

The Characterisation of Novel Dendritic Carriers with DNA by Isothermal Titration Calorimetry

Daniel Coles¹, Shu Yang², Anna Esposito¹, Deanne Mitchell², Rod Minchin² and Istvan Toth^{1*}

¹ School of Molecular and Microbial Sciences, The University of Queensland, St Lucia, Qld, 4072, Australia; ² School of Biomedical Sciences, The University of Queensland, St Lucia, Qld, 4072, Australia

E-mail: i.toth@uq.edu.au

Introduction

The formation of DNA complexes using polycations is important in understanding their role in gene delivery. While gene delivery is widely studied, little is known about the thermodynamic interactions between various carriers and DNA. The interaction involves two oppositely charged entities coming together to form a compact particle. This interaction is either specific or non-specific which is characterized by large or small binding affinities respectively [1]. By using isothermal titration calorimetry (ITC) the binding affinity (K_a), stoichiometry and enthalpy (ΔH), entropy (ΔS) and Gibbs free energy (ΔG) changes can be determined. ITC works by measuring the heat absorbed or released from the interaction of polycations with DNA. For our gene carriers we have used the cell penetrating peptide TAT, a nuclear localization signal (NLS) peptide and dendritic polylysine. The structures synthesized include: 1. TAT, 2. TAT-polylysine, 3. NLS, 4. NLS-polylysine, 5. polylysine, 6. TAT-Lys-NLS and 7. TAT-Lys(polylysine)-NLS [2]. By using ITC we aim to determine if the interaction is specific for each dendrimer or if it is determined by the number of positive charges the dendrimers have.

Results and Discussion

Dendrimers were synthesized by solid phase peptide synthesis on *p*-MBHA resin using Boc chemistry. Once purified, the dendrimers were analysed by analytical HPLC and ES-MS. To incorporate dendritic polylysine with the TAT and NLS peptide (Dendrimer 7), Boc-lysine(Fmoc) was used to link the two peptides. Once TAT-lysine-NLS was complete the N-terminus was acetylated to allow the dendrimer to be added to the side chain by deprotecting lysine N^ε-Fmoc with 20% piperidine [2].

Isothermal titration calorimetry (ITC) was performed using a MicroCal VP-ITC Microcalorimeter (MicroCal Inc, USA) with Origin software and VPViewer 2000. The addition of dendrimer into DNA results in an exothermic reaction which is shown by the negative spikes on the raw data graph (Fig 1). Once the DNA is saturated with dendrimer no more binding occurs and the following injections are either exothermic or endothermic heats of dilution. A blank titration was also performed where the dendrimer was titrated into 20 mM Hepes buffer. The area under each peak is converted to kcal/mol of dendrimer and plotted against the molar ratio (Fig 1). The resulting heats from the blank titration were subtracted from the

experimental heats. Binding curves were fitted to the data using a one sites binding model.

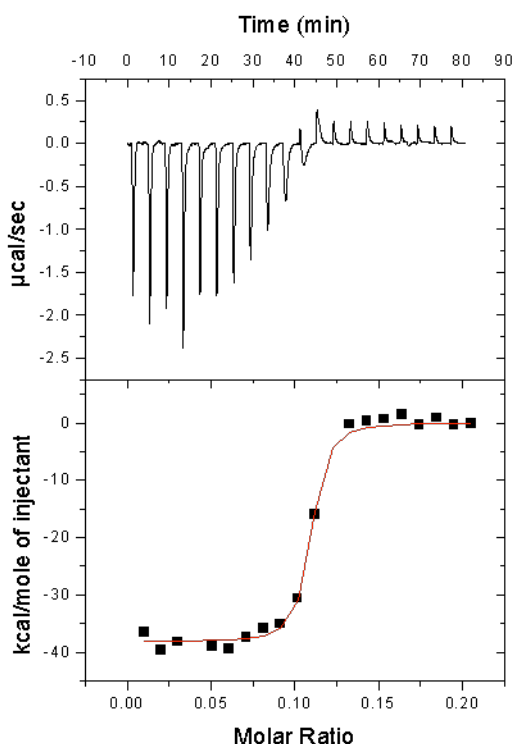


Fig. 1. Microcalorimetry Graph (dendrimer 7). In all titrations the micro-syringe contained the dendrimer solution (2 mg/mL), the sample cell contained salmon testes DNA (100 µg, 1.5 mL) and the reference cell contained 20 mM Hepes buffer. Injections of 3 µL were 4 min apart and reaction temperature was 25°C.

Table 1 shows the thermodynamic results. The charge ratio was around 1:1 for each dendrimer and was calculated from the binding stoichiometry which represents the moles of dendrimer / moles of DNA (in base pairs) at equilibrium. A strong binding affinity between each dendrimer and salmon testes DNA was found. This corresponds to dissociation constants (K_d) ranging from 23 nM for dendrimer 7 to 120 nM for dendrimer 5. The dissociation constant for the TAT peptide has recently been reported to be around 100 nM, which is close to our result of 36 nM [3]. The thermodynamic parameters show that the interaction between the dendrimers and DNA is

Table 1. Thermodynamic parameters from ITC experiments

Dendrimer	Charge Ratio (+ / -)	K_a (M^{-1})	K_d (nM)	ΔH (kcal/mol)	ΔG (kcal/mol)	$T \Delta S$ (kcal/mol)
1	0.80 / 1	2.76E+07	36	-16.27	-10.15	-6.13
2	1.09 / 1	3.37E+07	30	-29.83	-10.26	-19.57
3	0.90 / 1	1.17E+07	86	-8.97	-9.64	0.67
4	1.25 / 1	2.71E+07	37	-20.39	-10.14	-10.26
5	1.34 / 1	8.31E+06	120	-10.91	-9.44	-1.48
6	0.97 / 1	2.18E+07	46	-23.52	-10.01	-13.51
7	1.10 / 1	4.42E+07	23	-38.20	-10.42	-27.78

driven by favourable negative enthalpy accompanied by unfavourable negative entropy [4]. However, dendrimer 3 has a slightly positive entropy. Gibbs free energy changes are negative indicating the reaction proceeds spontaneously.

Further analysis of the results indicates that the thermodynamic parameters are correlated to the number of positive charges on the dendrimer (Fig 2). The correlation is positive indicating that when the number of positive charges increases, the binding affinity increases. While it has been shown that the binding affinity between our dendrimers and DNA is high, the ability of the complex to dissociate once it reaches the nucleus is essential to allow for successful gene expression.

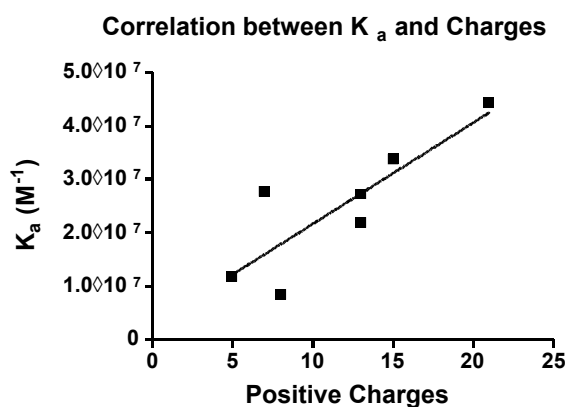


Fig. 2. Correlation between binding affinity and number of positive charges.

References

1. Ehtezazi, T., Rungsardthong, U., and Stolnik, S. (2003) *Langmuir*, **19**, 9387-9394.
2. Coles, D. J., Yang, S., Esposito, A., Mitchell, D., Minchin, R., and Toth, I. (2007) *Tetrahedron* doi: 10.1016/j.tet.2007.09.048
3. Ziegler, A., and Seelig, J. (2007) *Biochemistry*, **46**, 8138-8145.
4. Tan, J. F., Too, H. P., Hatton, T. A., and Tam K. C. (2006) *Langmuir*, **22**, 3744-3750.