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Design of a selective chemical probe for class I PDZ domains

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Abstract—Covalent labeling has been widely used for structural and functional analyses of proteins. To target a wide range of PDZ domains, we designed a chemical scaffold mimicking the E/D-T/S-XV peptide, which is a PDZ domain that binds ligands in higher occurrence. A chemical probe (**2**) that contained this moiety alkylated diverse PDZ domains, including NHERF-1 PDZ2, and differentially visualized the cellular proteome.

Chemical proteomics has been emerging as a novel approach to analyze the proteome of cells.[1] An essential step in this analysis is to covalently label protein residues with chemical probes. The selectivity of protein labeling that targets lysine or cysteine is defined solely by the accessibility of the protein residues, that is residues that are exposed to solvent are easier to label.[2] Therefore, this approach lacks the ability to represent the domain structure of proteins. The PDZ domain is a common protein–protein interaction module that binds to the carboxyl termini or sharp β turn.[3] PDZ domains are categorized on the basis of the peptide sequence of their preferred ligand. The class I PDZ domain, which preferentially binds to ligands having an S/T(-2) residue, is the most predominant subclass. Class I PDZ domains utilize a conserved histidine residue to recognize the S/T(-2) residue by hydrogen bond interaction.[4]

We have reported a chemical scaffold that targets the PDZ domain (**1**, Fig. 1A).[5] This scaffold was designed to mimic the hydrophobic M/I(-3) side chain by an *n*-pentyl group on the indole-2-position and hydroxyl group of S/T(-2) by an indole-3-methanol. Indole-3-methanol structures are prone to form carbocations, therefore show electrophilicity. Because of this chemical reactivity, the scaffold selectively alkylates the conserved histidine residue of the third PDZ domain of MAGI-3 protein. To the best of our knowledge, this is the only example of selective conjugation of a histidine residue.

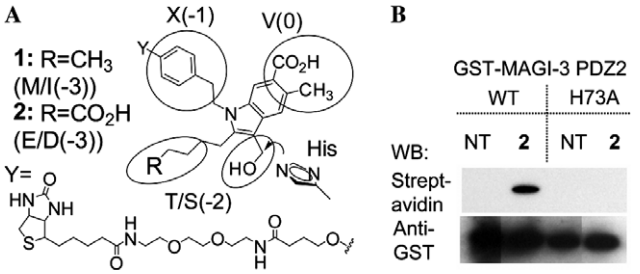

Figure 1. (A) Design of PDZ-targeting chemical probes. (B) Chemical probe **2** crosslinks to a histidine residue that is conserved in class 1 PDZ domains.

We have constructed a database in-house to search the human proteome for short peptide sequences of carboxyl termini. We searched proteins by querying two similar sequences that can be mimicked by our chemical scaffold: query M/I(-3)-T/S(-2)-X(-1)-V/I(0) (X is any amino acid) gave 228 hits, and query E/D(-3)-T/S(-2)-X(-1)-V/I(0) gave 677 hits (on May 2006). This finding suggests that the occurrence of the second sequence is about three times that of the first. Based on this observation, we hypothesized that a chemical scaffold mimicking the latter sequence would target a range of PDZ domains that is three times wider than that of the first (**2**, Figs. 1A and S1).

To confirm the specificity of the binding of **2** at the histidine residue of the class 1 PDZ domain, we reacted GST-fused MAGI-3 PDZ2 domain and its mutant, which contains an alanine substitution (H73A),[5] overnight with three equivalents of **2**, purified by

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SDS–PAGE and analyzed by streptavidin blotting. Only wild-type MAGI-3 PDZ2 domain showed the biotinylation, suggesting specificity of **2** against the histidine residue (Fig. 1B). Next, we compared the reactivity and binding diversity of **1** and **2** against class I PDZ domains. We successfully used a proteomic array of 96 distinct PDZ domains derived from a variety of cytosolic proteins.[6,7] These PDZ domains were selected from diverse candidates that might selectively associate with the carboxyl terminus of P2Y1 receptor (terminates D-T-S-L) or that of the β1-adrenergic receptor (terminates E-S-K-V), suggesting that they will associate with class I PDZ domains. To illuminate the selectivity, we overlaid the array with a limited concentration (1 μM) of **1** or **2**. After extensive washing, the labeled PDZ domains were visualized by streptavidin blotting. Probe **1** bound very weakly or not at all to various PDZ domains on the array (data not shown). In contrast, **2** exhibited strong binding to a number of the PDZ domains (Fig. 2A). In this assay, **2** bound most robustly to NHERF-1 PDZ2[8] (bin B4) and ZO-1 PDZ-2[9] (bin C12) and to several other PDZ domains, including ERBIN PDZ[10] (bin C10), Densin 180 PDZ[11] (bin E1), and γ2-synthrophin PDZ[12] (bin E12). All of these PDZ domains reportedly bind to proteins that have an E/D-T-X-V/I/L motif. Longer exposure of the array showed signals in many additional PDZ domains (data not shown), suggesting their weaker reactivity to **2**. MAGI-3 PDZ2 (bin A11), which binds to **2** (Fig. 1B), did not exhibit good signal in this assay. These data suggest that **2** has a much higher affinity (or reaction kinetics) for a number of the other PDZ domains on the array, relative to its affinity for MAGI-3 PDZ2. Because nonspecific binding occurs equally across proteins spotted at equal densities on this array, unequal binding of the PDZ domains to **2** suggests that these bindings are specific. Additionally, results from another array (Panomics PDZ domain Array III, Fig. S2) showed that Scribble PDZ1,[13] which reportedly binds to ETAL, also bound **2**. No PDZ domains from other classes were detected as major hits in this assay.

The binding of **2** to the NHERF-1 PDZ2 domain in the array (Fig. 2A) encouraged us to isolate NHERF-1 from the proteome mixture. The crude proteome of HEK293 cells stably expressing HA-tagged NHERF-1[14] was treated with **2**. The sample was immunoprecipitated using anti-HA antibody, and the precipitate was analyzed by streptavidin blotting. In the presence of **2**, a band with the expected molecular weight (50 kDa) was clearly observed, thereby showing the NHERF-1 labeling in proteome (Fig. 2B, arrowhead).

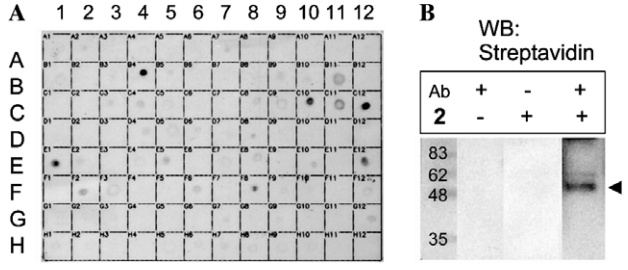

Figure 2. (A) Probe **2** selectively binds to those PDZ domains that bind to E/D-T/S-X-V/L/I motifs. (B) NHERF-1-HA (arrowhead) was isolated from crude lysate by **2** and anti-HA antibody. Numbers shown in the left column are molecular weight markers (kDa).

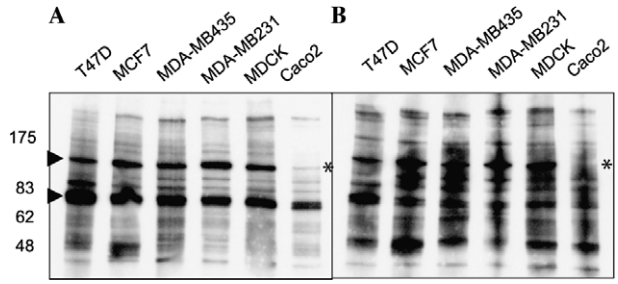

Figure 3. Analysis of proteomes treated with PDZ domain-targeting chemical probes **1** (A) and **2** (B). Probes (50 μM) were incubated overnight with 0.5 mg/mL of each proteome in PBS (pH 7.4).

To investigate the utility of **2** as a chemical proteomics tool, we next carried out differential analysis of cellular proteomes. We precleared the crude proteomes harvested from several cell lines with streptavidin agarose, quantified their protein concentrations to normalize them, and then reacted them with **1** or **2** (50 μM). Samples were isolated by SDS–PAGE and then compared by streptavidin blotting. Both compounds afforded a wide range of biotinylated bands, showing irreversible labeling of diverse proteins. Probe **1** highlighted two major bands (Fig. 3A, arrowheads) from all proteome samples, thereby indicating the selective character of **1**. Meanwhile, **2** showed weaker signal intensity in these two bands but demonstrated broader spectrum than **1** (Fig. 3B); which is consistent with its mimicry for E/D(-3). Interestingly, one band was clearly missing in Caco-2 cells (*), a finding that indicates downregulation of this protein. Together, these results demonstrate that the biotinylated probes can be used to analyze the PDZ domain-containing proteins in proteomes. Because of the flexibility of our chemical scaffold's design, we will be able to tailor it to analyze other PDZ domains of interest that show pathologic regulation of class I PDZ domains (e.g., NHERF-1/EBP50 in tumors).[15]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.10.027.

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