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Side chain elongation causes a change from enthalpy driven to entropy driven binding in the molecular recognition of tetraanionic peptides†

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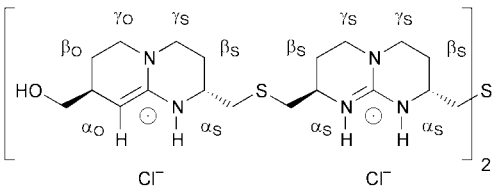
In binding to tetraguanidinium compounds, the average side chain length of tetraanionic peptides determines the thermodynamics of binding, the degree of helix induction and the rigidity of the complex formed.

Protein–protein interactions play an essential role in the regulation of biochemical events in living organisms.[1] Although the increasing number of structures deposited in the structural databases reveals some interesting trends in the biophysical properties of protein–protein interfaces,[2] the design of protein surface receptors able to disrupt such interactions remains a challenge. However, peptide surface binding by synthetic receptors has been studied intensively and is providing interesting candidates for protein surface recognition.[3,4] We have reported the association between tetraguanidinium receptor **1** and peptide **2** with an *i*, *i* + 3, *i* + 6, *i* + 9 arrangement



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of aspartate residues in 10% aqueous methanol.[4] Although side chain length has been shown to affect important properties of amino acids such as helical propensity[5] and the ability to make side chain-to-backbone hydrogen bonds[6] little attention has been paid to its importance in peptide and protein recognition.

† Electronic supplementary information (ESI) available: isothermal titration calorimetry, ^{1}HNMR and NOE data. See http://www.rsc.org/suppdata/cc/b0/b003728l/

With the aim of further developing the approach to protein surface receptors we have now investigated the effect of increasing side chain length. We have sequentially replaced aspartate by glutamate so that the Asp–Glu ratio spans gradually from the all aspartate peptide **2** to the all glutamate peptide **6**. This family of peptides has been studied by CD, NMR and isothermal titration calorimetry (ITC) in order to explore which molecular recognition parameters are affected by increasing side chain length. As reported below we have found that lengthening the side chain has a dramatic effect on the thermodynamics of binding, which shifts from enthalpically driven to entropically driven.

The helical content of the peptides in solution was assessed by circular dichroism spectroscopy and showed (Table 1) that replacement of aspartate by glutamate gradually increases the helical content of the peptide (Hel_i) as predicted from their helix propensities.

Binding of **1** to **2** causes a large increase in the helicity of the peptide because the alignment of atoms necessary for intermolecular hydrogen bond formation requires that the peptide adopts a helical conformation. CD binding[7] titrations of **3** to **6** were carried out to assess the effect of side chain length on helix induction. The results (Table 1) show that the effect of the stepwise replacement of aspartate by glutamate is a gradual decrease in the helical induction due to complex formation (ΔHel). The absolute increase of fractional helicity decreases from 24% for **2** to nearly no conformational change for **5** and **6**.

ITC was used to detect binding and measure affinity constants in the cases where there was no change in ellipticity at 222 nm. The data confirm the 1 : 1 stoichiometry of complexation and give an independent measurement of K_a for peptide **2**, validating the results previously reported by CD. Peptide **2** shows an overall negative enthalpy change (−5.64 kcal mol^{-1}) whereas **6** shows a positive enthalpy change upon binding (3.68 kcal mol^{-1}). Peptide **4**, which has an equal number of aspartate and glutamate residues in its surface shows an enthalpically

Table 1 Thermodynamic and binding data for **1** with tetracarboxylate peptides[14]

Sequence	Hel_i	Hel_f	ΔHel	K_a (M^{-1})	ΔG_a (kcal mol^{-1})	ΔH_a (kcal mol^{-1})	$T\Delta S_a$ (kcal mol^{-1})
2 Ac-A-A-A-**D**-Q-L-**D**-A-L-**D**-A-Q-**D**-A-A-Y-NH_2	21	45	24	CD 3.4 ± 1.2 × 10^5 ITC 1.2 ± 0.2 × 10^5	−7.23	−5.64	1.29
3 Ac-A-A-A-**E**-Q-L-**D**-A-L-**D**-A-Q-**D**-A-A-Y-NH_2	20	40	20	CD 5.6 ± 1.0 × 10^4			
4 Ac-A-A-A-**E**-Q-L-**E**-A-L-**D**-A-Q-**D**-A-A-Y-NH_2	26	36	10	CD 5.3 ± 2.2 × 10^4 ITC [a]	−6.44	0	6.44
5 Ac-A-A-A-**E**-Q-L-**E**-A-L-**E**-A-Q-**D**-A-A-Y-NH_2	27	26	−1	CD 2.2 ± 1.5 × 10^4			
6 Ac-A-A-A-**E**-Q-L-**E**-A-L-**E**-A-Q-**E**-A-A-Y-NH_2	33	30	−3	CD [a] ITC 1.5 ± 0.4 × 10^5	−7.06	3.68	10.74

[a] No observed change by the noted technique.

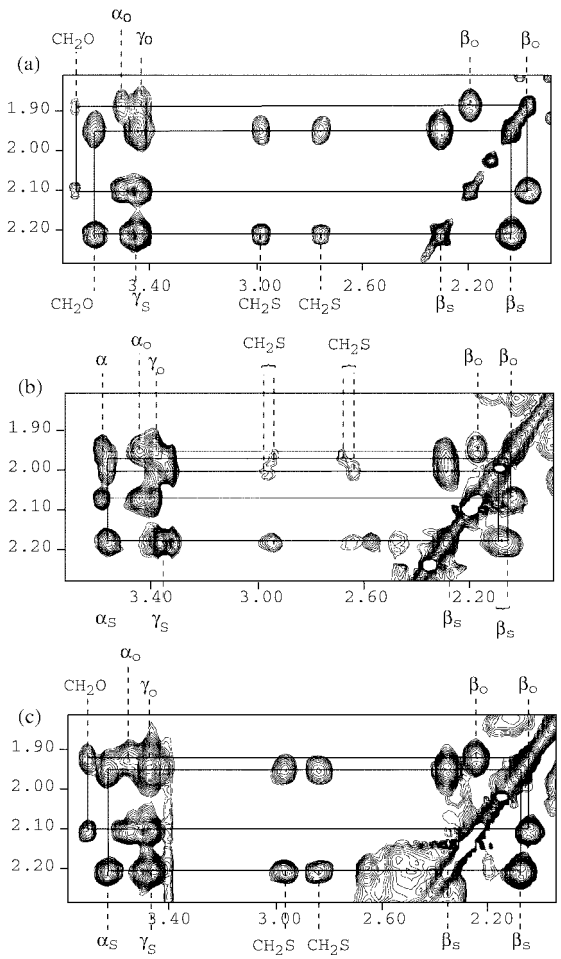

Fig. 1 Expansion of the TOCSY spectrum of **1** (a). Equivalent spectrum in the presence of peptide **2** (b), and in the presence of peptide **6** (c).

neutral binding curve. Despite this variation in thermodynamic behavior, the affinity constants obtained by the combination of CD and ITC methods reveal that affinity does not greatly depend on the individual nature of the residues involved with complex formation, as shown in Table 1.

Chemical shift changes in **1** upon complexation of **2** and **6** have been monitored by NMR spectroscopy.[8] Unbound **1** has a C_2 axis of symmetry which simplifies its spectrum and is no longer present in the complex with tetra acid peptides. Fig. 1(a) shows an expansion of the TOCSY spectrum of unbound **1**. The splitting of resonances in **1** due to the asymmetry of the **1**:**2** complex can be observed in Fig. 1(b). However, this splitting is not observed in the NMR spectrum of **1** bound to **6**. Although chemical shift changes occur, no loss of symmetry is detected as shown in Fig. 1(c). Further evidence for the different rigidity of the complexes is provided by the existence of intermolecular NOEs between the CH_2S and α_S protons of **1** and the methyl groups of leucine 6 and/or leucine 9 of **2** which are not observed in the complex formed between **1** and **6**.

Recognition of **2** is enthalpy driven. Because of the relative rigidity of the aspartate side chains in contact with the receptor, a change in the backbone conformation of **2** towards higher helicity is necessary as shown in Table 1. Despite the entropic penalty associated with preorganization, complex formation takes place due to the large negative enthalpy of α-helix formation[9] and the entropy of liberation of counterions and solvent. Complexation of **6** is however entropy driven. The longer side chain of glutamate allows complex formation to take place without major re-organization: the overall conformation does not change as observed by CD (Table 1). The large entropy change on complexation is probably due to the liberation of solvent and counterions initially bound to the peptide and the receptor. The rigidity of the complex, related to the change in entropy, is also side chain length dependent: close proximity of **1** and the all aspartate peptide **2** in the **1**:**2** complex is indicated by the existence of intermolecular NOEs and the extent of chemical shift changes compared to **1**. The lack of intermolecular NOEs and the less pronounced chemical shift changes suggest that **1**:**6** is significantly less rigid. Binding of **4**, which displays two Asp and two Glu residues on its anionic surface, is enthalpically neutral (Table 1), confirming that side chain length modulates to a great extent the features of recognition between **1** and tetraanionic peptides.

Although the binding of sulfate anions by guanidinium receptors has recently been reported to be entropy driven,[10] in our case the thermodynanic behavior can be tuned by changing side chain length. These results can be analyzed in the context of enthalpy–entropy compensation.[11] This phenomenon has often been described in ligand–protein interactions and is defined as a compensating behavior of the relative changes to ΔH and $T\Delta S$ of binding within a series of ligands or receptors of similar structure.[12]

In summary, we have shown how recognition of *i*, *i* + 3, *i* + 6, *i* + 9 tetra-carboxylate peptides by **1** is a general phenomenon that takes place both with Asp and Glu containing peptides but that length of the side chain has a dramatic impact on the thermodynamics of binding. A recent survey of 'hot spots' shows that Asp is 2–3 times more likely than Glu to contribute to the stability of protein–protein complexes.[13] Our results suggest that this may be due to the tighter nature of Asp *vs.* Glu complexation.

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7 CD experiments were carried out by increasing the concentration of **1** while keeping the concentration of peptide constant (T = 278 K). Ellipticity at 222 nm (Θ_{obs}) was used to calculate the percentage helicity f, $f = 100[(\Theta_{obs} - \Theta_0)/(\Theta_{100}-\Theta_0)]$ where Θ_0 is the ellipticity of the denatured peptide and Θ_{100} is the ellipticity of the 100% helical peptide [$\Theta_{100} = -40\,000(1 - 2.5/n)$; n is the number of amide bonds].
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