → **A.B**) increases with increasing exothermicity of the association; this exothermicity must of course reflect not only interactions at the interface between **A** and **B**, but also the change in bonding throughout **B** (if **B** represents the receptor). We illustrate cooperativity and anti-cooperativity by reference to effects of ligand binding (as models of classical agonists and antagonists) on the dimerisation of vancomycin-group antibiotics. Since dimerization of receptors (promoted by ligand binding) is a common theme in biological signalling, it must presumably have an advantage in natural selection. We suggest that concurrent demands of ligand binding and receptor dimerization may permit a more specific control of those ligand structures which can cause signal transmission. In this way, specificity in biological signalling might be aided.

INTRODUCTION

Partitioning free energy contributions to binding in aqueous solution

In recent work (1-4), we have factorised the free energy of binding (ΔG, kJ mol^{-1}) for an association **A** + **B** → **A.B** into four terms that reflect various "costs" and "benefits" to binding in aqueous solution (equation 1):

$$\Delta G = \Delta G_{T+R} + \Delta G_r + \Delta G_h + \Sigma \Delta G_p \qquad \text{equation (1)}$$

This equation has been used as an approximation (for cases where the components **A** and **B** can associate in their conformational energy minima in the complex **A.B**, and where the van der Waals contacts between **A** and **B** in **A.B** are as good as those of separated **A** and **B** with solvent) in the hope that an attempted semi-quantitation will give insights into the complexities of weak interactions. The conclusions reached so far are now summarised:

1. The change in translational and rotational free energy (ΔG_{T+R}), opposes a bimolecular association up to a maximum value of *ca* 50-60 kJ mol^{-1} for a highly exothermic interaction (5) (analogous to covalent bond formation between **A** and **B**, where the relative molecular mass of **A** is *ca* 100-400, and **B** >100); but that this term [essentially an adverse entropy term, $300\Delta S_{T+R}$ near room temperature (300 K)] tends to zero as the exothermicity of association $\Delta H_{ass} \rightarrow 0$ (6). The experimental relationship between ΔH_{ass} and $300\Delta S_{ass}$ ($\approx 300\Delta S_{T+R}$) for many "one-point" associations in non-polar solvents is presented in Fig. 1 (4).

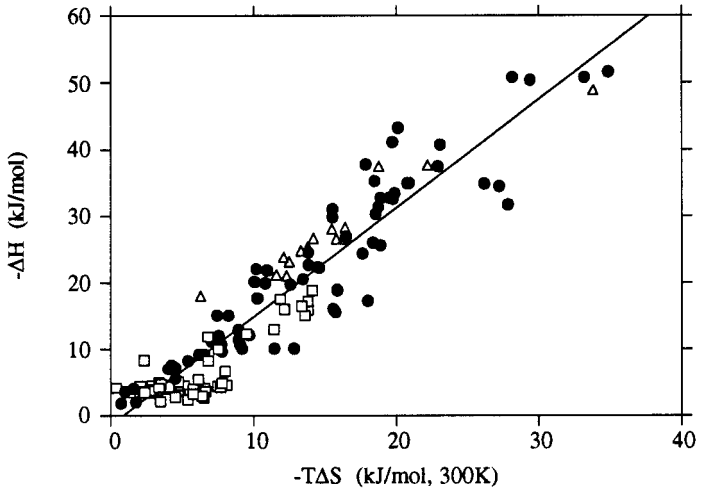


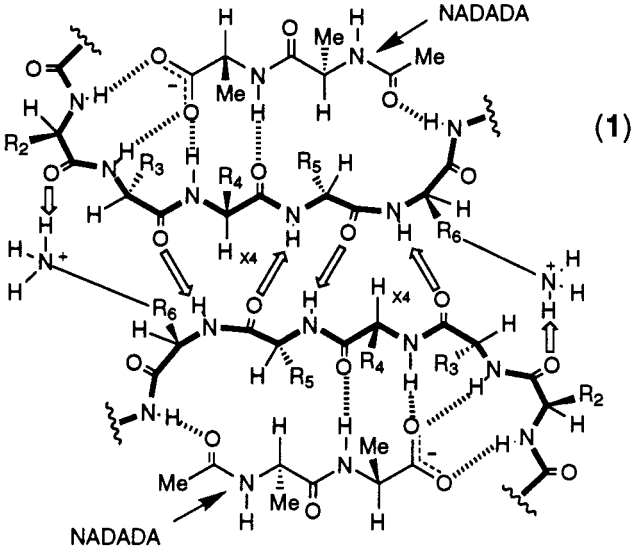
Fig. 1. Experimental enthalpy of association (ΔH_{ass}) versus entropy of association at 300 K (300 ΔS_{ass}) for weak associations in non-polar solvents. Data include charge transfer complexes, collision complexes and lactam dimerisations.

2. The change in free energy due to the restriction of internal rotations (ΔG_r), also opposes binding and appears to lie in the range 2-6 kJ mol^{-1} per restricted rotation at room temperature (5-7). The smaller value is applicable for the restriction of internal rotations where the adjacent interactions which give rise to the restriction are weakly exothermic (allowing appreciable residual torsion) (6). However, ΔG_r will increase gradually to the upper limit as the residual torsion is "frozen" due to strongly exothermic interactions presenting large barriers to internal rotation (5).

3. The promotion of the association due to the hydrophobic effect in aqueous solution (ΔG_h) is usefully expressed in terms of its surface area dependence and lies in the range -0.20 ± 0.05 kJ mol^{-1} Å^{-2} (2). Thus, if a total of x Å^{-2} of hydrocarbon surface area is removed from accessibility to water in the association **A** + **B** → **A.B**, then the favourable free energy change will be x(0.20 ± 0.05) kJ mol^{-1}. This value is in reasonably good agreement with the value (-0.23 kJ mol^{-1} Å^{-2}) for the hydrophobic effect determined from protein engineering experiments (8); and with that derived from solvent transfer data when corrected for the favourable entropy of mixing (9) - although it is not universally agreed whether such a correction is necessary.

4. The promotion of binding through pairs of polar functional group interaction energies ΔG_p (summed over all such pairs of interactions), has been estimated for the amide-amide hydrogen bond in aqueous solution to lie in the range -(1-7) kJ mol^{-1} (2). Again, this value is in reasonable agreement with the corresponding values [-(2-8) kJ mol^{-1}] from protein engineering experiments (10, 11).

In summary, it is clear that since the entropic cost of motional restriction (expressed in ΔG_{T+R} and ΔG_r) increases with increasing exothermicity of the association of **A** and **B** (which is expressed in $\Sigma\Delta G_p$), the three terms ΔG_{T+R}, ΔG_r and $\Sigma\Delta G_p$ interact. Consequently, the maximum (intrinsic) binding energy which can be obtained from the interaction of a pair of specified functional groups (12) is the binding energy observed when the introduction of the interaction is carried out without any cost in entropy (i.e., when the effects on ΔG_{T+R} and ΔG_r of the new interaction are zero). In general, for weak interactions this latter requirement will not be met and ΔG_p values will be less than their intrinsic values. An additional problem is that ΔG_p values will depend, in a cooperative manner, on the total extended array of interactions involved in binding. We address the problem of an extended array of interactions by analysing the cooperative and anti-cooperative effects of the binding of cell wall analogues to vancomycin group antibiotics upon antibiotic dimerisation. The extended agreggate is illustrated in **1**.



(1)

DISCUSSION

Cooperativity and dimerisation of vancomycin-group antibiotics

The binding constant for the interaction of **A** and **B** cannot necessarily be understood by examining the immediate interface between **A** and **B** in **A.B**, and the immediate solvent surroundings of those parts of **A** and **B** which interface in **A.B**. If **A** represents a ligand, and **B** a trans-membrane receptor, then any change in motion within the whole of **B** (affecting its entropy) or any change in bonding within the whole of **B** (affecting the heat change of the association) can affect the binding constant. A paradigm for illustrating this problem has long been available in the cooperative binding of oxygen to hemoglobin (13).

In the light of the complexity of the problem, how much progress can we hope to make in the semi-quantitation of weak interactions? A system that throws some light on the problem is found in the vancomycin group antibiotics, which are rather well defined conformationally (1), and are in many ways analogous to a receptor site in a protein.

The antibiotics bind to cell wall analogues, such as acetate, N-Ac-D-Ala, N-Ac-D-Ala-D-Ala (NADADA, see **1**), and di-N-Ac-L-Lys-D-Ala-D-Ala, with binding constants that can be measured by (for example) UV difference spectroscopy (14,15), solid phase binding assays (15), NMR spectroscopy (16), and calorimetry (17, 18). The analysis of the binding data to yield binding constants is however complicated by dimerisation of the antibiotics (19, 20). Pertinent to the present discussion is the finding that dimerisation of the antibiotics (Table 1) can be enhanced by binding of cell wall analogues (21).

TABLE 1. Dimerisation constants for vancomycin-group antibiotics with and without bound ligands (KAA = di-N-Ac-Lys-D-Ala-D-Ala; NAD = N-Ac-D-Ala).

Antibiotic	Ligand	K_{dim} (M^{-1})
Vancomycin	none	700
Vancomycin	NAD	1400
Eremomycin	none	$\approx 10^6$
Eremomycin	KAA (**2**)	$\approx 10^8$
Ristocetin-Ψ	none	50
Ristocetin-Ψ	KAA (**2**)	700
Ristocetin A	none	500
Ristocetin A	KAA (**2**)	360
Ristocetin A	**3**	≈ 20
Ristocetin A	**4**	< 1
Ristocetin A	**6**	600

The structure of the ligand-bound dimer has been deduced (see **1**, where the bold outline indicates the peptide backbone of the antibiotics at the dimer interface, dashed lines indicate hydrogen bonds between cell wall analogue NADADA and antibiotic, and arrows indicate hydrogen bonds between the two halves of the dimer). Thus, in these cases dimerisation is cooperatively enhanced by cell wall analogues which, on the basis of a thermodynamic cycle, implies that binding of cell wall analogues to antibiotic is cooperatively enhanced by dimerisation. These data constitute an illustration of the fact that the free energy of interaction (in this case the dimerisation constant) is not simply a function of the interactions made at the interface, but depends also of course on changes in interactions which are removed in space from this interface.

Although the enhancement of dimerisation by the cell wall analogues is unambiguous, it is not large (up to a factor of 2 in K_{dim} for vancomycin, a factor of 10 for ristocetin-Ψ, but a factor of 100 for eremomycin). A possible physical description of the origin of the cooperative interaction lies in the fact that the amide dipoles of the antibiotic are probably ordered more strongly by ligand than solvent, and thus hydrogen bonding at the interface between the two halves of the dimer is strengthened enthalpically but with a compensating cost in entropy. It is striking that the amino group of the amino-sugar which is attached to the benzylic possition of residue 6 of ristocetin and eremomycin (see **1**) can make a salt bridge to the carboxylate anion of the cell wall analogues which is mediated through an amide bond. Such an effect would be consistent with the cooperativity that is normally observed between cell wall analogue binding and dimerisation. Therefore, it may be that the benzylic sugar is so located precisely because it can cooperatively promote both dimerisation and the binding to cell walls. If this view is correct, then it may imply that dimerisation can be of significance in antibiotic action.

Dimers in biological signalling

The above observations may be relevant to a general mechanism involved in biological signalling. A remarkably large number of biological recognition, and control and signalling processes involve the use of dimerisation of the ligand or receptor (22-29). Why might this be the case? A receptor must undergo a conformational change (in order to transmit a signal) in passing from a monomer to a ligand-bound dimer. One possibilty is that a high specificity for a ligand able to accomplish this task is available by virtue of the requirement for the ligand to **simultaneously** bind to the receptor **and** promote its dimerisation. Put in another way, it is a much less stringent condition for a ligand simply to bind to a receptor without simultaneously promoting its dimerisation, and thereby failing to induce signal transmission. Compounds satisfying the former and more stringent condition are agonists, and those satisfying the latter condition are antagonists. Since trans-membrane receptors necessarily involve extended arrays of interactions, the above principle can apply (although perhaps as a less stringent requirement) even in the case of receptors which transmit signals without dimer formation. Thus, even in these cases, ligand binding has to concurrently satisfy a precise set of changes in bonding within the receptor.

Agonist/antagonist binding

The binding of agonists to β-adrenergic receptors has been shown to be enthalpy driven (30). In contrast, antagonists (often synthetic drug molecules), have similar binding constants but exhibit entropy driven binding with small negative, or even positive, ΔH values. The enthalpy/entropy relationship for agonists, partial agonists, and antagonists binding to β-adrenergic receptors is summarised in Fig. 2. It is evident from the data that ΔG for binding does not vary greatly ($\approx 10^6$ to 10^9 M^{-1} in binding constant), but highlights why binding energy is unrelated to physiological response. Agonists bind most exothermically to the active form of the receptor and are capable of eliciting a biological response. Signalling appears to be achieved as a consequence of strongly exothermic interactions with the active form of the receptor. In contrast, antagonists do not fulfill the requirement of good complementarity of polar functional groups (to the receptor conformation induced by agonists), as evidenced by the criterion that their enthalpies of binding are small. As a consequence, the loss of translational and rotational entropy on binding is also small. It is possible to have similar binding constants for agonists and antagonists, but the "looser" interaction of the antagonists does not permit transmission of a conformation change (6). It is noteworthy that a positive entropy change (associated with antagonist binding) in the absence of a significant

hydrophobic effect (31), could be rationalised in terms of an entropic benefit to ligand binding associated with some degree of disordering of the receptor, analogous to the effects of a crystal impurity on the extended array of interactions.

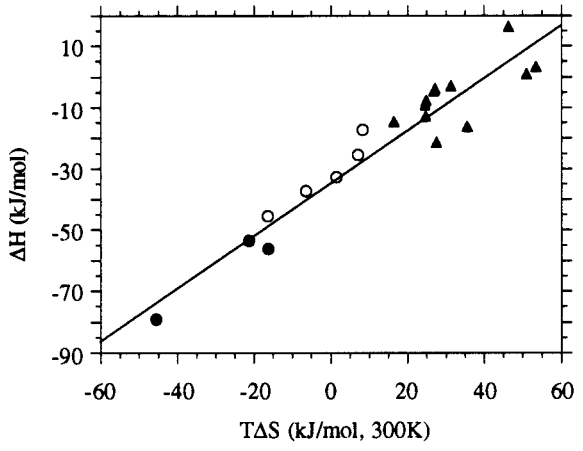
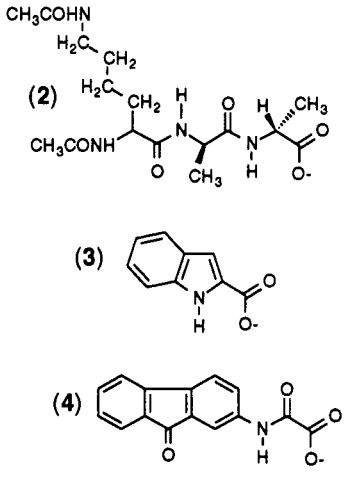
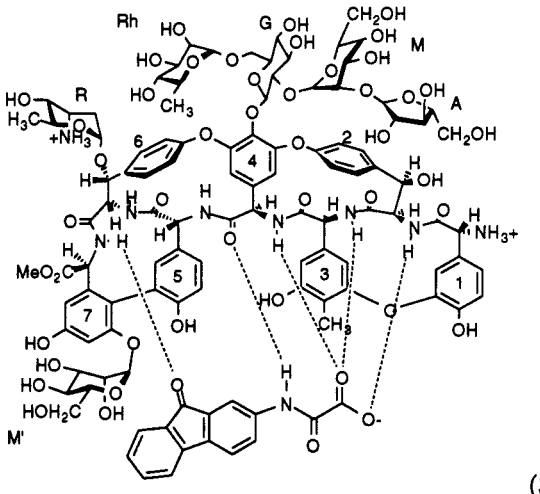


Fig. 2. Enthalpies versus entropies (TΔS at 300 K) of binding of agonists (●), partial agonists (O), and antagonists (▲) to β-adrenergic receptors. Data from ref. (30).

Anti-cooperative binding of cell-wall analogues to ristocetin A

We now discuss data on the dimerisation of ristocetin A as influenced by the binding of the antibiotic to some "unnatural" ligands. In the light of the preceding discussion, it might be expected that such artificial ligands, which bind into the cleft normally utilised by natural components of cell walls, will be unable to simultaneously satisfy the condition for continuing dimerisation of the antibiotic.

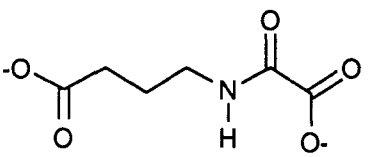




(5)

Ristocetin A is unique among the vancomycin group antibiotics so far examined in that binding of the natural cell-wall analogue di-N-Ac-Lys-D-Ala-D-Ala (**2**) is found to be anti-cooperative to a small extent (< factor of 2 in K_{dim}) (32) (Table 1). A series of unnatural structural motifs, that mimic many of the features of the natural cell wall analogues, but have hydrophobic aromatic ring systems, have been designed and investigated with ristocetin A. We highlight two of these ligands (**3** and **4**) which exhibit many of the features of classical antagonists insofar as, when they bind to the antibiotic, they do indeed fail to simultaneously satisfy the conditions required for a second sub-set of weak interactions, i.e., those promoting dimerisation (32).

While ristocetin A alone has a dimerisation constant of ≈ 500 M^{-1} at 300 K, K_{dim} is reduced to ≈ 20 M^{-1} by indole-2-carboxylate (**3**), and to < 1 M^{-1} in the case of the fluorenone ligand (**4**). NMR evidence (32), indicates that the structural basis for such large anti-cooperative effects on dimerisation stem from "unnatural" interactions between the aromatic components of these ligands, and the antibiotic tetrasaccharide and ristosamine sugars [see **5**; exploded view of the ristocetin A complex with the fluorenone ligand (**4**); dotted lines represent intermolecular hydrogen bonds. The rhamnose (Rh) and ristosamine (R) sugars are indicated]. Such interactions between ristosamine sugar and ligand are not found in the complex of the natural substrate (**1**), nor in the case of ligands that closely mimic the natural substrate, such as **6** (ligand binding constant of ≈ 10^3 M^{-1}, at 298 K), where effects on K_{dim} are also found to be small (see Table 1).



(**6**)

Although ligands **3** and **4** have substantial binding constants (> 7000 M^{-1}, greater than that of **6**), tight ligand binding alone does not appear to be compatible with strong antibiotic dimerisation.

CONCLUSIONS

If a man-made (synthetic) ligand is able only to bond into its nominal receptor site but not simultaneously to satisfy a second set of weak interactions, its binding will be anti-cooperative. Thus, synthetic compounds will often be antagonists in which the receptor/ligand system may be more disordered compared to the natural system; a useful analogy may be the introduction of an impurity in a crystal. We have illustrated both agonist and antagonist-like behaviour for the effects of cell wall analogues and cell wall mimetics on dimerisation of vancomycin-group antibiotics.The results of these studies emphasise that binding or dimerisation constants measured with these systems reflect the whole set of linked weak interactions within these extended aggregates, not just those at the interface (33).

REFERENCES

1. D. H. Williams *et al. J. Am Chem. Soc.* **113**, 7020 (1991).
2. M. S. Searle *et al. J. Am. Chem. Soc.* **114**, 10697 (1992).
3. D. H. Williams *et al. Proc. Natl. Acad. Sci. USA* **90**, 1172 (1993).
4. D. H. Williams *et al. Phil. Trans. R. Soc. Lond. A* **345**, 11 (1993).
5. M. I. Page & W. P. Jencks *Proc. Natl. Acad. Sci. USA* **68**, 1678 (1971).
6. M. S. Searle & D. H. Williams *J. Am. Chem. Soc.* **114**, 10690 (1992).
7. U. Gerhard *et al. Biomed. Chem. Lett.* **3**, 803, (1993).
8. L. Serrano *et al. Nature* **356**, 453 (1992).
9. K. A. Sharp *et al. Science* **252**, 106 (1991).
10. A. R. Fersht *Trends Biochem. Sci.* **12**, 301 (1986).
11. B. A. Shirley *et al. Biochemistry* **31**, 725 (1992).
12. W. P. Jencks *Proc. Natl. Acad. Sci. USA* **78**, 4046 (1981).
13. M. F Perutz *Proc. Roy. Soc. Lond. Ser. B* **208**, 135 (1980).
14. M. Nieto *et al. Biochem. J.***124**, 845 (1971).
15. P. W. Smith *et al. J. Org. Chem.* **53**, 1587 (1988).
16. J. B. Brown *et al. Mol. Pharmacol.* **11**, 126 (1975)
17. A. Rodriguez-Tebar *et al. J. Antibiot.* **39**, 1587 (1986).
18. A. Cooper *Phil. Trans. R. Soc. Lond. A* **345**, 43 (1993).
19. J. P. Waltho & D. H. Williams *J. Am. Chem. Soc.* **111**, 2475 (1989).
20 U. Gerhard *et al. J. Am. Chem. Soc.* **115**, 232 (1993).
21. J. P. Mackay *et al. J. Am. Chem. Soc.* submitted.
22. J. G. Pelton & D. A. Wemmer *et al. Proc. Natl. Acad. Sci. USA* **86**, 5723 (1989).
23. X. Gao & D. J. Patel *Biochemistry* **28**, 751 (1989)
24. P. S. Freemont *et al. Biochem. J.* **278**, 1 (1991).
25. A. Ullrich & J. Schlessinger *Cell* **61**, 203 (1990).
26. A. F. Russo & D. E. Koshland *Science* **220**, 1016 (1983).
27. F. Canals *Biochemistry* **31**, 4493 (1992).
28. M. V. Milburn *et al. Science* **254**, 1342 (1991).
29. D. M. Spencer *et al. Science* **262**, 1019 (1993).
30. G. A. Weiland *et al. Nature* **281**, 114 (1979).
31. A. Miklavc *et al. Biochem. Pharmacol.* **40**, 663 (1990).
32. P. Groves *et al. J. Chem. Soc. Perkin I* in press..

33. We are grateful to the SERC, Xenova and GLAXO for financial support. J.P.M. and D.A.B. acknowledge the British Council for postgraduate studentships, and M.F.C. thanks the National Institute of General Medical Sciences (Washington, U.S.A.).