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Investigation of the interaction between peanut agglutinin and synthetic glycopolymeric multivalent ligands†

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The interaction between synthetic glycoplymers bearing β-D-galactose side groups and the lectin peanut agglutinin (PNA) was investigated by UV-difference spectroscopy and isothermal titration calorimetry (ITC). UV-difference spectroscopy indicated that the polymer–lectin interaction was stronger than that between PNA and either the corresponding monomer, D-galactose or D-lactose. The thermodynamics of binding (K, ΔG, ΔH, ΔS and n) were determined from ITC data by fitting with a two-site, non-cooperative binding model. It was found that the glycopolymer displayed around a 50 times greater affinity for the lectin than the parent carbohydrate, and around 10 times greater than the monomer, on a valency-corrected basis. Binding was found to be entropically driven, and was accompanied by aggregation and precipitation of protein molecules. Furthermore, interesting differences between polymers prepared either from deacetylated monomers, or by deacetylation of pre-formed polymers, were found.

Introduction

Protein–carbohydrate interactions are involved in a myriad of human biological processes, including the initiation of infection and disease, trafficking and clearance of glycoproteins, immune defence, fertilisation, recruitment of leukocytes to inflammatory sites, and cancer malignancy and metastasis.[1,2] Synthetic saccharide ligands could therefore find wide employment in the investigation of key biochemical phenomena and in the design of new therapeutics able to, for example, inhibit pathogen[3] or toxin[4] carbohydrate-mediated adhesion or as efficient cell-specific macromolecular drug carriers.[5] However, attempts to synthesise such ligands have often been frustrated by the low intrinsic affinity of the protein–monovalent carbohydrate interaction. Typical binding constants of monovalent mono- and oligo-saccharide ligands to specific lectins[6] are in the range 10^3–10^6 M^{-1}. Nature has overcome this intrinsic weakness of binding through multivalency, both in ligand and receptor.[7] The activity enhancement of multivalent carbohydrate ligands has been termed the 'cluster glycoside effect'.[8,9] However, the physical origin of the phenomenon is still not well understood.[10] It has been repeatedly pointed out that both structural and energetic aspects of the lectin–carbohydrate interaction have to be elucidated, if a deep understanding of the process is to be achieved.[2,11] Of the techniques currently in use for protein–ligand association, only isothermal titration microcalorimetry (ITC) allows the direct determination of the delicate balance of thermodynamic binding parameters K, ΔG, ΔH, ΔS and n (stoichiometry of binding, carbohydrate : protein) in a single experiment (with techniques such as surface plasmon resonance, ΔH and ΔS are typically derived indirectly by means of the van't Hoff equation).

Despite their clear relevance to native interactions, few calorimetric studies of multivalent carbohydrate–protein interactions,[9,10,12–14] and indeed few for any multivalent ligand system,[15] have been reported so far. These ITC studies have been limited to small or dendritic glycoconjugates possessing six or fewer carbohydrate residues.[13] Remarkably, although haemagglutination inhibition studies (HIA),[16] enzyme-linked lectin assays (ELLA),[17,18] enzyme-linked immunosorbent assays (ELISA)[19] and surface plasmon resonance (SPR)[17] studies have shown the great potential of synthetic glycopolymers‡ as high-affinity polyvalent ligands, calorimetrically derived thermodynamic data of their association with lectins have never been reported. Reasons for this lack of study may reside in the complexity of the system and the difficulties associated with the exact knowledge of the ligand composition and structural features. Furthermore, polyvalent ligand–lectin interactions often proceed through an associated aggregative mechanism in which each ligand cross-links distinct protein molecules.[20] This phenomenon may result in the precipitation of the aggregates, which, in turn, may hamper the ITC experiment. As state functions the thermodynamic parameters thus determined are the sum of all processes occurring in the reaction cell.

We have recently reported[21] the synthesis of polymethacrylates bearing D-galactose units, poly-[(β-D-galactopyranosyl)oxyethyl methacrylate] (pGalEMA). As shown in Scheme 1, the glycopolymer pGalEMA was obtained following two complementary routes: novel polymerisation of the deprotected glycomonomer GalEMA (route A) and the more traditionally employed deprotection of the corresponding peracetylated polymer pAcGalEMA (route B). Here we report an ITC study of these polymers, which is, to the best of our knowledge, the first calorimetric study of the interaction between a lectin and a synthetic polymeric glycoconjugate.

Results and discussion

Peanut agglutinin, PNA,[22,23] was used as the receptor; the polymers bearing β-D-galactose units (pGalEMA-**A**,**B**; see

† Electronic supplementary information (ESI) available: model used to calculate thermodynamic binding parameters. See http://www.rsc.org/suppdata/ob/b4/b411555b/

‡ The term 'glycopolymers' relates to linear macromolecules bearing repeat units with pendant saccharide functionality, and does not include 'polymeric' multivalent ligands such as dendrimers.

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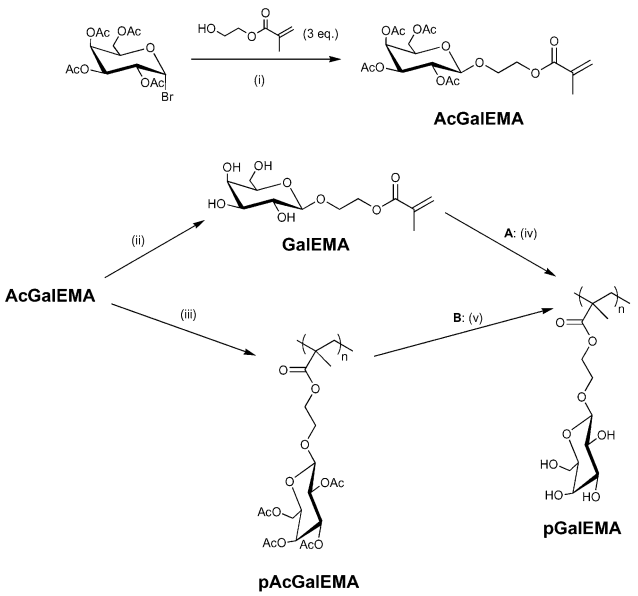

Scheme 1 Polymer and monomer synthesis.[21] *Reagents and conditions*: (i), AgOTf 1.2 equiv., dry DCM, under N_2, −40 °C, 48 h; (ii), $NaOCH_3$ [cat.], dry CH_3OH, RT, under N_2; (iii), AIBN, $CHCl_3$, 65 °C, 48 h; (iv), $K_2S_2O_8$, H_2O : CH_3OH (4 : 1), 65 °C, 48 h; (v), $NaOCH_3$ [cat.], CH_3OH : $CHCl_3$ (1 : 1), RT, under N_2.

Scheme 1) together with the corresponding deprotected monomer (GalEMA) and D-galactose itself, were tested as ligands. The carbohydrate–protein interaction was probed in two ways. Initially, qualitative information was obtained by UV-difference spectroscopy. Owing to the presence of tyrosine residues in the binding site,[22] PNA develops a strong UV-difference spectrum on ligand binding.[24,25] Spectra obtained using D-galactose, D-lactose, GalEMA and pGalEMA-**A** (Fig. 1) indicated clear binding interactions with increasing intensity in the order: D-galactose, GalEMA, D-lactose, pGalEMA-**A**. Consistent with a similar mode of binding within PNA, the addition of GalEMA and pGalEMA to the lectin solution generated a UV-difference spectrum showing a maximum at approximately the same wavelength (*ca.* 285 nm). Moreover, broad qualitative agreement between the spectroscopic and calorimetric measurements (*vide infra*) further confirmed observation of correlated binding events.

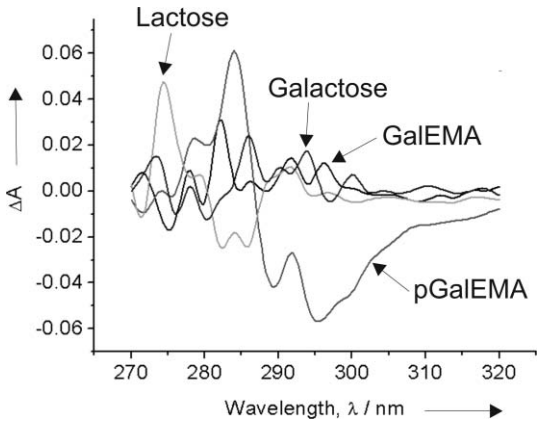

Fig. 1 UV-difference spectra of peanut agglutinin with galactose, GalEMA, pGalEMA-**A** and lactose. [PNA] = 2.12 mg ml^{-1}, [ligands] = 2×10^{-4} M. The solutions were in 0.01 M phosphate-buffered saline (PBS) at pH 7.4.

Next, isothermal titration microcalorimetric determinations of the binding affinity of D-galactose, GalEMA and pGalEMA-**A**,**B** for PNA were performed. After investigation of a wide variety of titration systems, optimal ITC data were obtained from titration of carbohydrate ligands *into* PNA solutions. Despite the limited solubility of PNA in citrate buffer, this medium was used in order to remove any possible contribution of buffer protonation effects to the enthalpic term (for citrate buffer,[26] $\Delta H_{ion} = 0$ kJ mol^{-1}). Thermodynamic parameters are state functions and protein–ligand complexation, changes in protonation state or coupled equilibria are all reported as a single binding enthalpy. Typical outputs of raw microcalorimetry data, binding isotherms and best curve fitting are shown in Fig. 2. The raw data are of rather poor quality, due to the relatively low protein concentration used. This leads to a lower than optimum value of c ([PNA monomer]·K), however one that for the polymeric systems is still within the accepted limits for ITC (2.6 for pGalEMA-**A**).[27] Vital to the determination of meaningful data in this glycopolymeric system, thermodynamic parameters (Table 1) were determined by non-linear least-squares analysis of the binding isotherms, assuming a non-cooperative, bimodal binding model.[28] Interestingly, within the model, changes in two enthalpic parameters (ΔH_1, ΔH_2) occur that may be best described as resulting from two distinct phases: an initial associative phase (ΔH_1, n) and a minor secondary (ΔH_2, n_s) phase. These two phases to date have not been distinguished in lectin binding, and their physical meaning is not yet known; however, we suggest that the first phase is the binding event between the polymer and the lectin, and the second is the precipitation of polymer–lectin conjugates. It is noteworthy that a single phase binding model could not be made to fit to the data. In this study the secondary phase contributes to a minor extent (*vide infra*) and the following results are largely considered in terms of ΔH_1 and n.

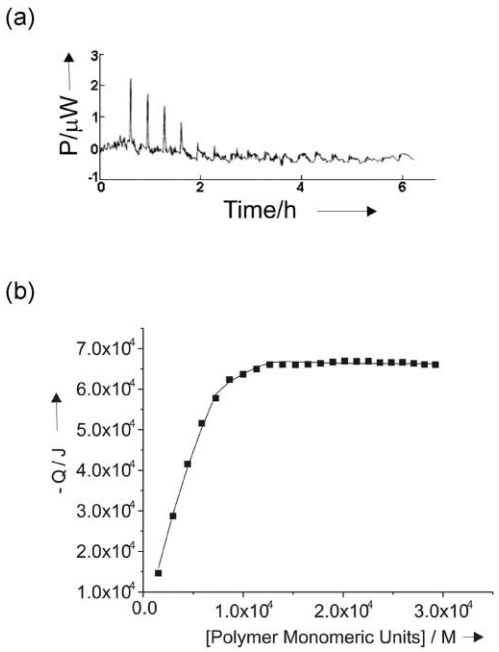

Fig. 2 (a) Experimental calorimetric data for the isothermal titration at 298 K of pGalEMA into PNA. [PNA] = 3 mg ml^{-1}, [ligand] = 0.55 mg ml^{-1}. The solutions were in 20 mM citrate buffer containing 150 mM NaCl, pH 7.4. (b) Corresponding binding isotherm and best fit curve to the data.

As expected, monovalent ligands gave rise to enthalpically driven protein–ligand interactions, exhibiting enthalpy–entropy compensation.[29] The favourable enthalpic contribution arises from the formation of direct and water-mediated hydrogen bonds and van der Waals interactions, while the negative entropic term may be mainly attributed to the loss of degrees of freedom of the ligand upon binding.[14] Values of K and n for D-galactose of approximately 1×10^3 M^{-1} and 1, respectively, showed good agreement with those previously reported.[25] GalEMA exhibited a 5-fold enhancement of binding affinity compared to D-galactose, due to an increase of $-\Delta H^0$

Table 1 Thermodynamic binding parameters of D-galactose, GalEMA and pGalEMA-**A**,**B** to peanut agglutinin.[a]

Ligand	n	$K/M^{-1} \times 10^{-3}$	ΔH_1^0/kJ mol^{-1}	ΔG_{obs}^0/kJ mol^{-1}	$T\Delta S_{obs}^0$/kJ mol^{-1}	n_s	ΔH_2^0/kJ mol^{-1}
Galactose[b]	1.00 ± 0.42	0.95 ± 0.06	-22.97 ± 0.87	-16.98 ± 0.16	-5.99 ± 1.03	0.96 ± 0.35	-0.74 ± 0.31
GalEMA[b]	1.13 ± 0.04	5.65 ± 0.10	-39.41 ± 0.36	-21.41 ± 0.05	-18.00 ± 0.41	1.21 ± 0.16	-7.58 ± 0.10
pGalEMA-**A**[b]	1.56 ± 0.39	46.36 ± 8.81	-5.79 ± 0.19	-26.62 ± 0.48	20.83 ± 0.67	1.95 ± 0.1	-1.39 ± 0.01
pGalEMA-**B**[b]	11.04 ± 0.50	9.40 ± 1.05	-19.84 ± 0.52	-22.67 ± 0.27	2.83 ± 0.79	9.19 ± 2.85	-0.93 ± 0.02
Galactose[c]	2.08 ± 0.22	0.78 ± 0.03	-23.94 ± 0.75	-16.51 ± 0.09	-7.43 ± 0.84	1.04 ± 0.04	-1.30 ± 0.03
pGalEMA-**A**[c]	1.62 ± 0.11	6.42 ± 0.37	-10.80 ± 0.19	-21.72 ± 0.14	10.92 ± 0.33	1.44 ± 0.17	-2.36 ± 0.007
pGalEMA-**A**[c]	1.35 ± 0.34	5.17 ± 0.79	-8.30 ± 0.29	-21.19 ± 0.38	12.89 ± 0.67	1.94 ± 0.58	-1.46 ± 0.002
pGalEMA-**A**[d]	1.00 ± 0.36	4.17 ± 0.57	-6.42 ± 0.18	-20.65 ± 0.34	14.23 ± 0.52	1.85 ± 0.62	-1.20 ± 0.01
pGalEMA-**A**[e]	4.03 ± 0.08	1.37 ± 0.03	-15.76 ± 0.18	-17.90 ± 0.05	2.14 ± 0.23	4.99 ± 0.66	-1.53 ± 0.01
pGalEMA-**A**[f]	4.07 ± 0.31	1.84 ± 0.14	-42.64 ± 2.57	-18.63 ± 0.19	-24.01 ± 2.76	2.99 ± 0.73	-4.56 ± 0.07

[a] Since molar concentrations of the reactants rather than their activities were used, the subscript 'obs' is used to denote to the calculated binding parameters. [b] [PNA] = 1.5 mg ml^{-1}, 20 mM citrate buffer, pH 7.4. [c] [PNA] = 3 mg ml^{-1}, 20 mM citrate buffer, containing 150 mM NaCl, pH 7.4. [d] [PNA] = 3 mg ml^{-1}, 20 mM citrate buffer, containing 150 mM NaCl, pH 7.4, heats of dilution have been subtracted. [e] [PNA] = 5.3 mg ml^{-1}, 50 mM Tris-HCl buffer, containing 500 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 6.9. [f] [PNA] = 5.3 mg ml^{-1}, 50 mM Tris-HCl buffer, containing 500 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 6.9, heats of dilution have been subtracted.

uncompensated by $-\Delta S_{obs}^0$. This result is in agreement with the previously reported specificity of PNA for sugars in the pyranose form[30] and may be due to a locked β-pyranoside configuration and/or hydrogen bond acceptor and hydrophobic interactions between the methacrylate aglycone and the lectin.

Significantly different thermodynamics were determined for polymeric ligands. On a valency-corrected basis, pGalEMA-**A** exhibited a 50-fold affinity gain compared to D-galactose and a 10-fold enhancement with respect to the corresponding monovalent ligand, GalEMA. There is a significant error associated with the K value for pGalEMA-**A** (entry 3 in Table 1). This is due to the aggregation process (*vide infra*) that hampers integration of the peaks in the ITC trace. The error is considerably larger than those associated with the K values for the binding of low molar mass species, for which precipitation does not occur. Interestingly, a significant diminution of enthalpy was determined with the polymeric ligands: $-\Delta H^0$ decreased from *ca.* -23 kJ mol^{-1} for D-galactose to *ca.* -6 kJ mol^{-1} for pGalEMA-**A**. By contrast, the entropic contribution became favourable, varying from negative for the monovalent ligands to positive for the polymer. In particular, for pGalEMA-**A** approximately 75% of ΔG_{obs}^0 arose from the favourable entropy of binding. The determination of these thermodynamic data thus confirms directly the predicted significance of entropic contributions in such multivalent systems.[31]

It is interesting to compare the results obtained for the two polymeric ligands pGalEMA-**A** and pGalEMA-**B**. Affinity enhancements were still detected for pGalEMA-**B** when compared to D-galactose and monomeric GalEMA of approximately 10-fold and 2-fold, respectively. These interactions were similarly characterised by a decrease of $-\Delta H^0$ and a favourable ΔS_{obs}^0. However, the overall affinity for PNA by the less well-defined[21] glycopolymer pGalEMA-**B** was much lower than that of pGalEMA-**A**. The binding constant diminished by approximately 5-fold, due to the reduction of the favourable entropic term, $T\Delta S_{obs}^0$, from *ca.* 20 kJ mol^{-1} for pGalEMA-**A** to *ca.* 3 kJ mol^{-1} for pGalEMA-**B**. Concomitantly, ΔH^0 decreased to *ca.* -20 kJ mol^{-1}, giving a more favourable contribution than that reported for pGalEMA-**A**. The stoichiometries observed for the protein–carbohydrate interactions were also, intriguingly, dramatically different, being 1.5 and 11 for pGalEMA-**A** and pGalEMA-**B**, respectively. The polymer deprotection step involved in the synthesis of pGalEMA-**B** is non-quantitative,[21] resulting in a polymer material possessing partially and/or fully protected sugar residues, unable to bind efficiently to the lectin. The lower affinity of pGalEMA-**B** relative to pGalEMA-**A** and the apparently higher stoichiometry required for the interaction may therefore be understood by considering that a smaller proportion of the Gal residues can, in principle, be recognised by the protein. This confirmed our expectations[21] that precisely and fully deprotected polymers and less precisely deprotected, partially acetylated ones would possess different functional properties.

A decrease of the binding enthalpy can be considered as the thermodynamic signature of an endothermic intermolecular aggregative process superimposed on an exothermic ligand binding event,[9,13] since the latter may be assumed to proceed with thermodynamic parameters equivalent to those of the corresponding monovalent saccharide.[10] This study for the first time confirms this mode of action for a synthetic glycopolymer. It is worthwhile to note that, for experiments performed in citrate buffer, thermodynamic data determined by subtracting heats of dilution (evaluated by means of blank titrations) from each binding heat or by subtracting the average dilution heat determined from the end part of the release heat titration profile[32] were substantially identical. Only a slight decrease of ΔG_{obs}^0 was observed, due to a slight decrease of the enthalpic term (Table 1). Therefore, the concomitancy of the polymer deaggregation with the binding event did not prevent the determination of thermodynamic binding parameters. The thermodynamic parameters evaluated for polymeric ligands, and especially those for pGalEMA-**A**, can be related to an affinity enhancement mainly achieved through the formation of cross-linked ligand–protein complexes,[10,33] which phase separate during the titration. Clear parallels can be drawn with the hydrophobic effect responsible for the micellisation of surfactants in aqueous media.[34] The significant favourable entropic contributions are possibly due to the release of structurally ordered water molecules from the hydration shells of protein tetramers brought close to one another by the aggregation, as well as beneficial ligand degeneracy.[32] Indeed, each PNA tetramer is surrounded by 518 molecules of water.[23] The aggregation hypothesis was also supported by dynamic light scattering (DLS) measurements, which showed a large increase in scattered light intensity (*ca.* 1000 → 2500) after addition of the first aliquot of polymer to the lectin solution. In contrast, only a slight increase in scattered light intensity (*ca.* 350 → 500) was observed in analogous titrations with either D-galactose or GalEMA, and this occurred at a ligand : lectin binding site stoichiometry of around 1.[35] Precipitation of the glycopolymer–lectin complex may preclude biomedical applications of these synthetic glycopolymeric ligands.

At least three phenomena have to be taken into account in the search for a justification of the determined protein-pGalEMA-**A** binding stoichiometry of approximately 1.5. First of all, the proximity of carbohydrate residues to each other within the polymer might prevent the interaction of all the sugars, due to steric hindrance. Secondly, binding of segments of the polymer chain may alter the flexibility of the unbound segments, impeding the attainment of full binding site saturation. Thirdly, the observed precipitation of the aggregates would decrease the

number of binding sites available in solution. Rao *et al.*[15] recently measured the thermodynamics of the interaction of a trivalent derivative of DADA (D-Ala-D-Ala) and of Lac-R′$_d$-Lac with a trivalent and a divalent ligand of vancomycin, respectively. The observed affinity enhancements compared to the monovalent analogues were in both cases certainly reached by means of an intramolecular mechanism. Both $-\Delta H$ and $-\Delta S$ were found to scale proportionally to the number of epitopes, behaviour not observed in this study. Despite the possibility that the glycopolymers might complex different binding sites on the same receptor PNA multimer molecule,[36] the thermodynamic data, the stoichiometry of the interaction, DLS measurements and the relative lack in the polymeric structure of portions that could lead to favourable interactions with protein surfaces in a chelation process, all indicate that the polymers analysed in this study most likely act mainly by aggregating distinct PNA tetramers.[13]

Finally, addition of salt was used to probe contributory electrostatic effects. While D-galactose binding was virtually unaffected, the presence of NaCl critically affected the interactions of GalEMA and pGalEMA. PNA–GalEMA binding was no longer detectable[27] while the binding constant of pGalEMA-**A** decreased *ca.* 7-fold to *ca.* 6×10^3 M^{-1}. The enthalpy change was not strongly affected, while $T\Delta S^0_{obs}$ almost halved indicating reduced entropically-driven, aggregation processes. Indeed, a further increase in salt concentration (50 mM Tris-HCl, 500 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM $MnCl_2$) further reduced binding to $K \approx 1 \times 10^3$ M^{-1}. It may be that electrostatic protein surface–surface interactions[23] are decreased at higher ionic strengths. It should be noted that, although Tris-HCl exhibits $\Delta H_{ion} = 46.0$ kJ mol^{-1} and buffer protonation effects may affect ΔH and ΔS values, favourable enthalpic and entropic contributions were still observed. An increase of n (which is not buffer dependent) up to a value of 4 was determined; a weaker interaction with little or no cross-linking would require an excess of polymer to reach protein binding site saturation.

Conclusions

For the first time the interaction of a lectin with synthetic glycopolymeric ligands was investigated by isothermal titration microcalorimetry. A 50-fold affinity enhancement of the polymer compared to the monovalent saccharide D-galactose was observed. The thermodynamics confirm, at least in the case of PNA, previous suggestions that polyvalent carbohydrates bind to lectins mainly by cross-linking distinct protein molecules. The polymer activity was strongly influenced by the presence of salt, indicating that electrostatic interactions were involved in the binding process, either by affecting polymer nanoparticle dissociation, or by neutralising charges on the protein surface somehow involved in the chelate/aggregative process. It should be stressed that the findings described here provide initial clues as to the nature of the binding between lectins and synthetic glycopolymers. Further experiments probing the influence of glycopolymer valency on binding, using techniques such as haemagglutination assays and surface plasmon resonance in addition to ITC, are required to provide a fuller understanding. In addition, studies involving a monovalent lectin such as galectin-3 [37] should be conducted to determine the influence of precipitation on the binding process.

Experimental

The polymers pGalEMA-**A**,**B** were prepared as described previously.[21] Molecular weight data§ were determined by aqueous size exclusion chromatography: pGalEMA-**A**, number-average molecular weight (M_n) = 461 000 and polydispersity index (M_w/M_n) = 2.21; pGalEMA-**B**, M_n = 2984 and polydispersity index = 2.15. UV-difference spectra were recorded on a Unicam UV2-100 spectrometer, using masked semimicro cells of 1 cm path-length. Aliquots of 500 μl of peanut agglutinin (PNA, Sigma, affinity-purified, salt-free lyophilised powder, activity < 0.1 μg ml^{-1}) solutions (*ca.* 2 mg ml^{-1}, *ca.* 75 μM when 27 000 g mol^{-1} was used as the molecular weight of PNA monomer, in PBS at pH 7.4) were added to both sample and reference cuvettes, and the baseline recorded. An aliquot (4 μl) of a 2.5×10^{-2} M solution of the ligand was added to the sample cell, while the reference received the same amount of buffer solution. Isothermal titration microcalorimetry (ITC) was performed using a Thermal Activity Monitor (TAM 2277, Thermometric AB, Sweden) operated at 298 K. Two sets of experiments were carried out preparing samples of PNA and ligands in 20 mM citrate buffer, containing or not 150 mM NaCl. The samples were adjusted to pH 7.4 (±0.05) using dilute NaOH. The addition of salt allowed the doubling of the lectin concentration in solution. In each experiment a 2.5 ml sample of a solution of PNA (1.5 or 3 mg ml^{-1}) was placed in a sample cell and inserted into the instrument. Once thermal equilibrium was reached, the titration was performed by consecutive injections (25 injections of 20 μl each) of ligand solution (0.275 or 0.55 mg ml^{-1}). The titrant was added by means of a Hamilton microlab syringe mounted on a computer-operated syringe driver (Lund 6100 syringe pump). The experimental method set up *via* the Digitam® 4 software allowed for data collection over a 15 min period for the injection and a 5 min baseline period before the next injection. This was found to be adequate for the interaction to proceed to completion at each injection and reach the baseline before the next addition. Data presented here are the mean of a minimum of three replicate titrations for each experiment. Heats of dilution/mixing were determined in blank titrations injecting aliquots of ligand solution into the appropriate buffer without the lectin. The signs of the released heat values were reversed for data analysis since the output from the instrument is from the perspective of the equipment and not the system under study. The equilibrium binding parameters K, ΔH^0 and n were determined by non-linear least-squares fitting using the routines available within Origin 5.0 (MicroCal Software). The integrated heats of binding were corrected for the enthalpy of dilution, ΔH_d, before calculation of the binding parameters. The heat of dilution can be evaluated either by performing a blank titration, *i.e.* injection of ligand solution into buffer without PNA, or from the end part of the released heat titration profile. In the latter case an average dilution heat is calculated and subtracted from the observed heat value at each data point.[32] In this study both methods were employed. When pGalEMA was used as the titrant, c values ([PNA monomer]·K) were ≥ 0.9.[27] Concentrations of polymer and PNA are both expressed in terms of monomeric units. The free energy of complex formation (ΔG^0_{obs}) was calculated from eqn. (1):

$$\Delta G^0_{obs} = -RT \ln K_{obs} \quad (1)$$

and the entropy of binding determined by eqn. (2):

$$T\Delta S^0_{obs} = \Delta H^0 - \Delta G^0_{obs}. \quad (2)$$

§ Number- and weight-average molecular weights (M_n and M_w, respectively) are used to characterise synthetic polymers which are obtained as a distribution of chain lengths. The breadth of the distribution is indicated by the polydispersity (M_w/M_n).

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