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COMMUNICATIONS

Published on Web 03/14/2002

The Role of Configurational Entropy in Biochemical Cooperativity

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Received October 8, 2001

Cooperativity is a common biochemical phenomenon in which two or more otherwise independent processes are thermodynamically coupled. Because cooperative processes are usually attended by changes in molecular conformation, thermodynamic coupling is usually attributed to an enthalpy-driven mechanism. In the family of glycopeptide antibiotics that includes vancomycin, however, cooperative phenomena occur that cannot be explained by conformational change. In this communication, we demonstrate that cooperativity in these systems can arise solely from changes in vibrational activity.

Glycopeptide antibiotics form homodimeric complexes in which each monomer binds a polypeptide ligand terminating in −D-Ala-D-Ala. The processes of ligand binding and dimerization mutually enhance each other, i.e., they exhibit positive cooperativity.[1] However, high-resolution X-ray crystallographic studies of vancomycin have demonstrated that neither process significantly alters the structural framework that is common to glycopeptide antibiotics.[2] It has been suggested that cooperativity arises from the tightening of the dimeric interface as ligands bind to an antibiotic dimer.[3] However, crystallographic studies also show that the H-bonds across the dimeric interface are not shortened. The average H-bond length across the dimeric interface of a vancomycin dimer with a single bound acetate ion is 2.17 Å, while it is slightly longer at 2.19 Å for a vancomycin dimer with two D-Ala residues.[2] Therefore, we have examined the role of altered dynamic behavior as the mechanism underlying this cooperativity.

The consequences of ligand binding and dimerization on the dynamic behavior of glycopeptide antibiotic complexes were assessed by conducting molecular dynamics simulations of vancomycin (VMN), chloroeremomycin (CEM), and their various complexes with the ligand Ac-D-Ala-D-Ala. VMN and CEM were chosen because they exhibit similar ligand-binding affinities, yet CEM has a much higher dimerization constant and it exhibits greater cooperativity in binding.[3] Prior studies of VMN using molecular dynamics simulation and published parameter sets have demonstrated that this approach can yield accurate thermodynamic predictions.[4]

Simulations were performed using CHARMM[5] with previously described atom types and parameters.[4] Electrostatic interactions were computed using the particle mesh Ewald algorithm.[6] Dynamics trajectories were generated at constant pressure (1.0 atm) and temperature (300 K)[7] with a 1 fs time step, shake constraints,[8] and fully solvated periodic boundary conditions. Initial coordinates for doubly liganded dimers of VMN and CEM were generated from the crystallographic coordinates of a dimeric vancomycin:acetate complex,[3] and these two systems were equilibrated for 1.7 ns. The final coordinates for each simulation were then used to generate starting structures for 12 additional simulations (two free monomers, two liganded monomers, ligand-free dimers, and double-liganded dimers of VMN and CEM). Each simulation was propagated for 1 ns, and one of the simulations was repeated seven times with different starting coordinates and conditioning protocols to assess the reproducibility of the results. Quasiharmonic analysis[9] of the non-hydrogen atoms of the macrocycles (atoms depicted in Figure 1) was performed on the final 800 ps of dynamics. The contribution of vibrational modes to configurational entropy was calculated using the relationship

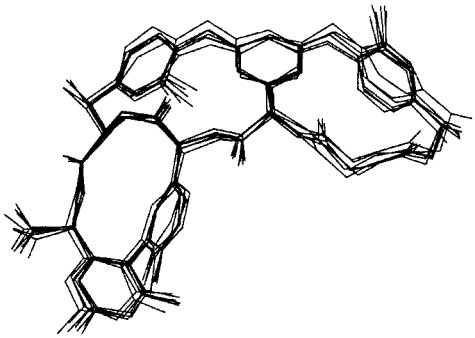

Figure 1. Macrocycle dynamics. Seven superimposed snapshots of the chloroeremomycin macrocycles over a 50 ps interval showing that significant fluctuations occur while a single overall conformation is retained.

$$TS_{\mathrm{vib}} = k_{\mathrm{B}}T \sum_i \left[\left(\frac{\Theta_i}{e^{\Theta_i} - 1} \right) + \ln\left(\frac{1}{1 - e^{-\Theta_i}} \right) \right] \qquad \Theta_i = \frac{h\omega_i c}{k_{\mathrm{B}}T}$$

where k_{B} is Boltzmann's constant, T is the absolute temperature, h is Plank's constant, c is the speed of light, and ω_i is the ith frequency (in cm^{-1}).

As expected, the RMS fluctuations of macrocycle atoms decrease upon binding. For example, the average RMS fluctuations for ligand-free monomeric CEM were 0.44 Å. They decreased to 0.29−0.30 Å upon dimerization or ligand binding, and further decreased to 0.25 Å for simultaneous dimerization and ligand binding. Quasiharmonic normal-mode analysis was performed to gauge the thermodynamic significance of these changes (Table 1). Ligand binding and dimerization broadly increased the frequencies of low-frequency modes ranging from 6 to 650 cm^{-1}. Increased mode frequencies imply a reduction in configurational entropy, and indicate that both processes incur an entropic "cost". The key result to emerge from this analysis was that the entropic costs of dimerization and ligand binding were not additive: the cost of forming a dimer with two bound ligands was significantly *less* than the sum of the cost of binding ligands by two monomeric antibiotics and the cost of combining two ligand-free monomers to form a dimer (Figure 2). In other words, cooperativity is positive because the energetic cost of the configurational entropy associated with forming a dimeric complex is less when the monomers are ligand-bound.

10.1021/ja017259h CCC: $22.00

Table 1. Vibrational Entropy Changes for the Processes of Dimerization and Ligand Binding[a]

process type	VMN	CEM
dimerization of two monomers	15	51
two monomers each binding one ligand	32	39
dimerization and binding of two ligands	38	65

[a] Data are shown as $-T\Delta S$ in kJ/mol at 300 K with the configurational entropy of free monomers set to zero. A standard deviation uncertainty of ±2 kJ/mol was estimated by repeating a simulation of the vancomycin dimer eight times using different preconditioning protocols.

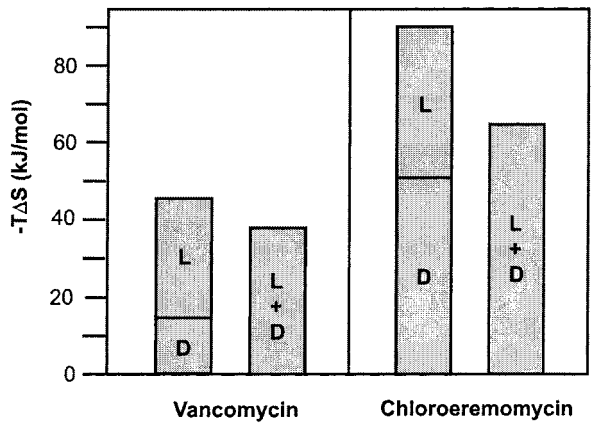

Figure 2. Nonadditive configurational entropies. The combined entropic cost of dimerization and ligand binding ($D + L$) is less than the sum of the separate costs of dimerization (D) and of ligand binding (L) for both vancomycin (by 9 kJ/mol) and chloroeremomycin (by 25 kJ/mol).

Ligand binding decreased the entropic cost of dimerization by 9 kJ/mol for VMN and by 25 kJ/mol for CEM (Table 1 and Figure 2). This demonstrates that the changes in vibrational activity are of sufficient thermodynamic magnitude to account for experimentally observed cooperative phenomena. The simulation-derived values are somewhat larger than the experimentally measured decreases of 5 kJ/mol for VMN and 12 kJ/mol for CEM,[3] but the simulations nevertheless correctly reproduced the roughly 2.5-fold difference between VMN and CEM.

Spin relaxation studies using NMR spectroscopy have been employed to estimate the contribution of subnanosecond motions to configurational entropy in other systems,[10] and have provided experimental evidence for entropic contributions to the cooperativity of calcium ion binding in calbindin D_{9k}.[11] As with glycopeptide antibiotics, ligand binding in calbindin induces thermodynamically significant stiffening of the polypeptide backbone with limited overall changes in conformation. Although NMR relaxation studies may be confounded by correlated motions that lead to overestimates of configurational entropy, recent investigations using molecular dynamics simulation of ubiquitin suggest that the effect of correlated motion on the assessment of changes in configurational entropy by NMR is relatively small.[12]

These results demonstrate the likelihood that ligand binding and dimerization in these two glycopeptide antibiotics are thermodynamically coupled through their effects on configurational dynamics. The extent of coupling appears to be more than sufficient to account for the magnitude of observed cooperative effects. Both processes stiffen the molecule, increasing the frequencies of soft vibrational modes and reducing configurational entropy. Increased frequencies imply that fluctuations away from an average structure are reduced in amplitude. In the absence of conformational change, therefore, the dynamic consequences of ligand-binding and dimerization are structurally congruent (i.e. they converge on the same average structure) and cooperativity should be positive.

Such a mechanism may also operate in much larger systems. It has been suggested that ligand-induced modulation of periodic motions in a macromolecular system is energetically significant, and that this is a plausible means of communicating the presence of bound ligand over long distances.[13] Indeed, the function of large membrane proteins such as those involved in transmembrane signal transduction may require a mechanism based on configurational entropy changes because the enthalpy changes generally involved in the binding of small ligands are small compared to the magnitude of potential energy fluctuations one would expect in systems of this size.[14]

Our investigation has focused exclusively on the thermodynamic effects of altered vibrational activity on cooperative behavior. Obviously, other factors not considered in this analysis will also contribute to the overall free energy changes that ultimately determine equilibrium behavior. For example, entropy changes are typically accompanied by compensatory enthalpy changes. We have not explored enthalpy changes in this study, but they may well account for the difference between the cooperativities we attribute to configurational entropy changes and the somewhat smaller cooperativities that are experimentally observed and that reflect changes in overall free energy. Moreover, enthalpic contributions arising from conformational change cannot be excluded even though the conformational changes we observe are small compared to the magnitude of structural variations within a crystal. Nevertheless, these results are significant because they demonstrate that changes in vibrational activity are of sufficient magnitude *by themselves* to account for the experimentally observed positive cooperativity between ligand binding and dimerization.

Acknowledgment. This work was supported by AI43412, GM54617, and the American Heart Association.

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JA017259H