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Design and Characterization of Non-Phosphopeptide Inhibitors for Src Family SH2 Domains

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Abstract—The development of novel non-phosphopeptide inhibitors for the Src family SH2 domain is described. Several commercially available hydroxyl aromatic acids have been appended off the N-terminus of pYEEIE and the potent phosphopeptide inhibitors of GST-Lck-SH2 were identified via ELISA. The most potent inhibitor, caffeic acid-pYEEIE, exhibited approximately 30-fold more binding activity than Ac-pYEEIE. Non-phosphopeptides were synthesized by replacing phosphotyrosine of caffeic acid-pYEEIE with tyrosine or 3,4-dihydroxyphenylalanine (DOPA). Caffeic acid-DOPA-EEIE that did not contain phosphotyrosine and its isosteres exhibited less than 20 times decreased binding affinity for GST-Lck-SH2 than Ac-pYEEIE. Moreover, it had a similar binding affinity for the GST-Lck-SH2, GST-Src-SH2, and GST-Fyn-SH2 domains. This study showed that the pY-1 positions of the phosphopeptide inhibitors and of the non-phosphopeptide inhibitors played an important role in the binding for the SH2 domain and that the non-phosphopeptide inhibitor must be a new lead in the development of SH2 inhibitors.

Introduction

Src homology 2 (SH2) domains play a critical role in organizing coherent signal transducing complexes that are essential for the appropriate cellular response to extracellular stimuli.[1] As inhibitors of the formation of SH2 domain-dependent signal transducing complexes are able to disrupt inappropriately hyperstimulated pathways,[2–4] the SH2 domains are regarded as attractive targets for drug development in the fields of oncology and immunology. Several researches have covered the design of ligands, which interact with the Lck SH2 domain, in attempts to develop novel drug candidates for autoimmune disease and T cell-based leukemias and lymphomas.[3,4]

Recent studies on the SH2 domains have revealed that they exhibit a marked preference for the -pYEEIE- sequence, and that short peptides bearing this sequence exhibit a reasonably high affinity for Src family SH2 domains.[3–5] Thus, the synthesis of several phosphopeptide-based ligands for SH2 domains has been reported.[3,4] However, the development of phosphopeptide-based ligands as therapeutic agents remains obscure. In general, phosphopeptide-based species exhibit poor bioavailability, for example, they generally have poor cell penetration properties. Furthermore, the pTyr moiety, which is essential for recognition by the SH2 pocket, is hydrolytically unstable in the presence of tyrosine phosphatase. To overcome this problem, several researches have focused on the design and synthesis of phosphatase-resistant surrogates of phosphotyrosine such as phosphonomethyl-Phe, difluorophosphonomethyl-Phe, carboxymethyl-Phe, and monocharged phosphinate analogue (PO_2HR) of phosphotyrosine.[3,4,6] However, the peptide ligands containing non-hydrolysable pTyr surrogates have exhibited disappointingly low binding affinities for several SH2 domains and required tedious syntheses of non-hydrolysable pTyr surrogates.[3,4]

Recently, Lawrence et al. reported that the affinity of Ac-pYEEIE for SH2 domains was significantly enhanced by appending non-amino acid substituent to the N-terminus of the peptide (i.e., 7-hydroxy-coumarin acid-pYEEIE).[7] However, the interactions responsible for the increased binding potency caused by the introduction of such a large aromatic compound at the N-terminus of the phosphopeptide are not fully understood with the available structural information.[8]

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In this study, several commercially available hydroxyl aromatic acids were introduced to the N-terminus of pYEEIE and high affinity Lck SH2 binding peptides were identified via an enzyme-linked immunosorbent assay (ELISA). Furthermore, the phosphotyrosine of the most potent inhibitor, caffeic acid-pYEEIE, was replaced with Tyr or 3,4-dihydroxyphenylalanine (DOPA), and we have investigated the possibility for developing non-phosphotyrosine peptide inhibitors of SH2 domains. In this study, we discovered novel non-phosphopeptide inhibitors of the SH2 domain and the function of the pY-1 position of the phosphopeptides and of the non-phosphopeptides in terms of interactions with Src family SH2 domains.

Results and Discussion

Several commercially available hydroxyl aromatic acids were introduced to the N-terminus of pYEEIE and potent phosphopeptide inhibitors of GST-Lck-SH2 were identified via ELISA.[9] Caffeic acid-pYEEIE (Ca-pYEEIE) was found to exhibit the most potent binding affinity for the GST-Lck-SH2 domain. As shown in Figure 1, Ca-pYEEIE and Ac-pYEEIE inhibited the binding of EPQpYEEIPIYL with the GST-Lck-SH2 domain in a concentration-dependent manner. As shown in Table 1, the IC_{50} value of Ca-pYEEIE was just 42 nM, whereas the IC_{50} value of Ac-pYEEIE was 1.3 μM, which is consistent with a previously reported value.[10] The phosphopeptide (Cm-pYEEIE),[7] which contained a 7-hydroxycoumarin-4-acetic acid at the N-terminus, was synthesized and its binding affinity for

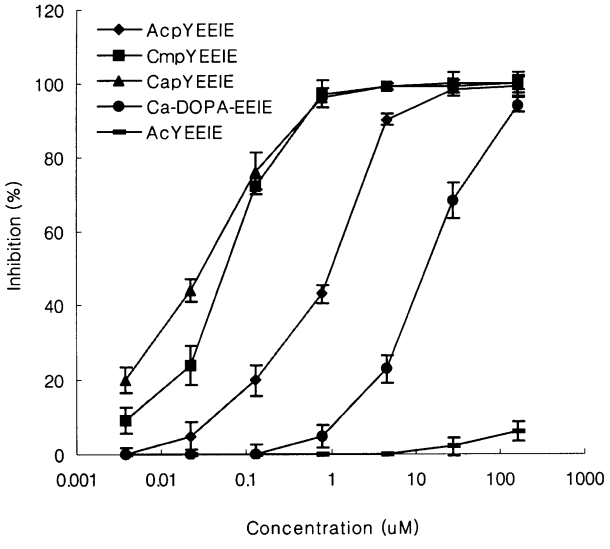


Figure 1. Peptide inhibition of the GST-Lck-SH2 domain by using ELISA. Each of the synthesized peptides was added to a solution containing the GST-Lck-SH2 domain and incubated for 10 min at room temperature. The mixture was added to biotinyl-ε-aminocaproyl-EPQpYEEIPIYL coated wells. After washing, the degree of interaction between SH2 domain and biotinyl-ε-aminocaproyl-EPQpYEEIPIYL was detected by incubating with an anti-GST polyclonal antibody, and this was followed by incubation with a peroxidase-conjugated anti-rabbit antibody and peroxidase substrate solution. The color development was monitored at 450 nm. Each value was calculated from three independent experiments performed in duplicate, which provided a standard deviation less than 20%.

GST-Lck-SH2 domain was found to be a half of that of Ca-pYEEIE.

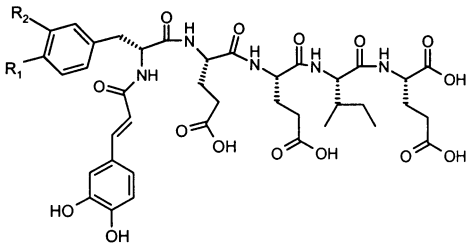
To develop non-phosphopeptide inhibitors for the SH2 domain, pTyr of Ca-pYEEIE was replaced with Tyr or DOPA. DOPA was reported to serve as a non-phosphorylatable mimetic of tyrosine and the pK_a value of DOPA (pK_a, 8.7) is lower than that of Tyr (pK_a, 9.2).[11,12] As shown in Table 1, the binding affinity of Ca-YEEIE (IC_{50} value: 27 μM) for the GST-Lck-SH2 domain was about 20-fold lower than that of Ac-pYEEIE, whereas the replacement of phosphotyrosine with phosphonomethylphenyl in Ac-pYEEIE was reported to decrease the binding affinity for the Src SH2 domain by a factor of 40.[10] To confirm the function of caffeic acid at the pY-1 position of non-phosphotyrosine peptides, the binding affinities of Ac-YEEIE, Ac-DOPA-EEIE, caffeic acid-DOPA-EEIE, and caffeic acid-YEEIE were compared. Ac-YEEIE did not show inhibitory activity up to 200 μM, while the introduction of caffeic acid to the N-terminus of YEEIE and DOPA-EEIE endowed potent inhibitory activity (IC_{50} value: 27 and 18 μM, respectively). The effect of incorporating caffeic acid at the N-terminus of DOPA-EEIE was similar to that of YEEIE. The minor difference between the inhibitory activities of Ca-DOPA-EEIE and Ca-YEEIE must be due to the different pK_a values of DOPA and Tyr.[11]

To confirm the inhibitory activity, the binding activities of the peptide inhibitors with SH2 domain were also measured by competitive assay using BIAcore.[13] First, the control phosphopeptide (EPQpYEEIPIYL) was immobilized on chip surface. The mixture containing the peptide inhibitors and SH2 protein was passed over the surface and refractive index change was measured. As shown in Figure 2, the peptide inhibitors inhibited the binding of SH2 to immobilized phosphopeptide in a concentration dependent manner. Ca-pYEEIE exhibited a more than 30-fold increased binding affinity than Ac-pYEEIE, whereas Ca-DOPA-EEIE had a 40-fold lower binding affinity than Ac-pYEEIE, which was consistent with the inhibition activity measured via ELISA.

As shown in Table 1, the binding affinities of the peptide inhibitors for the Fyn and Src SH2 domains were also investigated. The effect of caffeic acid at the pY-1 position was observed to be similar in terms of peptide binding with the Lck, Fyn, and Src SH2 domains. Ca-pYEEIE exhibited the most potent binding affinity for the Fyn and Src SH2, whereas Ca-DOPA-EEIE as a non-phosphopeptide also showed considerable binding affinity for the Fyn and Src SH2 domains. However, the selectivity of the peptides for the Lck, Fyn, and Src SH2 domains was not observed.

Finally, we investigated whether the non-phosphopeptide, Ca-DOPA-EEIE, could interrupt Lck SH2-mediated signaling and subsequent IL-2 promoter activation upon T-cell receptor stimulation (TCR). Jurkat T-cells were transfected with IL-2 promoter receptor plasmid for 24 h, and then incubated with various concentrations

Table 1. The binding affinities of the peptides for the Lck, Fyn, and Src SH2 domains



Ca-pYEEIE; R_1 = -OPO(OH)$_2$, R_2 = H

Ca-DOPA-EEIE; R_1 = OH, R_2 = OH

Ca-YEEIE; R_1 = OH, R_2 = H

Peptide	IC_{50} (μM, Lck)	IC_{50} (μM, Fyn)	IC_{50} (μM, Src)
Ac-pYEEIE	1.3	1.1	2.0
Cm-pYEEIE	0.081	0.10	0.090
Ca-pYEEIE	0.042	0.084	0.064
Ac-DOPA-EEE	120	>200	160
Cm-DOPA-EEIE	58	77	65
Ca-DOPA-EEIE	18	33	55
Ac-YEEIE	>200	>200	>200
Ca-YEEIE	27	45	54
Ac-pYAEIE	3.8	11	7.0

Ca: 3,4-dihydroxycinnamic acid, Cm: 7-hydroxycoumarin-4-acetic acid, DOPA: 3,4-dihydroxyphenylalanine. Average IC_{50} values were calculated from three independent experiments performed in duplicate, which provided a standard deviation below 20%.

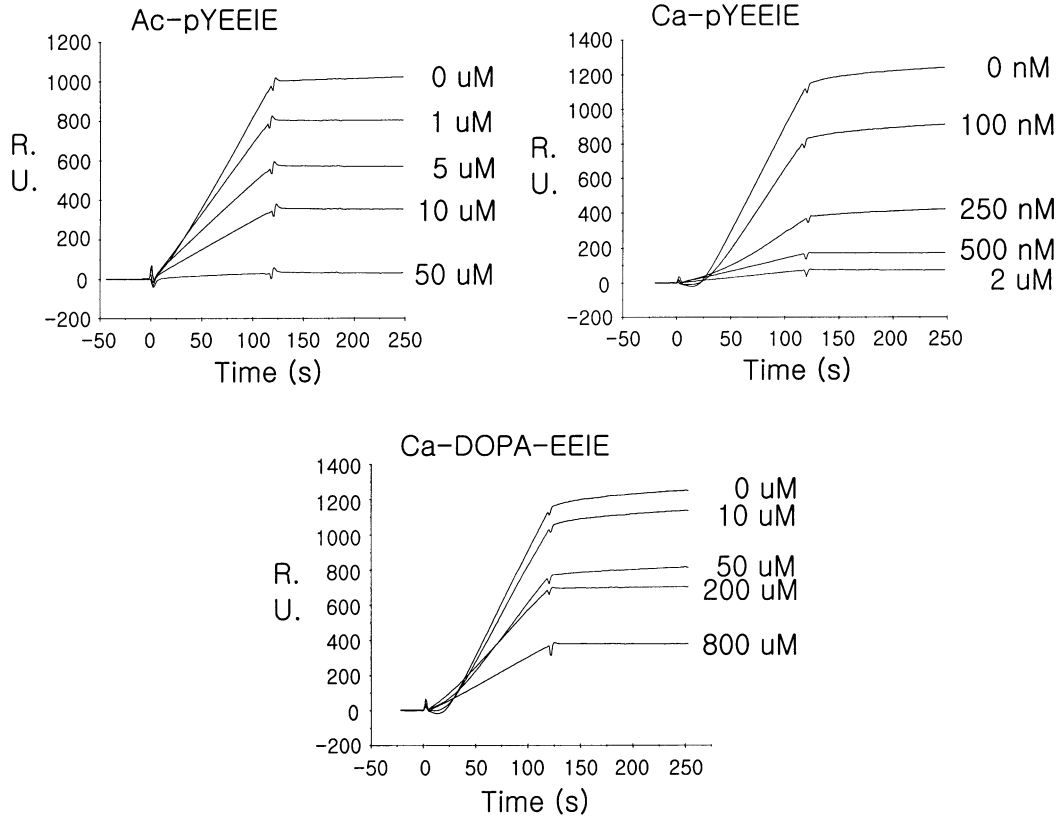


Figure 2. Peptide inhibition of the GST-Lck-SH2 domain using BIAcore. The BIAcore instrument used in this study was manufactured by Biacore AB (BIACORE 3000, Uppsala, Sweden). First, to immobilize the phosphopeptide on the chip surface, 10 μg/mL of biotinyl-ε-aminocaproyl-EPQ-pYEEIPIYL in the running buffer (50 mM Tris, 150 mM NaCl pH 7.5) was injected at a flow rate of 5 μL/min onto a streptavidin coated chip surface. For the competitive binding assay, the GST-Lck-SH2 domain was diluted to 100 nM in the running buffer. Peptide was titrated into the GST-Lck-SH2 domain from 800 to 100 nM and the reaction mixture containing peptide and SH2 protein was equilibrated in the running buffer. And then 10 μL of each reaction mixture was then passed over the surface at a flow rate of 5 μL/min and refractive index change was measured in the running period.

of Ca-DOPA-EEIE for 2 h, and stimulated with anti-CD3 antibody. The TCR-induced activation of IL-2 promoter was not inhibited by Ca-DOPA-EEIE at concentrations up to 100 μM (data not shown). The lack of inhibition of TCR-induced IL-2 promoter activation was presumably due to the poor cell penetration of the peptide, because even though the peptide did not have a phosphate group, it had a high negative charge under physiological conditions.

The present study indicates that the hydroxyl aromatic acid at the pY-1 position of phosphopeptides and of non-phosphopeptides plays an important role in Src family SH2 domain binding and the non-phosphopeptides employed in this work can provide a novel lead for the development of SH2 inhibitors.

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References and Notes

1. Cantley, L. C.; Auger, K. R.; Carpenter, C.; Duckworth, B.; Graziani, A.; Kappeller, R.; Soltoff, S. *Cell* **1991**, *64*, 241.
2. Botfield, M. C.; Green, J. *Ann. Rep. Med. Chem.* **1995**, *30*, 227.
3. Garcia-Echeverria, C. *Curr. Med. Chem.* **2001**, *13*, 1589.
4. Vu, C. B. *Curr. Med. Chem.* **2000**, *10*, 1081.
5. Songyang, Z.; Shoelson, S. E.; Chaudhuri, M.; Gish, G.; Pawson, T.; Haser, W. G.; King, F.; Roberts, T.; Patnofsky, S.; Lechleider, R. J.; Neel, B. G.; Birge, B.; Cantley, L. C. *Cell* **1993**, *72*, 767.
6. Broadbridge, R. J.; Sharma, R. P. *Current Drug Targets* **2000**, *1*, 365.
7. Lee, T. R.; Lawrence, D. S. *J. Med. Chem.* **1999**, *42*, 784.
8. Beaulieu, P. L.; Cameron, D. R.; Ferland, J. M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Llinas-Brunet, M.; Betageri, R.; Cardozo, M.; Hickey, E. R.; Ingraham, R.; Jakes, S.; Kabcenell, A.; Kirrane, T.; Lukas, S.; Patel, U.; Proudfoot, J.; Sharma, R.; Tong, L.; Moss, N. *J. Med. Chem.* **1999**, *42*, 1757.
9. Lee, T. R.; Lawrence, D. S. *J. Med. Chem.* **2000**, *43*, 1173.
10. Gilmer, T.; Rodriguez, M.; Jordan, S.; Crosby, R.; Alligood, K.; Green, K.; Kimery, M.; Wagner, C.; Kinder, D.; Charifson, P.; Hassell, A. M.; Willard, D.; Luther, M.; Rusnak, D.; Sternbach, D. D.; Mehrotra, M.; Peel, M.; Shampine, L.; Davis, R.; Robbins, J.; Patel, I. R.; Kassel, D.; Burkhart, W.; Moyer, M.; Bradshaw, T.; Berman, J. *J. Biol. Chem.* **1994**, *269*, 31711.
11. Niu, J.; Lawrence, D. S. *J. Am. Chem. Soc.* **1997**, *119*, 3844.
12. Siraki, A. G.; Smythis, J.; O'Brien, P. J. *Neuroscience Lett.* **2000**, *298*, 37.
13. Morelock, M. M.; Ingraham, R. H.; Betageri, R.; Jakes, S. *J. Med. Chem.* **1995**, *38*, 1309.