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Calculation of Ionic Bridge Contributions to Homospecific Interactions Mediated by Proteoglycans

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Calculation of Ionic Bridge Contributions to Homospecific Interactions Mediated by Proteoglycans#

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Abstract

Motivation. Adhesion proteoglycans containing the g200 acidic glycan are responsible for species–specific cell aggregation in some sponges. Their homospecific cohesion requires a physiological concentration of calcium ions, but does not take place in the presence of magnesium. This suggests that Ca^{2+}–mediated ionic bridges between the carboxylate groups of the glycan domains (g200–arms) are responsible for the homophilic interactions of adhesion proteoglycans. Here we apply computational methods to predict the strength of such ionic interactions and to explain the metal ion specificity in the aggregation of adhesion proteoglycans.

Method. Ionic bridge stabilities in water are calculated for model systems using the solvated interaction energy approach, which combines a molecular mechanics force field (AMBER) with a continuum model of solvation (BEM).

Results. Solvated interaction energy calculations show a preference for the formation of the Ca^{2+}–mediated ionic bridge between two acetate ions in water in comparison to the Mg^{2+}–mediated interaction, with a difference in binding free energy of 11.7 kcal/mol. Addition of the estimated translational entropy of the metal ion to the calculated solvated interaction energy results in –5.5 kcal/mol per Ca^{2+}–mediated ionic bridge formation between two carboxylate groups fixed on interacting g200–arms. The energetic cost due to the loss of conformational entropy during g200 homodimerization could reach 1200 kcal/mol at room temperature, while the carboxylate content is about 250 groups per g200–arm.

Conclusions. Binding free energy calculations applied to model systems reproduced the observed metal ion specificity in the aggregation of adhesion proteoglycans. The strength and the number of Ca^{2+}–mediated ionic interactions between glycan domains are sufficient to overcome the high conformational entropy costs incurred during homophilic cohesion in order to produce proteoglycan aggregation.

Keywords. Adhesion proteoglycan; homophilic interaction; metal ion specificity; ionic bridge; model system; solvated interaction energy; conformational entropy.

Abbreviations and notations

AP, adhesion proteoglycan	BEM, boundary element method

Dedicated on the occasion of the 70th birthday to Professor Alexandru T. Balaban.

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1 INTRODUCTION

Adhesion proteoglycans (APs) are responsible for species–specific cell aggregation in some sponges [1,2]. The AP–supramolecular complex consists of a protein core, and about 20 irradiating arms of the g200 glycan responsible for the homospecific AP–AP aggregation [1,3]. An autocomplementarity model based on C_2–symmetry has been proposed for the AP structure in order to explain the homophilic specificity of AP aggregation and cell recognition [4]. In this model, a maximal number of contacts between g200–arms can be established only for identical–type APs at the surface of adhering cells.

Glucuronic acid has been identified as the major acidic component of the g200 acidic glycan [3]. In addition, it has been shown that the homospecific AP aggregation requires a physiological concentration of calcium ions, but does not take place in the presence of magnesium [1,3]. It is therefore highly probable that Ca^{2+}–mediated ionic bridges between carboxylate groups, $–COO^-(Ca^{2+})^-OOC–$, are responsible for the interactions between the g200–arms of APs. Here we apply computational methods in order to predict the strength of such ionic interactions and to explain the metal ion specificity in AP–AP aggregation. Ionic bridge stabilities in water are calculated for model systems using the solvated interaction energy approach, which combines a molecular mechanics force field with a high–quality, implicit solvation model. We also estimate the costs in conformational entropy of the glycan chain upon g200–g200 cohesion as well as the number of ionic bridges that need to be formed in order to produce homophilic AP aggregation.

2 MATERIALS AND METHODS

2.1 Model Systems

The metal ion (M^{2+})–mediated interaction between two glucuronate residues from different g200–arms was simulated by the M^{2+}–mediated interaction of two acetate molecules (Ac^-):

$$2Ac^- + M^{2+} \rightarrow Ac_2M \quad (1)$$

The Ac_2Ca and Ac_2Mg complexes were constructed in the geometry corresponding to the symmetrical tetrahedral coordination of the metal ion by the Ac^- oxygen atoms, using SYBYL 6.6 molecular modeling software (Tripos, Inc. St. Louis, MO). Complexes were energy minimized in SYBYL 6.6 using the AMBER 4.1 force field [5] and a distance dependent (4*R*) dielectric constant. The van der Waals parameters (R^* and ε) for Ca^{2+} and Mg^{2+} were adapted from Åqvist [6]. The partial atomic charges of Ac^- were obtained by a two–stage RESP fit [7] to the molecular electrostatic potential calculated with the 6–31G* basis set in Gaussian 94 (Gaussian, Inc., Pittsburgh, PA).

2.2 Binding Free Energy Calculations

The free energy of binding in water, ΔG_{bind}, is calculated with the solvated interaction energy approach [8,9] as the sum of a molecular mechanics energy term, $\Delta E_{\text{bind}}^{\text{MM}}$, and an implicit solvation free energy term, $\Delta G_{\text{bind}}^{\text{solv}}$:

$$\Delta G_{\text{bind}} = \underbrace{E_{\text{inter}}^{\text{vdw}} + E_{\text{inter}}^{\text{coul}} + \Delta E_{\text{intra}}^{\text{strain}}}_{\Delta E_{\text{bind}}^{\text{MM}}} + \Delta G_{\text{bind}}^{\text{solv}} \quad (2)$$

$\Delta E_{\text{bind}}^{\text{MM}}$ is composed of three terms. $E_{\text{inter}}^{\text{vdw}}$ and $E_{\text{inter}}^{\text{coul}}$ are the van der Waals intermolecular interaction energy and Coulomb electrostatic intermolecular interaction energy, respectively, in the bound state. $\Delta E_{\text{intra}}^{\text{strain}}$ represents the change in intramolecular energy between the bound and free states (i.e., the internal strain introduced in Ac^- upon complexation). These molecular mechanics terms were computed using the AMBER 4.1 force field [5] with infinite cutoff and a dielectric constant of 2.

$\Delta G_{\text{bind}}^{\text{solv}}$ is the change in solvation free energy between the bound and free states. For the highly charged chemical species investigated here, only the electrostatic contribution to solvation was calculated. This was done using a continuum dielectric model with a solute interior dielectric constant of 2 and a solvent dielectric constant of 78.5. Reaction field energies were computed by solving the Poisson equation with the boundary element method (BEM) implemented in the BRI BEM program [10,11] and using the SIMS molecular surface program [12]. AMBER–like atomic radii [9] were used for Ac^- (1.908 Å for carbon; 1.387 Å for non–polar hydrogen; 1.500 Å for carboxylate oxygen). Born radii for metal ions (1.712 Å for Ca^{2+}; 1.364 Å for Mg^{2+}) were derived as described previously [9]. RESP2–fitted 6–31G* partial atomic charges were used for Ac^-. The calculated hydration free energies of Ca^{2+}, Mg^{2+} and Ac^- (i.e., BEM reaction field energies for transferring the solute from vacuum to water, with a solute interior dielectric constant of 2) obtained with these atomic parameters (radii and charges) agree well with the experimental ones (Table 1).

Table 1. Calculated and Experimental Hydration Free Energies (kcal/mol)

Ion	$\Delta G_{\text{hydration}}^{\text{calc}}$	$\Delta G_{\text{hydration}}^{\text{exp}}$
Ca^{2+}	–383.1	–380.8[a]; –362.0[b]; –366[c]
Mg^{2+}	–480.8	–455.5[a]; –439.2[b]; –459[c]
Ac^-	–78.4	–79.9[d]

[a] From reference [15a] [c] From reference [15c]
[b] From reference [15b] [d] From reference [16]

3 RESULTS AND DISCUSSION

The results of binding free energy calculations for model systems (Table 2) clearly show a preference for the formation of the Ac_2Ca complex in water in comparison to the Ac_2Mg complex, with a difference in binding free energy of 11.7 kcal/mol. This specificity to the metal ion is primarily dictated by the difference in the net electrostatic term (9.1 kcal/mol) that results from a

delicate balance between the two opposing electrostatic contributions to binding affinity: the favorable intermolecular electrostatic interaction in the complex, $E_{\text{inter}}^{\text{coul}}$, and the desolvation costs incurred upon binding, $\Delta G_{\text{bind}}^{\text{solv}}$. The favorable electrostatic interactions in the Ac_2Ca complex overcome substantially the corresponding desolvation costs, with a net electrostatic binding free energy of –12.8 kcal/mol. In contrast, the Coulomb attraction in the Ac_2Mg complex just recovers the desolvation penalty, leading to a net electrostatic binding affinity of only –3.7 kcal/mol. The non–electrostatic terms, $E_{\text{inter}}^{\text{vdw}}$ and $\Delta E_{\text{intra}}^{\text{strain}}$, oppose binding in both cases, but again favor the Ac_2Ca over the Ac_2Mg ionic bridge formation. Addition of these contributions to the net electrostatic binding free energy results in a favorable binding free energy for the Ac_2Ca complex (–8.5 kcal/mol) and unfavorable binding free energy in the case of the Ac_2Mg complex (3.1 kcal/mol). Overall, our binding free energy calculations in model systems reproduce the observed metal ion specificity in the homophilic aggregation of adhesion proteoglycans [1,3]. The present results are also qualitatively consistent with those of a previous computational study, which however, overestimated the hydration free energy of metal ions [13].

Table 2. Contributions to Binding Free Energy (kcal/mol)[a]

Ionic bridge	$E_{\text{inter}}^{\text{vdw}}$	$E_{\text{inter}}^{\text{coul}}$	$\Delta E_{\text{intra}}^{\text{strain}}$	$\Delta G_{\text{bind}}^{\text{solv}}$	$E_{\text{inter}}^{\text{coul}} + \Delta G_{\text{bind}}^{\text{solv}}$	ΔG_{bind}
Ac_2Ca	3.74	–237.55	0.50	224.77	–12.78	–8.54
Ac_2Mg	5.04	–288.36	1.75	284.69	–3.67	3.12
Δ[b]	1.29	–50.80	1.25	59.92	9.12	11.66

[a] See Materials and Methods section for the description of energy terms
[b] Relative energies, with Ac_2Ca ionic bridge formation taken as reference

Estimation of the g200–g200 aggregation free energy is more difficult. We recall that in calculating the ionic bridge stability in model systems of the type (1) and using the solvated interaction energy function (2), the contributions arising from the loss of rotational and translational entropy upon binding were not included. Since the carboxylate groups are fixed on the g200–arms, only the loss of translational entropy, ΔS_{tr}, for binding of Ca^{2+} ions has to be taken into account. This amounts to approximately –10 e.u. and gives a $-T\Delta S_{\text{tr}}$ contribution to binding at room temperature in the range of 3.0 kcal/mol [14], thus leaving about –5.5 kcal/mol per $–COO^{-}(Ca^{2+})^{-}OOC–$ ionic bridge formation. In addition, the g200–arms, of about 180 nm in length and 10^3 carbohydrate residues each, are highly flexible according to atomic force microscopy and electron microscopy images [1]. Aggregation will produce a formidable loss of conformational entropy, ΔS_{conf}, of the glycan chain. Considering that during aggregation, each hexose residue would be fixed in one out of the 3 reciprocal hexose–hexose chain conformations, a ΔS_{conf} of –2 e.u. per hexose unit is expected. The $-T\Delta S_{\text{conf}}$ contribution to aggregation at room temperature for two interacting g200–arms could reach 1200 kcal/mol. More than 200 inter–arm ionic bridges would be needed to compensate this cost. Does a g200–arm contain sufficient carboxylate groups in order to establish such a high number of ionic interactions? The g200 acidic glycan has a molecular weight of 200 kDa and therefore, it contains approximately 10^3 carbohydrate residues. Glucuronic

acid has been determined to account for 20–25% of the carbohydrate residues of g200 [3]. Thus, the g200–arm would contain a total of 250 carboxylate groups, which could be engaged in a sufficient number of Ca^{2+}–mediated ionic bridges in order to lead to the g200–g200 cohesion and in turn, to cause homophilic AP aggregation.

4 CONCLUSIONS

Our computations on the strength of ionic interactions in water explain the Ca^{2+}–specificity in the aggregation of adhesion proteoglycans. The estimation of the carboxylate content of the g200 acidic glycan, and of the loss in conformational entropy of the glycan chain upon g200–g200 cohesion, suggest that a sufficient number of $–COO^{-}(Ca^{2+})^{-}OOC–$ ionic bridges can be formed in order to produce homospecific aggregation of adhesion proteoglycans.

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