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Selected Paper

Peptide-Assisted Enhancement of Inhibitory Effects of Small Molecular Inhibitors for Kinases

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Abstract

Conjugation of small molecular inhibitor with a site recognition peptide would generate highly potent peptide–drug conjugate inhibitors of protein kinases. A peptide selected by ribosome display with a tRNA carrying an inhibitor-conjugated amino acid exhibited up to 10- to 100-fold higher inhibitory effects towards different kinases than solely the inhibitor.

Kinases are involved in signal transduction pathways that regulate cell growth, proliferation, and death[1] and their deregulation results in a number of cancers. Thus, kinases serve as potential anticancer drug discovery targets. These proteins belong to the large family of phosphotransferases that catalyze transfer of the γ-phosphoryl group from the high-energy phosphate-donating molecule ATP to the hydroxy group on a serine, threonine, or tyrosine side chain of specific substrates. The phosphorylation results in changes in activity, reactivity, and binding ability of a substrate with other proteins. The human genome encodes 518 protein kinases.[2] Currently, 32 small-molecule protein kinase inhibitors are approved for clinical use and more than 100 are at different stages of clinical trials.

Most of the reported kinase inhibitors are small molecules that provide limited overall surface area available to react with a target protein.[3] An elegant strategy for increasing the overall surface area available to interact with a target protein is conjugation of an ATP-competitive small molecule with a peptide substrate analog.[4] Johnson and co-workers tethered

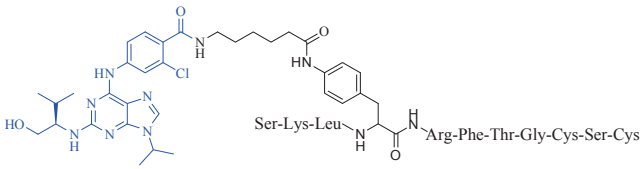

Figure 1. The structure of synthesized peptide–PVB conjugate A5, the blue part represents purvalanol B (PVB).

ATP-γS with a 30-residue cell division cycle 6 (CDC6) peptide to generate a bisubstrate inhibitor with high affinity for cyclin-dependent kinase 2 (CDK2).[5] Staurosporine-like compounds have also been conjugated to peptide to generate bisubstrate inhibitors for protein kinases, including cyclin-dependent kinase 1 (CDK1)[6] and cAMP-dependent protein kinase (PKA).[7] This strategy largely relies on the availability of the cocrystal structures of the inhibitor–kinase complex as well as prior knowledge of a pseudosubstrate. Many kinases of interest do not satisfy the above criteria and therefore this strategy cannot be applied to any arbitrarily selected protein target.

Recent progress of molecular evolutionary engineering has enabled us to create new artificially functional polypeptides to any arbitrarily selected target protein using non-canonical amino acids.[8–15] Using this methodology, we reported recently the introduction of a small molecule inhibitor into a random sequence peptide library for ribosome display using tRNA carrying the inhibitor and selection of the peptide containing the inhibitor.[16] Purvalanol B (PVB, Figure 1) and cyclin-dependent kinase 2 (CDK2) were chosen as the inhibitor and the target, respectively.[17] As a result, the selected peptide–PVB conjugate A5 (SKLXRFTGCSC, Figure 1, (X represents PVB carrying aminophenylalanine)) bound to CDK2 tightly and the interaction of the conjugated peptide with the kinase surface adjacent to the small molecule interaction site improved dramatically the potency of the inhibitor.

However, no negative selection using other non-CDK2 kinase proteins as target was performed during the previous selection process. Therefore, it was expected that the selected peptides inhibit other kinases more efficiently than PVB. In this study, we investigated the inhibitory effects of A5 against several kinases and performed molecular dynamics simulations of some of these protein–peptide complexes.

The interactions of PVB and cyclin-dependent kinases (CDKs) have been well studied, and a previous study indicated that the carboxylic acid of the 6-anilino substituent of the purine can be modified without affecting the inhibitor–kinase interaction.[17] We introduced PVB to a random sequence peptide library by coupling the carboxylic acid side chain to a non-natural amino acid (aminophenylalanine) and isolated the peptide–drug conjugates with higher inhibitory activity.[16] The selected A5 was the most abundant sequence identified (the same sequence was found in 31 of the 65 clones examined). By incorporation of the peptide, the inhibitory activity was enhanced 8-fold more than PVB, and the peptide alone had no activity.[16]

Results and Discussion

To investigate if the peptide improves the inhibitory potency against other kinases (Figure 2), we tested the abilities of A5, PVB, and the control peptide (CP, SKLFRFTGCSC) carrying

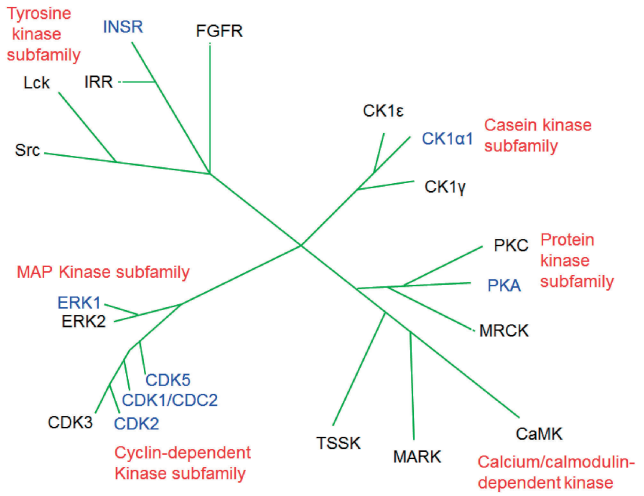

Figure 2. The human kinome. The kinases tested in this research are shown in blue.

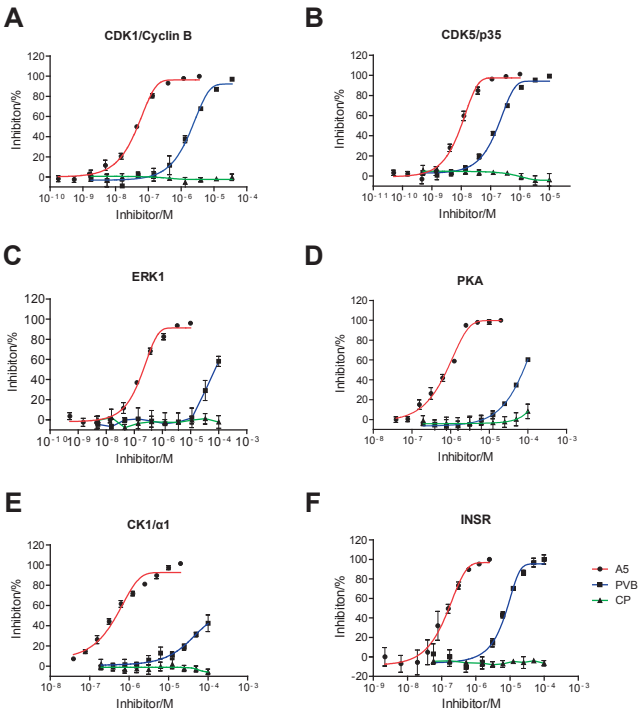

Figure 3. Interaction between PVB, A5, and CP with CDK1 (A), CDK5 (B), ERK1 (C), PKA (D), CK1α1 (E), and INSR (F). The results indicate that the peptide–PVB conjugate A5 exhibits higher inhibitory activity than the parent molecule PVB and the control peptide that carrying no PVB at the side chain has no inhibitory activity against all the tested kinases.

no PVB at the side chain to inhibit CDK1/cylcin B, CDK5/p35, ERK1, PKA, CK1α1, and INSR. The inhibitory effect was assayed by the FRET-based Z′-LYTE kit (Figure 3) and the results are summarized in Table 1. The CP exhibited no inhibitory activity against all of the above kinases. Interestingly, A5 showed higher inhibitory potency against all of the kinases when compared with the results from the parent inhibitor PVB. Not only CDK2, but also CDK1 and CDK5 were inhibited. The

Table 1. Inhibitory properties of A5, PVB, and CP on the tested kinase proteins

Kinase	IC_{50}/nM		
	A5	PVB	CP
CDK1/cyclin B	138	1,995	—[a)]
CDK2/cyclin A	34	263	—
CDK5/p35	9	170	—
ERK1	178	>33333	—
PKA	914	91000	—
CK1α1	355	>10000	—
INSR	150	7300	—

a) No inhibitory activity.

inhibitory potencies (IC_{50}) were 138 and 9 nM for A5 against CDK1 and CDK5, respectively, and those values are 14- and 18-fold higher than that of the parent molecule PVB (Table 1). The difference of IC_{50} between these CDKs might be explained by the A5 affinity; 287, 73, and 32 nM in K_d for CDK1, CDK2, and CDK5, respectively (Figure S1).

In addition, although the inhibitory effect of PVB on MAP kinase subfamily (ERK), protein kinase subfamily (PKA), casein kinase subfamily (CK1α1), and tyrosine kinase subfamily (INSR) is low, the inhibitory activity was also significantly improved by the conjugation of PVB with the selected peptide (Table 1). The IC_{50} values were 178, 914, 355, and 150 nM for ERK1, PKA, CK1α1, and INSR, respectively, and the inhibitory activities are 187-fold, 99-fold, 28-fold, and 48-fold higher than that of PVB. This may be because the small molecule PVB works as a warhead and the conjugated peptide increasing the biding affinity of the PVB. Therefore, the inhibitory activity of peptide–PVB conjugate was enhanced compare with that of PVB alone. The peptide binds to kinase proteins neither at ATP-binding site nor at substrate binding site, thus the peptide itself without PVB exhibits no inhibitory activity against any of the tested kinases. The enhancement on these non-CDK proteins was higher than that on CDKs. Because no negative selection round was performed during the previous selection process, it was considered that the selected peptides inhibit other similar kinases more than PVB.

We performed six 40 ns molecular dynamics simulations of protein (CDK2, CDK5, and PKA)–ligand (PVB) or –A5 peptide complex systems and analyzed the binding interface between the protein and ligand or peptide to investigate the role of the A5 peptide (Figures 4 and S2). The systems were constructed using the X-ray structures of CDK2 (PDB Entry 1CKP), CDK5 (PDB Entry 4AU8), and PKA (PDB Entry 1ATP). In CDKs, we observed that PVB interacted with the protein at the binding site and the A5 peptide made additional interactions (Figures 4A, 4B, S2A, and S2B). The interaction between A5 and CDKs generated intra-interactions inside A5 (Figures 4A and 4B). Our simulation results indicate that the A5 peptide has the ability to increase the binding affinity of PVB to CDKs by forming additional interactions and these additional contacts enable PVB to inhibit protein function. In the case of PKA, the protein–PVB interaction was weaker than that of the CDKs–PVB interaction. The position of PVB was slightly moved and PVB does not bind tightly during the simulations. However, A5 interacted with PKA, although the intra-interaction within A5

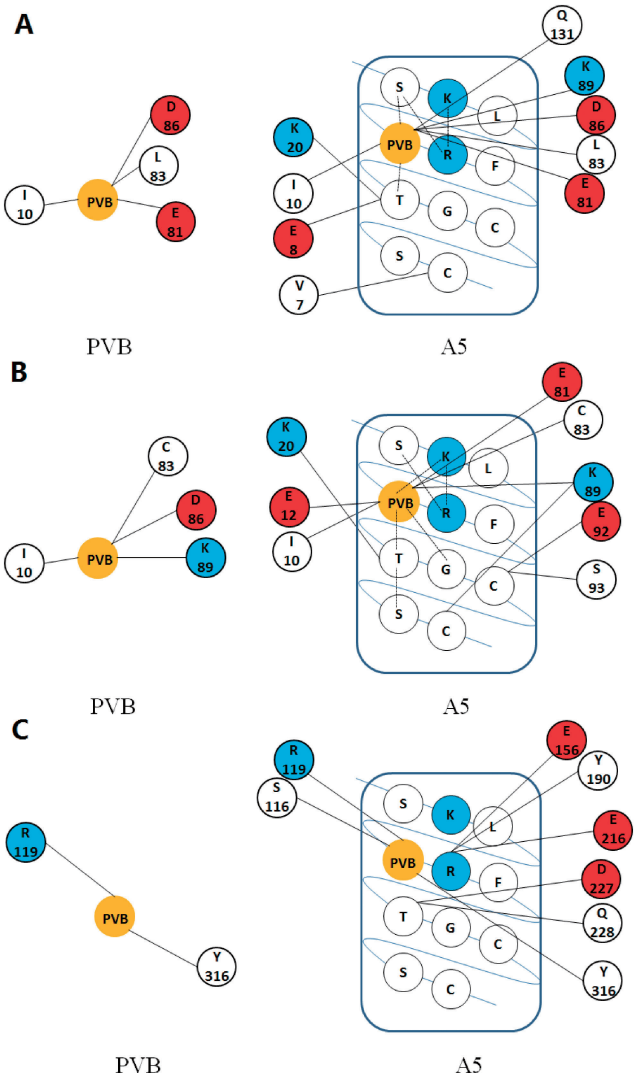

Figure 4. Scheme of the interaction between PVB and A5 with (A) CDK2, (B) CDK5, and (C) PKA by MD simulations. PVB and A5 peptide interacted with each protein. The number of protein–A5 interactions is larger than that of protein–PVB. Table 1 shows that inhibitory activities of A5–protein are stronger than that of PVB–protein. From our results, we believe that increase of interactions by A5 peptide should be a reason for differences of IC_{50} in A5 peptide and PVB only for the same protein. Red, blue, and white circles indicate acidic, basic, and other residues, respectively. Figure 4A is reported in our previous report.[16]

observed in the interactions with CDKs was not observed (Figures 4C and S2C).

A peptide selected by ribosome display with a tRNA carrying a small molecule showed synergetic effects on inhibition of kinases. This strategy should be useful for improving the activity of existing small molecule inhibitors.

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Supporting Information

Materials and Methods, Figures S1, S2, and S3. This material is available on http://dx.doi.org/10.1246/bcsj.20150414.

References

1 M. E. M. Noble, J. A. Endicott, L. N. Johnson, *Science* **2004**, *303*, 1800.
2 G. Manning, D. B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam, *Science* **2002**, *298*, 1912.
3 G. L. Verdine, L. D. Walensky, *Clin. Cancer Res.* **2007**, *13*, 7264.
4 D. Lavogina, E. Enkvist, A. Uri, *ChemMedChem* **2010**, *5*, 23.
5 K.-Y. Cheng, M. E. Noble, V. Skamnaki, N. R. Brown, E. D. Lowe, L. Kontogiannis, K. Shen, P. A. Cole, G. Siligardi, L. N. Johnson, *J. Biol. Chem.* **2006**, *281*, 23167.
6 S. Sasaki, T. Hashimoto, N. Obana, H. Yasuda, Y. Uehara, M. Maeda, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1019.
7 T. L. Schneider, R. S. Mathew, K. P. Rice, K. Tamaki, J. L. Wood, A. Schepartz, *Org. Lett.* **2005**, *7*, 1695.
8 T. Uzawa, S. Tada, W. Wang, Y. Ito, *Chem. Commun.* **2013**, *49*, 1786.
9 M. Liu, H. Jinmei, H. Abe, Y. Ito, *Chem. Lett.* **2008**, *37*, 102.
10 M. Liu, H. Jinmei, H. Abe, Y. Ito, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2964.
11 M. Liu, S. Tada, M. Ito, H. Abe, Y. Ito, *Chem. Commun.* **2012**, *48*, 11871.
12 S. Tada, Q. Zang, W. Wang, M. Kawamoto, M. Liu, M. Iwashita, T. Uzawa, D. Kiga, M. Yamamura, Y. Ito, *J. Biosci. Bioeng.* **2015**, *119*, 137.
13 M. R. Jafari, L. Deng, P. I. Kitov, S. Ng, W. L. Matochko, K. F. Tjhung, A. Zeberoff, A. Elias, J. S. Klassen, R. Derda, *ACS Chem. Biol.* **2014**, *9*, 443.
14 S. Bellotto, S. Chen, I. Rentero Rebollo, H. A. Wegner, C. Heinis, *J. Am. Chem. Soc.* **2014**, *136*, 5880.
15 W. Wang, T. Uzawa, N. Tochio, J. Hamatsu, Y. Hirano, S. Tada, H. Saneyoshi, T. Kigawa, N. Hayashi, Y. Ito, M. Taiji, T. Aigaki, Y. Ito, *Chem. Commun.* **2014**, *50*, 2962.
16 W. Wang, Y. Hirano, T. Uzawa, M. Liu, M. Taiji, Y. Ito, *Med. Chem. Commun.* **2014**, *5*, 1400.
17 N. S. Gray, L. Wodicka, A.-M. W. H. Thunnissen, T. C. Norman, S. Kwon, F. H. Espinoza, D. O. Morgan, G. Barnes, S. LeClerc, L. Meijer, S.-H. Kim, D. J. Lockhart, P. G. Schultz, *Science* **1998**, *281*, 533.