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Thermodynamics of phosphotyrosine peptide–peptoid hybrids binding to the p56^{lck} SH2 domain

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A frequently used approach to transform peptides into more drug-like compounds is preparation of the corresponding peptoids or peptide–peptoid hybrids. Although peptoids have advantages, there may also be some disadvantages such as their increased flexibility and the reduced ability for hydrogen bond formation due to alkylation of the backbone amide nitrogen, which might affect the free Gibbs energy (ΔG). To obtain more insight into these contributions to ΔG, we performed thermodynamic analyses on the interaction between peptide–peptoid hybrids, based on the sequence -pTyr-Glu-Glu-Ile-, and the p56^{lck} (Lck) Src homology 2 domain. van't Hoff analysis was performed on binding data obtained from surface plasmon resonance competition experiments in a temperature range of 10–40 °C. It is observed that amino acid–peptoid substitutions do not have a systemic negative effect on the entropic contributions to ΔG. However, loss in hydrogen-bonding capacity of the backbone may strongly reduce the binding enthalpy and contribute to the observed lower binding affinity. Copyright © 2010 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: surface plasmon resonance (SPR); peptidomimetics; van't Hoff analysis; *N*-alkylation; hydrogen bonding; protein–ligand interaction

Introduction

Peptide sequences play a central role in numerous biological processes and have therefore been recognised as potential lead compounds for drug development. However, administration of unmodified peptide drugs is in most cases not recommendable, because peptides exhibit poor oral bioavailability and have a short half-life due to proteolysis [1]. Assembly of peptoid and/or peptide–peptoid hybrids is a method to remain closely to the original peptide structure and to avoid the drawbacks of peptide drugs [2–6]. A peptoid is an analogue of a peptide in which the C-α side chains are shifted to the amide nitrogens of the amino acids. Potentially, peptoids have many advantages compared to peptides, such as resistance to proteolysis [4]. Nevertheless, peptoids have some limitations such as a reduced hydrogen bond-donating capacity, due to *N*-alkylation of the amide nitrogen in the peptide backbone. Furthermore, *N*-alkylation facilitates *cis/trans* isomerisation of the amide bond and might increase rotation of the amino acid ϕ and ψ angles, thus resulting in a larger conformational freedom, which might be disadvantageous for entropy changes upon binding (Figure 1). Insight into the binding thermodynamics of peptide–peptoid hybrids is important because it might guide the design of new ligands and drug candidates [7–9].

The p56^{lck} (Lck) Src homology 2 (SH2) domain was chosen as a model system to study binding thermodynamics of peptide–peptoid hybrids. The Lck SH2 domain binds with high affinity ($K_d \sim 10^{-7}$ M) to the short peptide sequence -pTyr-Glu-Glu-Ile-. SH2 domains are modular domains found in a wide range of signal transduction proteins, in which their most important role is recognition of specific tyrosine-phosphorylated peptide sequences [10–12]. SH2 domain containing proteins have been found in signal transduction pathways that have a relation to a number of diseases. Therefore, SH2 domains are considered as potential targets for therapeutic intervention [13–15].

Crystal structures of complexes with the Lck and Src SH2 domains give the structural basis for binding of the -pTyr-Glu-Glu-Ile- sequence to Src family SH2 domains [16,17]. These crystal structures reveal a very characteristic binding mode that can be denoted as a two-pronged plug engaging a two-holed socket [17,18], because the phosphotyrosine (pTyr) residue is buried in a deep positively charged pocket and the Ile residue in a hydrophobic pocket. The -Glu-Glu- motif lies across the surface of the protein and was initially expected to contribute little to binding. However, later studies showed that the -Glu-Glu- motif is equally important as the (pTyr + 3) Ile [19]. There is evidence that the Glu (pTyr + 1) residue is involved in interaction with a basic residue on the surface of the SH2 domain and that the Glu (pTyr + 2) residue and the peptide backbone are involved in a hydrogen-bonding network with water molecules [20]. Replacement of the -Glu-Glu- motif by -Ala-Ala- or -Gly-Gly- resulted in a major loss of binding enthalpy, which was partially counteracted by a more favourable binding entropy [21]. It has been shown that the Src and Lck SH2 domains do not change in conformation upon binding [16,17]. Therefore, changes in receptor conformation

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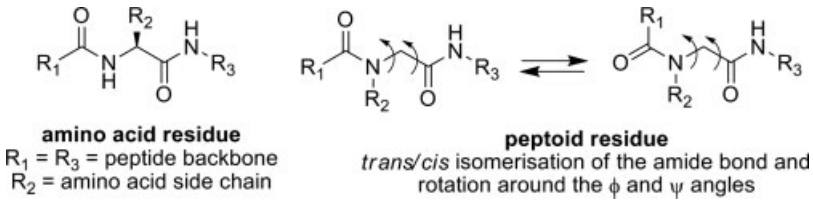

Figure 1. Peptide-to-peptoid conversions increase the flexibility of ligands. α-substituted amino acids have a limited rotational freedom of the ϕ and ψ angles in their backbone, whereas peptoid residues have a larger rotational freedom. Peptide amide bonds are normally present in the *trans*-configuration, whereas *N*-alkylated amide bonds undergo *trans*/*cis* isomerisation.

do not likely cause differences in binding thermodynamics for different peptide–peptoid hybrids.

Previously, our group demonstrated that a peptoid scan using peptide–peptoid hybrids is very useful to explore to what extent a peptide sequence can be transformed into a peptide–peptoid hybrid while retaining its affinity for the Syk tandem SH2 domain [6]. In this study, a peptoid-scan on the sequence pTyr-Glu-Glu-Ile was carried out, and binding of the synthesised ligands to the Lck SH2 domain was studied using a surface plasmon resonance (SPR) assay. The thermodynamic properties of binding to the Lck SH2 domain were investigated using van't Hoff analysis. It was found that peptoid substitution of the Glu (pTyr + 2) (**5**) resulted in the greatest reduction in affinity and the greatest reduction in binding enthalpy. The loss of affinity can be explained by a loss of hydrogen bonding to the receptor. Furthermore, it was observed that a double peptide–peptoid hybrid with substitution of Glu (pTyr + 2) and Ile (pTyr + 3) (**7**) binds with only a small loss in affinity and a slightly less favourable binding entropy, thus illustrating that the larger flexibility of the peptoid hybrids not *per se* causes a more unfavourable entropy.

Materials and Methods

SH2 Domain of p56^{lck}

The c-DNA corresponding to residues 119–226 of mouse Lck, amplified and cloned into the pGEX-3X vector [22] was kindly provided by Prof. Steven E. Shoelson, Harvard Medical School, Boston, MA, USA. The recombinant protein was expressed as *N*-terminal glutathione-*S*-transferase (GST) fusion protein in *Escherichia coli* strain DH10B.

Synthesis of Peptoid-building Blocks

The peptoid-building block of Ile (Fmoc–NIle–OH) was prepared as described by Elgersma *et al*. [4] starting from racemic *sec*-butylamine. The peptoid of the glutamate peptoid-building block [Fmoc–NGlu(OtBu)–OH] was prepared as described by Ruijtenbeek *et al*. [6]

Synthesis of Peptide–Peptoid Hybrids

The peptides and peptide–peptoid hybrids were synthesised using ArgoGel®-Rink-NH-Fmoc Resin (0.76 g, 0.25 mmol, load 0.33 mmol/g) in a reaction vessel through which nitrogen was bubbled for mixing. The coupling cycles were run manually. A typical cycle for coupling of an individual amino acid by the 9-fluorenylmethyl-oxycarbonyl (Fmoc) strategy was: (i) Fmoc deprotection with 20% piperidine in *N*-methyl-2-pyrrolidone (NMP; two times 5 ml, each 8 min); (ii) washing with NMP (three times 5 ml, each 2 min); (iii) washing with CH_2Cl_2 (three times 5 ml, each 2 min); (iv) coupling for 1 h of the Fmoc-protected amino acid by addition of a freshly prepared mixture of the amino acid (1 mmol), benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (1 mmol, 442 mg) and DiPEA (2 mmol, 0.36 ml) in NMP (5 ml) and (v) repeating steps (ii) and (iii). The coupling cycles were monitored with the Kaiser test [23] for primary amines or the chloranil test [24] for secondary amines. These coupling cycles were performed with Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(PO(OBzl)OH)-OH and the peptoid-building blocks of Glu and Ile, Fmoc-NGlu(OtBu)-OH **1** and Fmoc-NIle-OH **2**. HATU was used as a coupling reagent for coupling of an amino acid on the secondary amine of the peptoid residue. Finally, the *N*-terminus was acetylated by using a mixture of acetic acid anhydride (0.5 M), DiPEA (0.125 M) and HOBt (0.015 M) in NMP (5 ml) for 1 h. The peptide–peptoid hybrids were deprotected and cleaved from the resin by treatment with a mixture of TFA/1,2-ethanedithiol/triisopropylsilane/H_2O 3 ml/80 µl/80 µl/160 µl for 2.5 h, followed by precipitation in a mixture of methyl *tert*-butyl ether/hexanes (1 : 1). The precipitate was washed with diethylether (three times). The crude peptide was dissolved in a mixture of *tert*-butanol/H_2O (1 : 1) and lyophilised. The product was purified by RP preparative HPLC (C8), and the purity was verified by RP analytical HPLC (C8). The purity was more than 95%. The identity of the peptide hybrids was verified by ESI-MS analysis. Since Fmoc-NIle-OH was applied as racemate in the synthesis, the resulting peptide–peptoid hybrids **6** and **7** are diastereomeric mixtures, which were not separated by preparative HPLC.

Thermodynamic Analysis Using a SPR Assay

Experiments were performed with a double channel IBIS II SPR instrument (IBIS Technologies, Enschede, the Netherlands) as described before [21]. In short, the instrument was equipped with a CMD6 sensor chip (Xantec GmbH, Münster, Germany). The amino acid 6-aminohexanoic acid (Ahx) was used as a flexible spacer between the SH2 domain-binding peptide and the sensor chip. In the sample cell, the peptide Ahx-Glu-Pro-Gln-pTyr-Glu-Glu-Ile-Pro-Ile-Tyr-Leu-NH_2 was covalently coupled by EDC/*N*-hydroxysuccinimde chemistry. Affinity for the Lck SH2 domain was determined using methods described previously [25]. van't Hoff analysis was performed as described previously [21].

Molecular Modelling

Molecular modelling of the ligand–protein complex was carried out using Sybyl 6.8 (Tripos, Inc., St Louis, MO, USA) [26] on a Silicon Graphics workstation. Construction of the peptide–protein complexes was based on the crystal structure of the Lck SH2 domain complexed with an pTyr peptide inhibitor (PDB entry

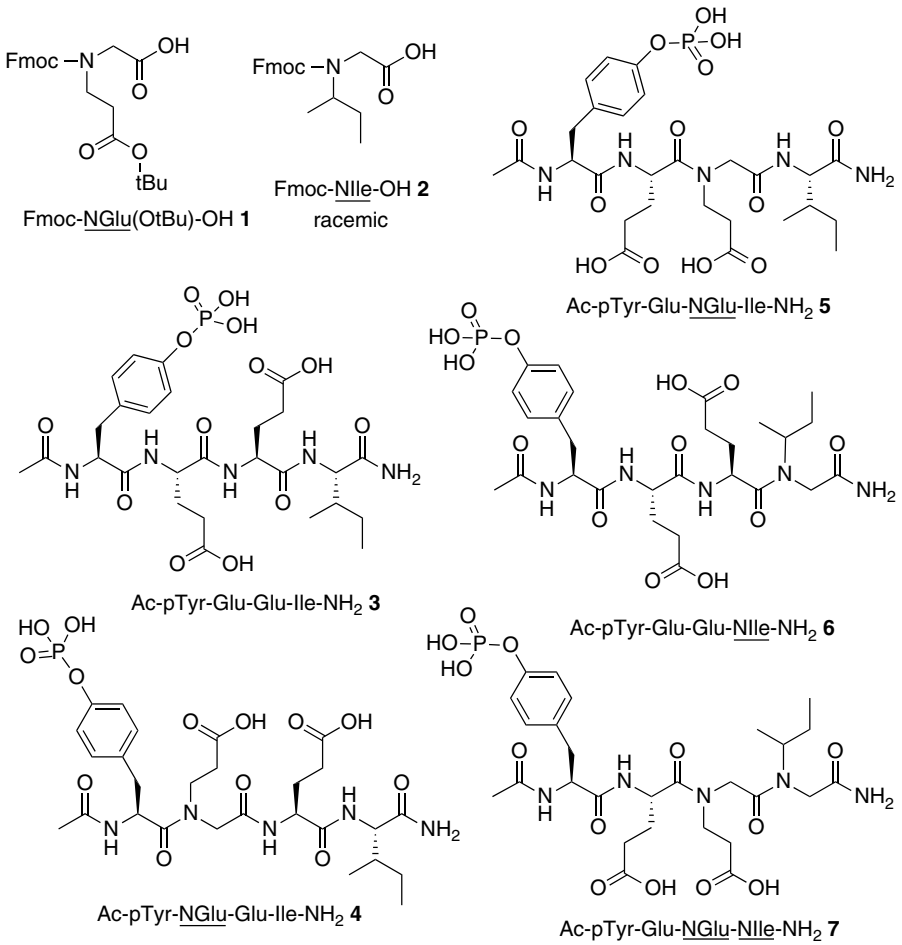

Figure 2. Peptoid-building blocks, peptides and peptide–peptoid hybrids that were used in this study.

code 1LKK) [27]. The water molecules were removed from the protein and hydrogen molecules were added to the protein using the biopolymer module in Sybyl. Peptide–peptoid hybrids were constructed starting from the pTyr peptide and energy minimised in the presence of the Lck SH2 domain for which the geometry was kept fixed. The energy minimisation was performed using a Powell gradient minimisation with the MMFF94s force field [28] in maximal 5000 steps until gradient convergence was reached. The minimised conformations of the peptoid–peptide hybrids were compared with the conformation of the native peptide Ac-pTyr-Glu-Glu-Ile-NH_2 **3** that was also energy minimised.

NMR Experiments

The NMR spectrum of the peptide–peptoid hybrid **4** was recorded as described previously [21].

Results and Discussion

Synthesis of Peptide–Peptoid Hybrids

A peptoid scan of the Lck SH2 domain-binding peptide Ac-pTyr-Glu-Glu-Ile-NH_2 **3** was performed to study the effect on binding of substitution of individual amino acids by the corresponding peptoid-building blocks (Figure 2). Substitutions of amino acids by peptoid-building blocks in the hybrid molecules are indicated by the prefix 'N' for the three letter code and are underlined, e.g. NIle for the peptoid substitution of Ile. It was decided not to substitute the pTyr residue by a peptoid residue, because previous work in our group with the Syk tandem SH2 domain revealed that this substitution abolished binding completely [6]. This effect was attributed to the crucial role of the Arg134 αA2 side chain in the Syk tandem SH2 domain. This Arg side chain is involved in a hydrogen bond to the carbonyl oxygen of the acetyl group and a hydrogen bond to a phosphate oxygen atom [16,17]. In the Lck SH2 domain, Arg134 plays an identical role [27], and it can therefore be expected that shifting the pTyr side chain from the C-α atom to amide nitrogen will strongly reduce binding affinity.

The peptoid-building blocks were synthesised using previously described procedures [4,6]. The Ile peptoid residue was synthesised starting from racemic *sec*-butylamine, thus resulting in a racemic peptoid-building block. The phosphopeptide–peptoid hybrids were synthesised using Fmoc solid-phase peptide synthesis. The crude peptides were purified using preparative RP HPLC. Finally, the purity was verified using analytical RP HPLC and the mass was verified using MS.

Affinity for the Lck SH2 Domain

The binding constants for the peptides and peptide–peptoid hybrids to the Lck SH2 domain were studied using a SPR competition assay [25]. An 11-mer peptide from the middle T

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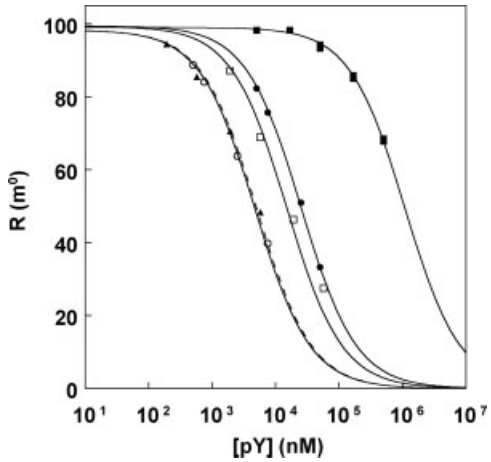

Figure 3. Affinity of peptide–peptoid hybrids as assayed with SPR competition experiments. The SPR signal at equilibrium (*R*) for binding of 25 nM Lck SH2–GST to immobilised Ahx-Glu-Pro-Gln-pTyr-Glu-Glu-Ile-Pro-Ile-Tyr-Leu-NH_2 peptide in the presence of various concentrations of peptoid-peptide hybrids: (○) Ac-pTyr-Glu-Glu-Ile-NH_2; (■) Ac-pTyr-NGlu-Glu-Ile-NH_2; (□) Ac-pTyr-Glu-NGlu-Ile-NH_2; (▲) Ac-pTyr-Glu-Glu-NIle-NH_2; (○) Ac-pTyr-Glu-NGlu-NIle-NH_2.

Ag (Glu-Pro-Gln-pTyr-Glu-Glu-Ile-Pro-Ile-Tyr-Leu) provided with an Ahx spacer was immobilised on the sensor surface. Direct SPR experiments yield the affinity of the Lck SH2 domain, as a GST fusion protein, to the immobilised ligand on the sensor surface (K_c). However, K_c is an apparent binding constant and is, for example, in this case affected by dimer formation of the GST fusion protein [25,29]. To avoid artefacts due to immobilisation, competition experiments were performed in order to obtain thermodynamic dissociation constants for the interaction in solution (K_d) [25]. Results from the SPR competition experiments are shown in Figure 3 and Table 1.

The position of the peptide-to-peptoid substitutions appeared to be critical for the binding affinity. Substitution of Glu (pTyr + 1) as in **4** lowered the binding affinity 100-fold corresponding to a reduction of free binding energy ΔG° of 2.8 kcal/mol, whereas substitution of Glu (pTyr + 2) as in **5** and Ile (pTyr + 3) as in **6** residues was well tolerated. Almost no loss in affinity was observed upon substitution of the Ile (pTyr + 3) residue to a peptoid (compound **6**) and little loss in affinity was observed in the double peptide–peptoid hybrid Ac-pTyr-Glu-NGlu-NIle-NH_2 **7**, which demonstrated that the affinity is retained for peptide-to-peptoid substitution in this part of the ligand. This indicates that the backbone amide NH functionalities of Glu (pTyr + 2) and Ile (pTyr + 3) are not involved in strong interactions with the SH2 domain.

Thermodynamic Analysis of the Binding to the Lck SH2 Domain

Thermodynamic analyses were performed to study the influence of replacement of amino acids by peptoid-building blocks on the thermodynamic parameters for binding to the Lck SH2 domain. Thermodynamic analysis was performed using the SPR competition assay, in which the affinity in solution (K_d) was determined at different temperatures ranging from 10 to 40 °C. These data were fitted with the integrated van't Hoff equation as described previously [18,21]. The van't Hoff plots for the various peptide–peptoid hybrids are shown in Figure 4, and the thermodynamic parameters derived from the fits are shown in Table 1. The previously published peptides **8** and **9** were included in Table 1 in order to compare their binding thermodynamics to the peptide–peptoid hybrids [21]. We previously demonstrated that van't Hoff analysis of K_d values obtained by SPR provides comparable enthalpy and entropy values as obtained by isothermal titration calorimetry (ITC) [21]. ITC is a more direct approach to determine enthalpy and entropy values for protein–ligand interactions. However, proton exchange events upon binding can contribute to the observed heat release. Furthermore, high affinity constants ($K_a > 10^7$ M^{-1}) cannot be assayed very accurately with ITC. Therefore, van't Hoff analysis and ITC-based studies can be considered as complementary techniques for thermodynamic analysis. The higher flexibility of peptoids compared with the corresponding peptides (Figure 1) was not directly reflected in the entropy values. The higher conformational freedom due to *cis*/*trans* isomerisation of the amide bond was confirmed in NMR experiments as shown for peptide–peptoid hybrid **4** (Figure 5). Peptide–peptoid hybrid **4** shows, for example, a more favourable entropy compared with peptide **3**, which indicates that the increased conformational freedom of this ligand in solution does not play a predominant role in binding. Peptide–peptoid hybrid **4** has the lowest affinity for the SH2 domain, which raises the notion that a crucial interaction with the Lck SH2 domain cannot be formed in this peptide–peptoid hybrid. The other peptide–peptoid hybrids show a modest

Table 1. Dissociation binding constant (K_d) for the interaction of peptide–peptoid hybrids with the Lck SH2 domain in solution at 25 °C derived from the data in Figure 3 and thermodynamic parameters for binding of peptide–peptoid hybrids to the Lck SH2 domain at reference temperature 25 °C as derived from van't Hoff analysis from the data in Figure 4

	Compound	K_d (10^{-6} M)[a]	ΔG° (kcal/mol)[b]	ΔH° (kcal/mol)	$T\Delta S^\circ$ (kcal/mol)	ΔCp (cal/mol K)
○ **3**	Ac-pTyr-Glu-Glu-Ile-NH_2	0.93 (± 0.08)	−8.2 ± 0.1	−5.1 ± 0.4	3.1 ± 0.4	−288 ± 86
■ **4**	Ac-pTyr-NGlu-Glu-Ile-NH_2	110 (± 20)	−5.4 ± 0.1	0.05 ± 0.1	5.6 ± 0.1	−305 ± 71
□ **5**	Ac-pTyr-Glu-NGlu-Ile-NH_2	3.3 (± 0.5)	−7.5 ± 0.1	−7.1 ± 0.4	0.4 ± 0.4	−336 ± 100
▲ **6**	Ac-pTyr-Glu-Glu-NIle-NH_2	1.0 (± 0.2)	−8.1 ± 0.1	−6.0 ± 0.4	2.1 ± 0.4	−490 ± 100
● **7**	Ac-pTyr-Glu-NGlu-NIle-NH_2	5.0 (± 0.7)	−7.2 ± 0.1	−5.0 ± 0.4	2.2 ± 0.3	−381 ± 90
8	Ac-pTyr-Ala-Ala-NH_2[c]	8.5 (± 1.8)	−6.9 ± 0.3	2.1 ± 1.1	8.9 ± 1.1	−141 ± 343
9	Ac-pTyr-Gly-Gly-NH_2[c]	390 (± 67)	−4.7 ± 0.1	6.0 ± 1.4	10.7 ± 1.4	501 ± 427

[a] $n = 3$, standard deviations of the non-linear curve fit are reported, $T = 25$ °C.
[b] Calculated from the K_d value at 25 °C, $n = 3$ standard deviations of the non-linear curve fit are reported.
[c] Values reported previously [21].

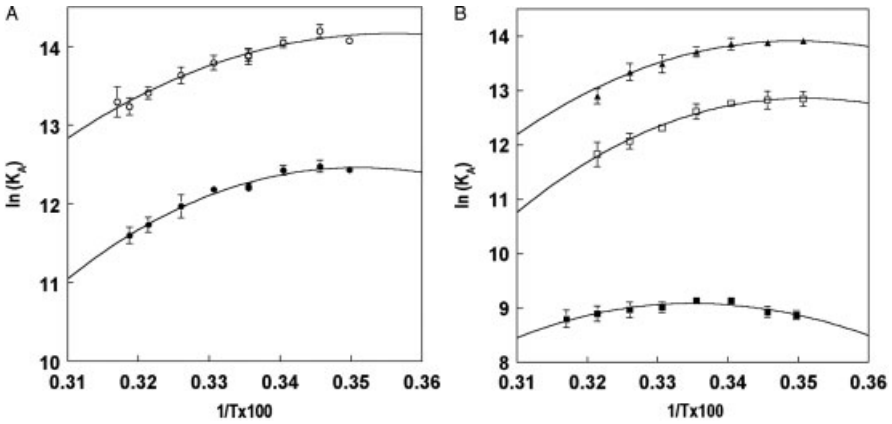

Figure 4. Van't Hoff plots for binding of peptide–peptoid hybrids to the Lck SH2 domain. Panel A: (○) -Ac-pTyr-Glu-NGlu-NIle-NH_2; (●) -Ac-pTyr-Glu-NGlu-NIle-NH_2. Panel B: (■) -Ac-pTyr-NGlu-Glu-Ile-NH_2; (□) -Ac-pTyr-Glu-NGlu-Ile-NH_2; (▲) -Ac-pTyr-Glu-Glu-NIle-NH_2. The error bars indicate the standard deviation non-linear curve fitting of binding data as shown in Figure 3.

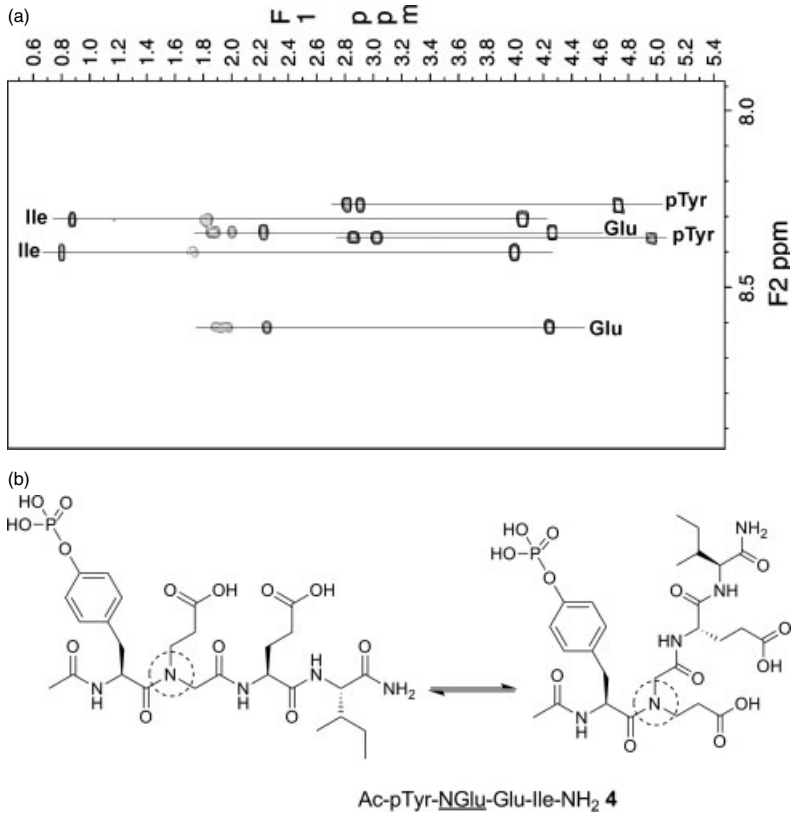

Figure 5. Five hundred megahertz ^{1}H NMR TOSCY spectrum of peptide–peptoid hybrid **4**. Two distinct resonances were observed for the pTyr, Glu and Ile residues. The NH resonances of these residues are marked with horizontal lines. On the horizontal lines, the resonance peaks of the α, β and γ protons of the corresponding amino acid residues are observed. Two NH resonance lines were observed for each residue, which indicates that *cis/trans* isomerisation on the peptoid amide bond occurs.

reduction in binding affinity compared with peptide **3** and the deviations in the enthalpy and entropy contributions are less pronounced than for peptide–peptoid hybrid **4**. This suggests that the most crucial interactions with the SH2 domain can be made by these peptide–peptoid hybrids.

Binding of Ac-pTyr-NGlu-Glu-Ile-NH_2 4 to the Lck SH2 Domain

Comparison of the Ac-pTyr-NGlu-Glu-Ile-NH_2 **4** peptide–peptoid hybrid with all-peptide Ac-pTyr-Glu-Glu-Ile-NH_2 **3** shows that the loss in binding affinity is caused by a less favourable enthalpy contribution of approximately 5 kcal/mol (Table 1). This loss was, however, partially compensated by a more favourable $T\Delta S^\circ$ of approximately 2.5 kcal/mol. Molecular modelling was performed to study the molecular basis for this. Modelling was performed based on the crystal structure of the Lck SH2 domain in complex with -pTyr-Glu-Glu-Ile- (PDB entry code 1LKK) [27]. The peptide ligand was converted to peptide–peptoid hybrid **4** followed by energy minimisation in presence of the Lck SH2 domain for which the geometry was kept fixed. The model suggests that the NGlu

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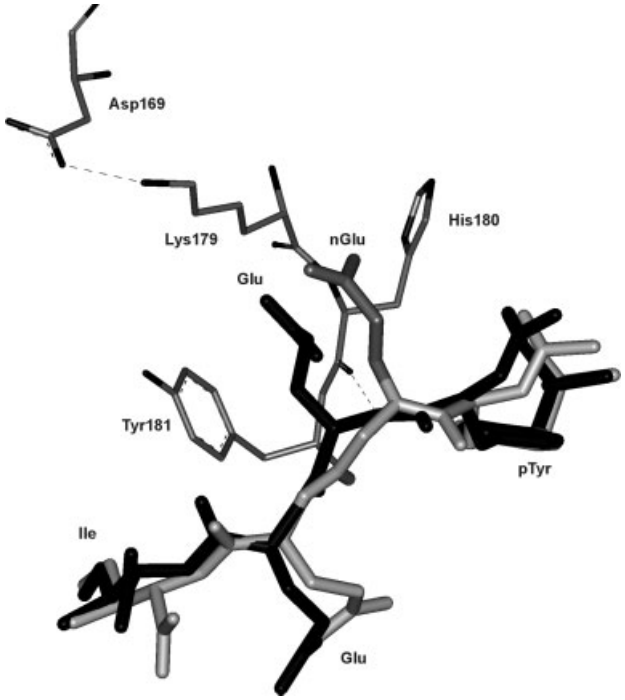

Figure 6. The structure of the peptide sequence -pTyr-Glu-Glu-Ile- bound to the Lck SH2 domain (PDB code 1LKK) [27] is shown in black thick sticks. The modelled structure of the peptide–peptoid hybrid Ac-pTyr-NGlu-Glu-Ile-NH_2 **4** is shown in grey thick sticks. Lck residues are shown as thin sticks. A hydrogen bond between the NH of the pTyr + 1 Glu and the Lck SH2 domain and the electrostatic interaction between Lys 179 and Asp 169 are shown as dashed lines.

residue of **4** lies on the surface of the protein just like in the native peptide; however, it is shifted somewhat in the direction of pTyr (Figure 6). The backbone nitrogen of the Glu (pTyr + 1) residue in the native peptide forms a hydrogen bond with the backbone carboxyl oxygen of His 180 (His βD4) (Figure 6), which is very well conserved in various SH2 domains [30]. This hydrogen bond is lost upon substitution of the Glu (pTyr + 1) amino acid residue to an NGlu peptoid residue in which the nitrogen is alkylated. This is probably the most important factor in the decreased binding enthalpy caused by NGlu peptoid substitution on the Glu (pTyr + 1) position. Furthermore, the model shows that the carboxylate of the Glu (pTyr + 1) residue in the native peptide or NGlu in peptide–peptoid hybrid **4** is close to the amine of SH2–Lys 179 (βD3 K). The Lys 179 ammonium is involved in an electrostatic interaction with the carboxylate of Asp 169 (βC8 D) (Figure 6). This was possibly an artefact in the crystal structure, because Lys 179 is at the surface of the protein and there is enough space to allow bending of the Lys ammonium group toward the carboxylate group of the residue at the Glu (pTyr + 1) position and make an electrostatic interaction. However, the total energetic contribution of this electrostatic interaction will be very small, because interaction with Asp 169 has to be disrupted for interaction between Lys 179 and Glu (pTyr + 1) carboxylate.

Binding of Ac-pTyr-Glu-NGlu-Ile-NH_2 5 to the Lck SH2 Domain

Binding of Ac-pTyr-Glu-NGlu-Ile-NH_2 **5** at 25 °C showed a more favourable enthalpy and a less favourable entropy compared with **3**, which resulted in a slightly lower affinity (Table 1). There are no obvious interactions between the Glu (pTyr + 2) residue side chain and the SH2 domain [27]. The Glu (pTyr + 2) residue is most likely involved in interactions with a network of water molecules, very similar to that described for the Src SH2 domain [27]. The Glu (pTyr + 2) residue carboxylate side chain forms apparently a hydrogen bond with an ordered water molecule [27]. For the closely related Src SH2 domain, there is evidence that upon binding, a rearrangement in the size and strength of hydrogen bonds with such water molecules occurs [31]. Considering this, the unfavourable entropy of NGlu (pTyr + 2)-substituted peptoid hybrid **5** is consistent with less distortion of the water network upon binding, i.e. less water molecules, present in the apo SH2 protein, are exchanged upon binding.

Binding of Ac-pTyr-Glu-Glu-NIle-NH_2 6 to the Lck SH2 Domain

Although the affinity of the Ac-pTyr-Glu-Glu-NIle-NH_2 **6** peptide–peptoid hybrid at 25 °C was similar to that of the all-peptide **3**, deviations in binding thermodynamics are observed (Table 1). The binding of the Ac-pTyr-Glu-Glu-NIle-NH_2 **6** peptoid hybrid was characterised by a less favourable entropy and a more favourable enthalpy. In modelling studies, it was observed that the NIle (pTyr + 3) peptoid residue in peptoid hybrid **6** can bind in the hydrophobic-binding pocket for pY + 3 Ile. Moreover, the Ile (pTyr + 3) residue in the native peptide forms a hydrogen bond with a structured water molecule, which is lost upon *N*-alkylation in the peptide–peptoid hybrid **6** [20,27]. It is remarkable that the loss of this hydrogen bond, and the higher flexibility of a peptoid amide bond, does not lead to lower affinity compared with the all-peptide **3**.

Binding of Ac-pTyr-Glu-NGlu-NIle-NH_2 7 to the Lck SH2 Domain

The peptoid substitutions in the -Glu-Ile- part of the peptide Ac-pTyr-Glu-Glu-Ile-NH_2 **3** are very well tolerated by the Lck SH2 domain and are thus valuable conversions to make more drug-like compounds. The fivefold lower affinity of the double peptide–peptoid hybrid pTyr-Glu-NGlu-NIle-NH_2 **7** can be completely ascribed to an unfavourable entropy contribution compared to the all-peptide **3** (Table 1). This might originate from the increased flexibility of the peptoid backbone. However, it can be expected that other factors contribute to this decrease as well, as outlined for the single peptide–peptoid substitutions in compounds **5** and **6**.

Conclusions

This thermodynamic study of a peptoid scan of ligands to the Lck SH2 domain gives additional insight into the role of increased flexibility of peptoids and the effect of *N*-alkylation compared to affinity data alone. The study shows that substitutions of single amino acids in peptides by the corresponding peptoid residues are a valuable approach to transform peptides to more drug-like compounds. High-affinity binding is retained for peptide-to-peptoid substitutions in regions of the peptide backbone where no strong interactions with the receptor occur. This is demonstrated by the relatively high affinity of the peptide–peptoid hybrid Ac-pTyr-Glu-NGlu-NIle-NH_2 **7**. Moreover, the results show that a potential loss in binding entropy due to the higher flexibility of the peptoid residues in comparison to α-amino acids is limited and that numerous other contributions have to be taken into account. In contrast, the loss of hydrogen bonding with the

peptide backbone upon replacement of an α-amino acid for a peptoid residue can reduce the affinity significantly, as observed for the peptide–peptoid hybrid Ac-pTyr-NGlu-Glu-Ile-NH_2 **4**.

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