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Enthalpy versus Entropy-Driven Binding of Bisphosphonates to Farnesyl Diphosphate Synthase

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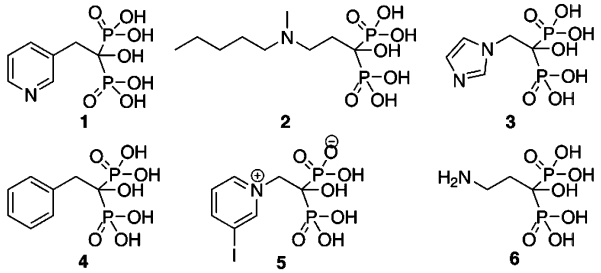
Bisphosphonates are an important class of drug molecules, used to treat osteoporosis, Paget's disease, and hypercalcemia due to malignancy.[1,2] They act by inhibiting the enzyme farnesyl diphosphate synthase (FPPS, EC 2.5.1.10), which produces farnesyl diphosphate from dimethylallyl diphosphate and two molecules of isopentenyl diphosphate (IPP), the FPP being used in cholesterol, heme a, and dolichol biosynthesis, and in the post-translational modification of signaling proteins. It has also recently been shown that the IPP which would be expected to accumulate on FPPS inhibition is converted to the isopentenyl ester of ATP (ApppI), a strongly pro-apoptotic molecule which inhibits the mitochondrial ADP/ATP transporter.[3] Bisphosphonates are also of interest in the context of cancer therapy since they stimulate human $\gamma\delta$ T cells containing the Vγ2Vδ2 T cell receptor to proliferate and release large amounts of TNFα and IFNγ,[4] leading to potential uses in immunotherapy,[5] plus they have potent effects[6−8] against the parasites responsible for sleeping sickness, Chagas' disease, malaria, and the leishmaniases.

In early work,[9] we suggested that bisphosphonates bind to the allylic (DMAPP) site of FPPS, acting as transition state/reactive intermediate analogues. This general model has recently been confirmed by X-ray crystallography.[10,11] We proposed the importance of electrostatic and attractive van der Waals interactions between the bisphosphonate side chains and the protein; however, the model ignored ligand hydration/dehydration (hydrophobic) effects, and whether binding is enthalpy or entropy-driven. The importance of the hydrophobic effect has been detected in more recent computational studies,[12,13] and here, we investigate this topic experimentally by using ITC (isothermal titration calorimetry).

We investigated binding of the following six bisphosphonates:



to *T. brucei* FPPS in the presence of Mg^{2+} (5 mM) at 300 K and pH = 7.4 and 8.5 using a Microcal (Northampton, MA) VP−ITC.

As can be seen in Figure 1A and Table 1, the binding of risedronate (**1**, Actonel) is strongly exothermic at pH = 7.4 with $\Delta H = -8.87$ kcal mol^{-1}, $\Delta S = 2.83$ cal deg^{-1} mol^{-1}, and $\Delta G = -9.71$ kcal mol^{-1}, clearly indicating enthalpy-driven binding.

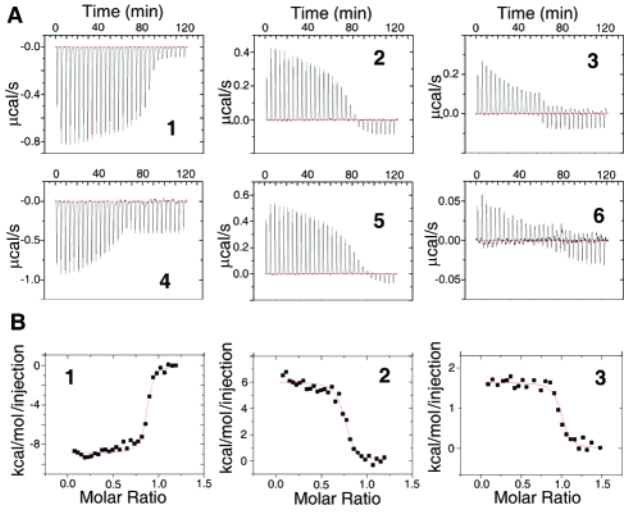


Figure 1. (A) ITC data for the six bisphosphonates binding to *T. brucei* FPPS. Binding of **1** and **4** is enthalpy-driven; binding of **2**, **3**, **5**, and **6** is entropy-driven. (B) Representative fitting curves for compounds **1**, **2**, and **3**.

Table 1. Thermodynamic Parameters for Bisphosphonate Binding

compound	pH	ΔG (kcal)	ΔH (kcal)	ΔS (e.u.)	K_b (M^{-1})	IC_{50} (μM)
1	7.4	−9.71	−8.87	2.83	1.31×10^7	0.5
	8.5	−8.68	−10.41	−5.79	2.31×10^6	2
2	7.4	−9.14	6.03	50.91	5.07×10^6	3.1
	8.5	−8.39	2.91	37.93	1.43×10^6	12
3	7.4	−9.93	1.65	38.85	2.04×10^7	0.4
	8.5	−9.05	−4.76	14.39	4.60×10^6	1
4	7.4	−7.46	−9.98	−8.44	3.13×10^5	19.8
	8.5	−7.48	−10.24	−9.27	3.20×10^5	22.9
5	7.4	−9.21	7.21	55.08	6.02×10^6	1.3
	8.5	−8.67	7.37	53.85	2.44×10^6	2.5
6	7.4	−8.85	0.48	31.21	3.12×10^6	4.0
	8.5	*a*	∼0	*a*	*a*	*a*

[a] Not determined.

Surprisingly, however, the binding of ibandronate (**2**, Boniva) is strongly endothermic ($\Delta H = 6.03$ kcal mol^{-1}), and binding is entropy-driven ($\Delta S = 50.91$ cal deg^{-1} mol^{-1}; $-T\Delta S = -15.27$ kcal mol^{-1}) although the overall ΔG of −9.14 kcal mol^{-1} is very similar to that seen with risedronate (−9.71 kcal mol^{-1}). Since the configurational entropy of ibandronate can be expected to decrease on binding to FPPS, these results indicate the key importance of hydrophobic effects, that is, water molecules which are ordered around **2** increase their entropy on movement of **2** into the FPPS active site, and ordered water molecules in the active site also increase their entropy as they transfer to the bulk solvent on drug binding. In the case of zoledronate (**3**), binding is also entropy

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driven at pH 7.4 ($\Delta H = 1.65$ kcal mol^{-1}; $\Delta S = 38.85$ cal deg^{-1} mol^{-1}; $-T\Delta S = -11.7$ kcal mol^{-1}; $\Delta G = -9.93$ kcal mol^{-1}). However, at pH = 8.5, binding switches to being enthalpy-driven, with $\Delta H = -4.76$ kcal mol^{-1}, $-T\Delta S = -4.32$ kcal mol^{-1}, and $\Delta G = -9.05$ kcal mol^{-1} (Table 1). At pH = 8.5, the ΔH for risedronate binding becomes slightly more negative ($-8.87 \rightarrow -10.41$ kcal mol^{-1}, Table 1), and likewise, the much less potent phenylethane bisphosphonate inhibitor, the de-aza analogue of risedronate (**4**), also binds in an enthalpy-driven mode at both pH values (Figure 1A and Table 1). When taken together, these results strongly support the idea that bisphosphonates which have charged side chains bind in an entropy-driven manner, due to the high enthalpic cost of desolvating the ligand (due to strong ion−dipole interactions) and a corresponding large increase in ΔS, due to the increased disorder of the previously ligand (and protein)-bound water molecules on ligand binding to FPPS. At pH = 7.4, the pyridine side chain of risedronate (**1**) in solution is mostly nonprotonated, an effect which increases slightly at pH = 8.5, resulting in a small increase in ΔH. However, with zoledronate (**3**), at pH = 7.4, the imidazole side chain is expected to exist in a ~1:4 protonated/nonprotonated form (given a pK_a of ~6.7 for imidazole), so the ΔH of binding is less favorable than with risedronate and is in fact positive ($\Delta H = 1.65$ kcal mol^{-1}) but becomes negative ($\Delta H = -4.76$ kcal mol^{-1}) at pH = 8.5 (Table 1). In the case of the pyridinium-1-yl bisphosphonate **5**, the side chain is of course fully charged at both pH values, and ΔH is positive and has essentially the same value at both pHs ($\Delta H =$ 7.21 and 7.37 kcal mol^{-1}). With the phenyl-containing analogue of risedronate (**4**), there is again no change in ΔH and ΔS on changing pH, and ΔG is again enthalpy-driven (Figure 2A and Table 1). Likewise, for pamidronate (side chain alkylammonium pK_a ~ 10.6), there is almost no change in ΔH (~0) of binding at either pH value since the group remains essentially fully protonated. The increased activity of the longer alkyl chain length species ibandronate correlates with an increased hydrophobic effect (increase in ΔS), while the enhanced activity of risedronate and zoledronate over the phenyl species can be attributed to enhanced (electrostatic ≫ dispersion/pi) interactions in the FPPS active site.

These results clearly show that bisphosphonate binding to FPPS can be either enthalpy or entropy-driven, with neutral side chain species binding in an enthalpy-driven manner and charged side chain species binding in an entropy-driven manner, shown schematically in Figure 2A (colored surfaces). The results shown in Figure 2A also indicate the presence of an apparent "enthalpy−entropy compensation",[14] that is

$$(\Delta H)_i = a(\Delta S)_i + b$$

However, there is no need to invoke an "extra-thermodynamic" basis for the $\Delta H-\Delta S$ correlation observed experimentally since the range in ΔG ($\Delta\Delta G$) is small (2.47 kcal); ΔG (avg) = $-8.78 \pm$ 0.79 kcal mol^{-1}, while the range in ΔH is very large ($\Delta\Delta H \sim$ 17.8 kcal). Under this circumstance, taking $\Delta\Delta G$ to be a 0 results in

$$(\Delta H)_i = T(\Delta S)_i + b$$

with a slope $\partial(\Delta H)_i/\partial(\Delta S)_i = T$, and experimentally, the results shown in Figure 2A do in fact yield a slope $T = 300$ K, the temperature at which the ITC experiments were carried out. As expected, the IC_{50} values for enzyme inhibition (Table 1) are highly correlated with the ΔG and K_b values for bisphosphonate binding ($R^2 = 0.85$, $P < 0.001$) as measured by ITC, as shown in Figure 2B. Moreover, the fitted slope (1.21 kcal) is very close to that which would be expected theoretically ($2.303RT = 1.37$ kcal) for competitive inhibition of FPPS.

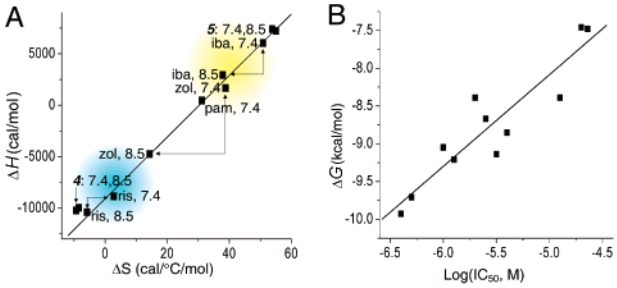

Figure 2. (A) Enthalpy versus entropy of binding of six bisphosphonates to *T. brucei* FPPS at pH 7.4 and 8.5. Yellow, entropy-driven; blue, enthalpy-driven. (B) Correlation between ΔG measured by ITC and log(IC_{50}) values for enzyme inhibition obtained by using a radiochemical assay ($R^2 = 0.85$; $P < 0.001$).

These results are of general interest since they represent the first detailed thermodynamic investigation of the binding of a widely used class of drug molecules, bisphosphonates, to their target, FPPS; the results were unexpected since they show both enthalpy and entropy-driven binding of drugs having the same activity, but can be rationalized in terms of the molecular structures involved, opening the way to the design of more potent and selective inhibitors using ITC.[15,16]

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References

(1) Fleisch, H. *Eur. Spine. J.* **2003**, *12* Suppl 2, S142−6.
(2) Fleisch, H. *Breast Cancer Res.* **2002**, *4* (1), 30−4.
(3) Mönkkönen, H.; Auriola, S.; Vepsäläinen, J.; Mönkkönen, J. *30th European Symposium on Calcified Tissues*; Rome, Italy, 2003; p P-134.
(4) Kunzmann, V.; Bauer, E.; Feurle, J.; Weissinger, F.; Tony, H. P.; Wilhelm, M. *Blood* **2000**, *96* (2), 384−92.
(5) Wilhelm, M.; Kunzmann, V.; Eckstein, S.; Reimer, P.; Weissinger, F.; Ruediger, T.; Tony, H. P. *Blood* **2003**, *102* (1), 200−6.
(6) Martin, M. B.; Grimley, J. S.; Lewis, J. C.; Heath, H. T., III; Bailey, B. N.; Kendrick, H.; Yardley, V.; Caldera, A.; Lira, R.; Urbina, J. A.; Moreno, S. N.; Docampo, R.; Croft, S. L.; Oldfield, E. *J. Med. Chem.* **2001**, *44* (6), 909−16.
(7) Yardley, V.; Khan, A. A.; Martin, M. B.; Slifer, T. R.; Araujo, F. G.; Moreno, S. N.; Docampo, R.; Croft, S. L.; Oldfield, E. *Antimicrob. Agents Chemother.* **2002**, *46* (3), 929−31.
(8) Rodriguez, N.; Bailey, B. N.; Martin, M. B.; Oldfield, E.; Urbina, J. A.; Docampo, R. *J. Infect. Dis.* **2002**, *186* (1), 138−40.
(9) Martin, M. B.; Arnold, W.; Heath, H. T., III; Urbina, J. A.; Oldfield, E. *Biochem. Biophys. Res. Commun.* **1999**, *263* (3), 754−8.
(10) Hosfield, D. J.; Zhang, Y.; Dougan, D. R.; Broun, A.; Tari, L. W.; Swanson, R. V.; Finn, J. *J. Biol. Chem.* **2004**, *279* (10), 8526−9.
(11) Gabelli, S. B.; McLellan, J. S.; Montalvetti, A.; Oldfield, E.; Docampo, R.; Amzel, L. M. *Proteins* **2005**, *62* (1), 80−8.
(12) Sanders, J. M.; Gomez, A. O.; Mao, J.; Meints, G. A.; Van Brussel, E. M.; Burzynska, A.; Kafarski, P.; Gonzalez-Pacanowska, D.; Oldfield, E. *J. Med. Chem.* **2003**, *46* (24), 5171−83.
(13) Sanders, J. M.; Ghosh, S.; Chan, J. M.; Meints, G.; Wang, H.; Raker, A. M.; Song, Y.; Colantino, A.; Burzynska, A.; Kafarski, P.; Morita, C. T.; Oldfield, E. *J. Med. Chem.* **2004**, *47* (2), 375−84.
(14) Ford, D. M. *J. Am. Chem. Soc.* **2005**, *127* (46), 16167−70.
(15) Ward, W. H.; Holdgate, G. A. *Prog. Med. Chem.* **2001**, *38*, 309−76.
(16) Velazquez Campoy, A.; Freire, E. *Biophys. Chem.* **2005**, *115* (2−3), 115−24.

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