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Positive ion pair cooperativity exhibited for the binding of phosphate under physiological conditions†

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The synthesis of a heteroditopic receptor which exhibits positive cooperativity for the binding of phosphate ion pairs under physiological conditions. Optimised complementarity between crown ether host and metal guest leads to increased binding affinity, K_a.

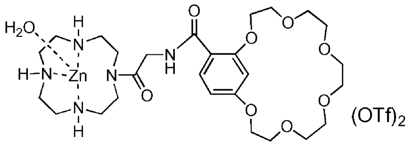

Fig. 1 Schematic structure of Zn**L**·(OTf)$_2$.

Introduction

Ion pair cooperativity[1] is an exciting facet of molecular recognition which has yet to be fully explored by biomimetic chemists. The thermodynamics of binding interactions in aqueous environments have come to prominence with the use of techniques such as isothermal titration calorimetry (ITC).[2] Through the determination of $\Delta H°$ and the affinity constant K_a it is possible to obtain a full thermodynamic profile of the binding event. In particular, calorimetric techniques have been used to great effect in biological research to measure the affinities of substrates for specific proteins and to elucidate the stoichiometries of the interactions.[3] More recently ITC, in conjunction with other spectroscopic methods, has been used to probe the thermodynamic contributions of artificial host–guest complexation.[4]

There have been a number of calorimetric investigations into the binding of ion pairs by artificial guest receptors in non-polar solvents,[5] but there are limited examples of such experiments in an aqueous environment.[6] The focus of this work was to determine the nature of the binding cooperativity between ions in established heteroditopic ion pair receptors.[7] Previous study by Anslyn into the origin of negative cooperativity was based around the findings of Jencks.[8]

$$\Delta G°_S = \Delta G°_A + \Delta G°_B - \Delta G°_{AB}$$

Jencks formulated an equation which considered the Gibb's free energies of ion pair binding.[1b] This stated that the Gibb's free energy of connection or tether between the two ions $\Delta G°_S$, is equal to the sum of the individual component parts, $\Delta G°_A$ and $\Delta G°_B$ and the overall free energy for the binding of the ion pair $\Delta G°_{AB}$. Therefore, if $\Delta G°_{AB}$ is more favourable than the combined free energies of $\Delta G°_A$ and $\Delta G°_B$, then a positive $\Delta G°_S$ value will result. Positive cooperativity is denoted by a positive $\Delta G°_S$ and counter-wise a negative $\Delta G°_S$ value represents negative cooperativity. Utilisation of this mathematical model, allows the calculation of $\Delta G°_S$ from $\Delta G°_{AB}$, $\Delta G°_A$ and $\Delta G°_B$ through ITC.

On this premise I now report the first example of positive ion pair cooperativity exhibited by an artificial hetero-ditopic receptor in an aqueous environment. It is important to remember the inherent difficulties associated with working in an aqueous environment where hydrophillic receptors must overcome their own solvation sphere and that of a polar substrate.

Fig. 1 gives the schematic structure of the ligand system. It has been shown in previous work[7] that the adoption of a modular synthetic approach was preferable in terms of ligand optimisation. The ligand was tested on $(X^+)(H_2PO_4^-)$, where X was either Na^+, K^+ or the Li^+ cation. The different sized metal cations offered the opportunity to determine the effect of the cation on binding cooperativity. Optimum dimensions for metal/crown ether coordination have been well-established[9] and the use of a 19-benzocrown-6[7b] specifically favours the binding of K^+ over Na^+ and Li^+. 19-Benzocrown-6 was synthesised with a pendant aldehyde using 2,4-dihydroxybenzaldehyde and 1,2-bis(2-chloroethoxy-)ethane with K_2CO_3 in 40% yield (Scheme 1). The aldehyde functional group was oxidized to the corresponding carboxylic acid through the use of NaO_2Cl and sulfamic acid to give **4**. Tri-*N*-Boc protected cyclen was coupled to Cbz protected glycine using DCC and DMAP in 78% yield, furnishing **2**. Cbz removal was mediated by 1,4-cyclohexadiene and 10% Pd/C. The deprotected primary amine **3** was then coupled to the pendant carboxylic acid of the 19-benzocrown-6 **4** in 85% yield. Boc deprotection in TFA–DCM yielded the ligand precursor **6**, which was complexed to zinc triflate in an equimolar ratio to form the desired ligand complex, Zn**L**·(OTf)$_2$.

Binding experiments were principally carried out using isothermal titration calorimetry (ITC), UV-vis titrations and ^{1}H NMR titrations.†

As has been stated, experimental calculation of the sign and value of $\Delta G°_S$ required the values of $\Delta G°_{AB}$, $\Delta G°_A$ and $\Delta G°_B$ individually. $\Delta G°_{AB}$ was found from the binding of the ion pair $(X^+)(H_2PO_4^-)$, and the binding of the individual cation and anion calculated through the use of a non-coordinating counter ion. In the case of the cation binding ($\Delta G°_A$), we used the corresponding metal perchlorate salt. With all three metal cations there was no observable metal–ligand interactions in water. This was experimentally confirmed using ^{1}H NMR titrations. Therefore $\Delta G°_A$ can be assumed to be 0 kcal mol^{-1}. Determining the individual phosphate anion binding contribution ($\Delta G°_B$) was problematic. Attempts to measure the binding affinity of $H_2PO_4^-$ with a *tert*-butyl ammonium non-coordinating counter cation were hindered by the salts lack of solubility in an aqueous environment. One can however, assume with reasonable accuracy that the binding of phosphate is principally achieved through an oxygen–zinc interaction.[6a,10] Therefore the binding of the phosphate anion and the determination of $\Delta G°_B$ can be obtained by using a model system which precludes cation binding and its effects. Through the use of a zinc(II) cyclen model we were able to measure the binding of the individual phosphate anion. It can be seen from Table 1 that the three different metal cations have negligible effects upon phosphate anion binding to zinc(II) cyclen. Therefore, the model gives the individual phosphate anion binding to the zinc metal centre. It is important to realise that the findings are based on a model

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† Electronic supplementary information (ESI) available: synthetic procedures, ITC data, NMR spectra. See http://dx.doi.org/10.1039/b510262f

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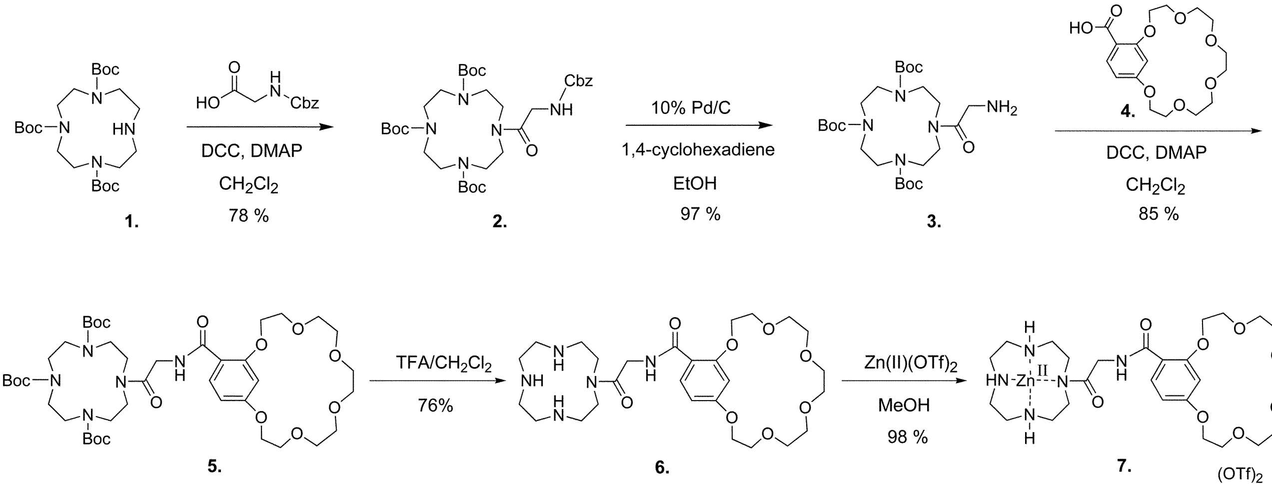
Table 1 Thermodynamic data for the binding of Zn**L** and Zn cyclen in HEPES buffer at pH 7.4 at 25 °C

Ligand	Ligand : substrate binding ratio[a]	Substrate	$K \times 10^4$	$\Delta G°$/kcal mol^{-1b}	$\Delta H°$/kcal mol^{-1}	$-T\Delta S°$/kcal mol^{-1}
ZnL	1 : 1	$Na[H_2PO_4]$	3.90 ± 0.81^c	−6.57	0.39 ± 0.02	6.96
ZnL	1 : 1	$K[H_2PO_4]$	5.10 ± 0.94^c	−6.42	0.29 ± 0.02	6.71
ZnL	2 : 1	$Li[H_2PO_4]$	3.13 ± 0.21^d	−6.16	1.71 ± 0.01	7.84
Zn cyclen	1 : 1	$Na[H_2PO_4]$	1.60 ± 0.09^c	−5.74	-3.25 ± 0.08	2.49
Zn cyclen	1 : 1	$K[H_2PO_4]$	1.52 ± 0.19^c	−5.70	-3.89 ± 0.24	1.81
Zn cyclen	1 : 1	$Li[H_2PO_4]$	1.34 ± 0.29^c	−5.13	-3.56 ± 0.05	1.57

[a] Binding stoichiometry was also confirmed by ^{1}H NMR titration experiments. [b] Calculated from $\Delta G° = \Delta H° - T\Delta S°$. [c] Units: mol^{-1} dm^3. [d] Units: mol^{-2} dm^6



Scheme 1 Synthetic procedure for the synthesis of Zn**L**·$(OTf)_2$.

system but offer an important insight into the mechanisms of binding and the thermodynamic origins of cooperativity.

Having calculated both the individual and combined thermodynamic contributions, it was possible to determine the three $\Delta G°_S$ values for the different metal phosphate salts. In the case of $Na[H_2PO_4]$, $\Delta G°_S$ was calculated to be +0.83 kcal mol^{-1}. For $K[H_2PO_4]$ the $\Delta G°_S$ value was +0.72 kcal mol^{-1} and $Li[H_2PO_4]$ was found to be +1.03 kcal mol^{-1}. In the three examples shown, cooperative binding of the ion pair was found to be positive. This is the first example in which a model system has exhibited ion pair positive cooperativity. The nature of the binding and cooperativity is assigned almost entirely to the entropy contribution. The favourable entropy is associated with desolvation of both the phosphate guest and the crown ether cavity. All three-phosphate species have significant entropy contributions, the largest belonging to $Li[H_2PO_4]$. This was attributed, principally with the desolvation of the crown ether cavity and also the dispersion of the highly organized water sphere around the Li^+ metal.[11] In terms of enthalpy, the interactions are endothermic and slightly unfavourable. Theoretically, the size of the crown ether favours the chelation of potassium. The higher, more disfavoured, endothermic values for the binding of the sodium and lithium phosphate were associated with the poor complementarity of the cation with the crown ether. The experimental results confirm that the $K[H_2PO_4]$ species is more tightly bound, exhibiting higher binding constants and the lowest unfavourable $\Delta H°$ of +0.29 kcal mol^{-1}.

In conclusion it can be seen that positive cooperativity can be achieved in model systems through a combination of good complementarity between host–guest and efficient desolvation effects. In the Zn**L**·$(OTf)_2$ ligand system, positive cooperativity was driven almost entirely by entropy. Despite the unfavourable $\Delta H°$ contributions, highly efficient solvent expulsion due to the ligand–ion pair interactions seems sufficient to facilitate positive cooperativity. These experiments reiterate the findings of other workers,[8] that shows that in an aqueous environment it is often the solvent effects which determine the nature of the binding. The design of a receptor which complements the ion pair, is not sufficient to guarantee high affinity between host and guests. Exploitation of solvent spheres through their disruption holds the key to highly efficient artificial hosts in water environments.[12]

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Notes and references

1 (*a*) D. H. Williams, A. J. Maguire, W. Tsuzuki and M. S. Westwall, *Science*, 1988, **280**, 711; (*b*) W. P. Jencks, *Proc. Natl. Acad. Sci. U. S. A.*, 1981, **78**, 4046.

2 K. S. Cameron, J. K. Clark, A. Cooper, L. Fielding, R. Palin, S. J. Rutherford and M.-Q. Zhang, *Org. Lett.*, 2002, **20**, 3403.

3 J. Kallen, J.-M. Schlaeppi, F. Bitsch, I. Delhon and B. Fournier, *J. Biol. Chem.*, 2004, **279**, 14033.

4 (*a*) B. R. Linton, M. S. Goodman, E. Fan, S. A. Van Arman and A. Hamilton, *J. Org. Chem.*, 2001, **66**, 7313; (*b*) S. L. Tobey and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 14807; (*c*) G. Müller, J. Riede and F. P. Schmidtchen, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 1516; (*d*) M. Haj-Zaroubi, N. W. Mitzel and F. P. Schmidtchen, *Angew. Chem., Int. Ed.*, 2002, **41**, 104; (*e*) P. D. Beer and P. A. Gale, *Angew. Chem., Int Ed.*, 2001, **40**, 486.

5 (*a*) D. B. Smithrud, T. B. Wyman and F. Diederich, *J. Am. Chem. Soc.*, 1991, **113**, 5420; (*b*) D. B. Smithrud, E. M. Sanford, I. Chao, S. B. Ferguson, D. R. Carcanague, J. D. Evanseck, K. N. Houk and F. Diederich, *Pure Appl. Chem.*, 1990, **62**, 2227; (*c*) B. Linton and A. D. Hamilton, *Tetrahedron*, 1999, **55**, 6027.

6 (*a*) E. Kimura, *J. Am. Chem. Soc.*, 1997, **119**, 3068; (*b*) E. Kimura, Y. Kodoma and T. Koike, *J. Am. Chem. Soc.*, 1995, **117**, 8304; (*c*) S. Aoki and E. Kimura, *Chem. Rev.*, 2004, **104**, 769.

7 (*a*) P. T. Gunning, A. C. Benniston and R. D. Peacock, *Chem. Commun.*, 2004, 2226; (*b*) A. C. Benniston, P. T. Gunning and R. D. Peacock, *J. Org. Chem.*, 2005, **123**, 123.

8 S. L. Tobey and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 10963.

9 C. J. Pederson, *J. Am. Chem. Soc.*, 1967, **89**, 7017.

10 J. C. Mareque-Rivas, R. Prabaharan and R. T. Martín de Rosales, *Chem. Commun.*, 2004, 76.

11 F. A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry*, John Wiley & Sons, Inc., New York, 1988, 5th edn., pp. 132.

12 F. P. Schmidtchen and M. Berger, *Chem. Rev.*, 1997, **97**, 1609.