Expression of Electrostatic Binding Cooperativity in the Recognition of Cell-wall Peptide Analogues by Vancomycin Group Antibiotics

Patrick Groves, Mark S. Searle, Martin S. Westwell and Dudley H. Williams*

Cambridge Centre for Molecular Recognition, University Chemical Laboratories, Lensfield Road, Cambridge, UK CB2 1EW

The strength of the binding interaction of the carboxylate group of peptide cell-wall analogues with ristocetin A is shown, by ^{1}H NMR studies of hydrogen bonded NHs, to increase towards a limiting value as the number of interactions on the same ligand template increases; the carboxylate electrostatic binding energy appears to reflect the whole set of linked weak interactions as a cooperative unit, and this cooperative effect is distinct from the classical chelate effect.

In recent work,[1–4] we have factorised the free energy change for the association **A** + **B** → **A·B** in aqueous solution into a number of terms representing various costs and benefits to binding [eqn. (1)].†

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

Using this approximation, and data on the binding of cell-wall analogues to vancomycin group antibiotics (particularly ristocetin A), we have established an experimental basis for the adverse free energy of restricting a σ-bond rotation ΔG_r, the benefit in free energy from the hydrophobic interaction ΔG_h and amide–amide hydrogen bonds ΔG_p. In the general case, $\Sigma\Delta G_p$ represents the sum over all pairs of polar functional group interactions in the complex. The term ΔG_{T+R}, the change in translational and rotational free energy (essentially the entropic cost of restricting freedom of motion on binding) reaches a limiting value for a highly exothermic association (analogous to covalent bond formation between **A** and **B**), but for weak associations this term tends to zero as the exothermicity of the association tends to zero. Such a relationship has been justified experimentally for one-point associations in non-polar solvents.[4,5] Thus, since the exothermicity of association is expressed in $\Sigma\Delta G_p$, then it is clear that ΔG_{T+R} and $\Sigma\Delta G_p$ strongly interact; as $\Sigma\Delta G_p$ contributes a greater free energy of binding, then the ΔG_{T+R} term opposing binding becomes large. Conversely, the maximum binding energy (defined by Jencks[6,7] as the intrinsic binding energy) that can be obtained from the interaction of a pair of specific functional groups will be observed when binding occurs with complete loss of translational and rotational entropy. For example, in binding ligand A–X to a receptor the binding energy of X will achieve its intrinsic value when the A portion of the ligand binds with the maximum value of ΔG_{T+R}, such that the introduction of X occurs without any additional cost in loss of translational and rotational entropy. Thus, eqn. (1) accommodates the cooperativity expressed through the operation of the classical chelate effect as described by Jencks.[6,7] In practice, for associations involving weak interactions, the maximum chelate enhancement of binding is rarely achieved.[4] We now discuss the situations where the binding energy of X will be less than the intrinsic value with reference to binding studies of cell-wall peptide analogues with ristocetin A. The data illustrate how multiple weak interactions on the same template act simultaneously in a cooperative fashion to enhance the binding of each interaction. Thus, the net binding interaction is greater than the sum of the parts even when the classical chelate effect is accommodated through application of eqn. (1).

The ^{1}H chemical shift of the backbone amide NH of residue 2 (w_2) of ristocetin A is a sensitive probe of the binding interaction of the carboxylate group of cell wall analogues. The chemical shift of w_2 moves downfield by ≈ 3.7 ppm in the presence of cell-wall tripeptide analogue di-N-Ac-L-Lys-D-Ala-D-Ala (Di-AcKDADA), and is strongly protected from solvent accessibility through hydrogen bonding.[8] An exploded view of the complex of ristocetin A with N-Ac-D-Ala-D-Ala (N-AcDADA) is shown in Fig. 1, with dotted lines representing key intermolecular hydrogen bonds. In the light of the above discussion, we have monitored the strength of the interaction of the ligand carboxylate group with the antibiotic as a function of the overall ligand binding energy within a series of cell-wall analogues (Fig. 2). That is, we have plotted (Fig. 3) the limiting chemical shift of w_2 (at ligand concentra-

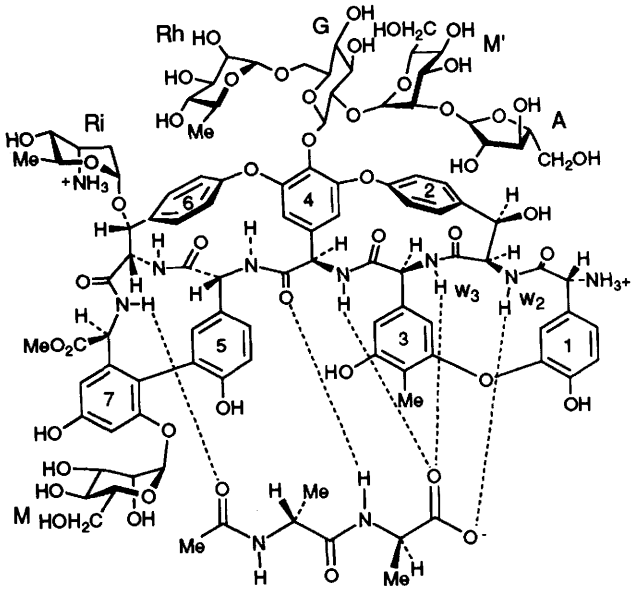

Fig. 1 Exploded view of the ristocetin A complex with N-Ac-D-Ala-D-Ala. Dotted lines represent intermolecular hydrogen bonds

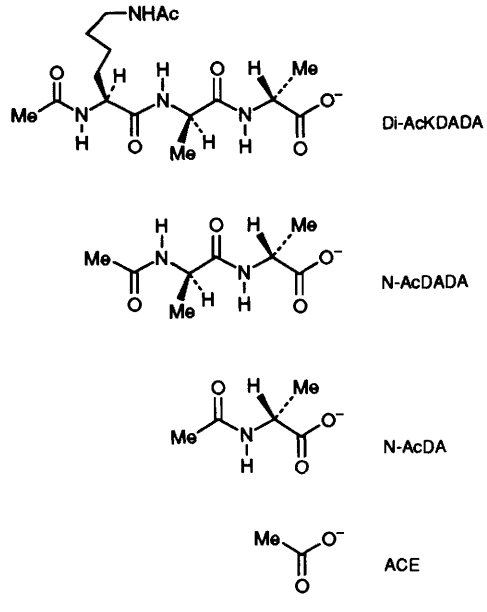

Fig. 2 Structure of cell-wall analogues used in this study

tions in which the antibiotic is >95% bound) for all of the ligands shown. It is evident that the limiting shift approaches an asymptotic limit as ΔG increases within the series of ligands. Larger binding shifts are taken to imply stronger electrostatic interactions (as opposed to structrual changes of different origin) in the complexes since the carboxylate anion is a constant structural unit in all ligands. In addition, a corresponding set of chemical shift changes are observed for the amide resonance w_3 in the same binding pocket (see Fig. 3).

Therefore, by tethering the carboxylate group to a strongly binding ligand forming multiple interactions with the receptor (and presumably where binding is associated with a large value for ΔG_{T+R}), the electrostatic binding energy of the carboxylate group is cooperatively enhanced. Thus, when the restriction of motion of the carboxylate group is aided by neighbouring interactions, then the average position of the carboxylate group in its electrostic (enthalpic) well will correspond to some larger binding exothermicity reflected in a larger limiting chemical shift of w_2. The binding of the rest of the ligand aids the binding of the carboxylate group, and the total binding exothermicity of the ligand will be greater than the sum of the parts.

In conclusion, eqn. (1) provides a basis for a semi-quantitative description of molecular recognition phenomena through an accommodation of cooperativity arising from the classical chelate effect. However, it does not allow for the additional cooperativity that arises from the mutual enhancement of electrostic interactions, which can give rise to an exothermicity that is greater than the sum of the parts.

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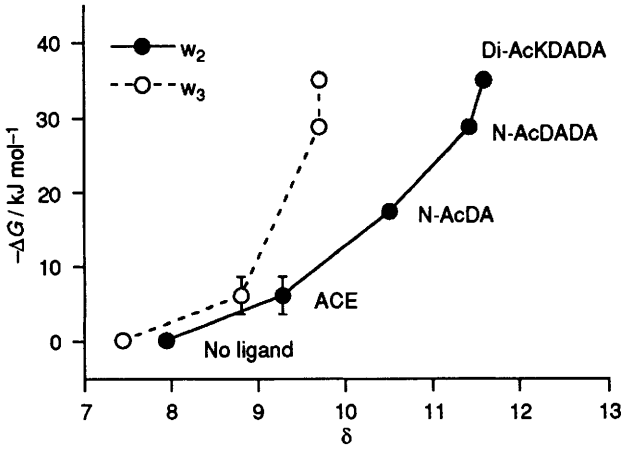

Fig. 3 Plot of ligand binding energy (ΔG at 298K, pH 7) *versus* limiting chemical shift of antibiotic amide NHs w_2 and w_3. ^{1}H chemical shifts were determined at 400 or 500 MHz and are referenced to trimethylsilylpropionic acid at 298K. In all cases the w_2 and w_3 signals were assigned on the basis of 2D NOE experiments. Ligand binding energies are those reported in earlier studies from UV titration measurements and from the calorimetry data of Rodriguez-Tebar *et al.*[9] (see ref. 2). Error bars for ligand binding energies and chemical shift values lie within the size of the data point, except in the case of the binding of acetate (ACE) where the uncertainty in ΔG is indicated.

Footnote

† 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 van der Waals contacts between **A** and **B** in **A**·**B** are as good as those of the separated components with solvent.

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