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A Chemical Library Approach to Organic-Modified Peptide Ligands for PDZ Domain Proteins: A Synthetic, Thermodynamic and Structural Investigation

D. Gomika Udugamasooriya,[a] Sudhir C. Sharma,[a] and Mark R. Spaller*[a, b]

PDZ domains are members of the *protein interaction domain* family,[1] semi-autonomous modules embedded within larger signaling proteins that impart a degree of exclusivity to the binding properties of their hosts. As mediators of mammalian protein–protein interactions that number in the hundreds, PDZ domains are party to a correspondingly large array of cellular processes, most notably those that regulate or support neuronal activities.[2] Specific, bioavailable molecular probes are needed to foster biological inquiries into their functions; towards this end, we report our recent progress in the discovery and development of PDZ domain inhibitors.

Here the focus is on the protein *postsynaptic density 95* (PSD-95), which bears three PDZ domains. Abundant in neurons, PSD-95 serves as a nexus for transient interactions that affect core synaptic events, such as transmission and plasticity.[3,4] Inhibitors that selectively uncouple these PDZ domain-promoted associations will greatly assist in determining their exact roles. Further, disrupting these interactions of PSD-95 could constitute novel therapeutic avenues for treatment of stroke and ischemic brain damage[5] and other excitotoxic disorders.[6]

We now expand upon prior work in which linear peptide ligands for the third PDZ domain (PDZ3) of PSD-95 were developed.[7] Data from that investigation, in conjunction with an X-ray crystal structure we solved of PDZ3 bound to the hexapeptide KKETWV (PDB ID: 1TP5), indicate that the binding site occupied by the side chain of the penultimate C-terminal residue (Figure 1, position P_{-1}) can accommodate a variety of organic substructures. We hypothesized that suitable elaboration of a side chain within that site—and perhaps even just beyond its perimeter—might enhance existing interactions and possibly accrue new ones.

A ligand design strategy was devised, built upon a parallel chemical synthesis platform, in which a diverse collection of organic acids was used to acylate individually an amino side chain at P_{-1} of a peptide ligand for PDZ3 (Scheme 1). This approach was inspired by a library-based peptide modification protocol, in which consensus sequences have been iteratively transformed into high-affinity ligands.[8] As originally reported, however, the methodology does not allow for display and screening of the unmodified peptide C terminus (a strict requirement for most PDZ domain binding events), and a significantly redeveloped scheme was implemented for our studies.

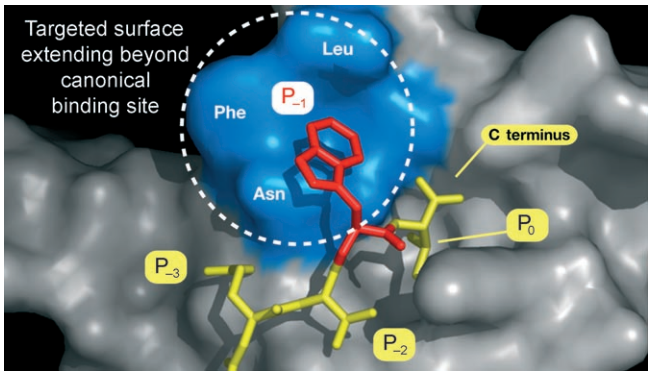

Figure 1. Structure-based design rationale for organic-modified libraries for PSD-95 PDZ3, based on the complex between PDZ3 and KKETWV. Positions P_0 and P_{-2} denote conserved primary binding determinants. P_{-1}, occupied by Trp, will be replaced by Lys acylated with organic acids. The blue surface represents a region of diameter 12 Å that encircles the Trp indole.

Two chemical libraries were prepared, templated upon a different hexapeptide—YKQTSV—that we had previously demonstrated to exhibit slightly higher affinity than KKETWV for PDZ3.[7] The acyl acceptor at P_{-1} was either lysine (Library I) or diaminopropionic acid (Dap; Library II), replacing the original residue at that position (Scheme 1). Sets of 92 (Library I) and 186 (Library II) organic acids were used, which were selected to reflect a range of functionality and carbogenic character (alkyl and aryl). Between the variable length of the donor arms and the organic structural diversity presented, the expectation was that modified ligands displaying improvements in affinity, target specificity, and in vivo (proteolytic) stability might be discovered. For this pilot inquiry, the focus was first to evaluate leads strictly on the basis of the criterion of binding strength.

An ELISA-type assay was developed to screen the two libraries, in which the direct binding of a GST-PDZ3 fusion protein was detected by use of a standard dual antibody system (see the Supporting Information). The top-scoring sequences (Scheme 2) were individually synthesized on preparative scale without N-terminal extensions, and isothermal titration calorimetry (ITC) was used to measure their solution binding parameters with PDZ3 (Table 1).

All six of the modified ligands exhibit improved affinity over the unmodified parent peptide YKQTKV, with dissociation constants in the low- to submicromolar range. The upper end is marked by an almost 20-fold enhancement for **6**, with the addition of an aryl bromide to Dap. Of note is that the next tightest binder, **1**, bears a chlorinated aryl sulfide, with the aromatic ring of almost equal bond count distance from the backbone.

[a] *D. G. Udugamasooriya, S. C. Sharma, Prof. M. R. Spaller*
Department of Chemistry, Wayne State University
Detroit, MI 48202 (USA)

[b] *Prof. M. R. Spaller*
Department of Molecular Pharmacology, Brown University
Providence, RI 02912 (USA)
E-mail: mspaller@brown.edu

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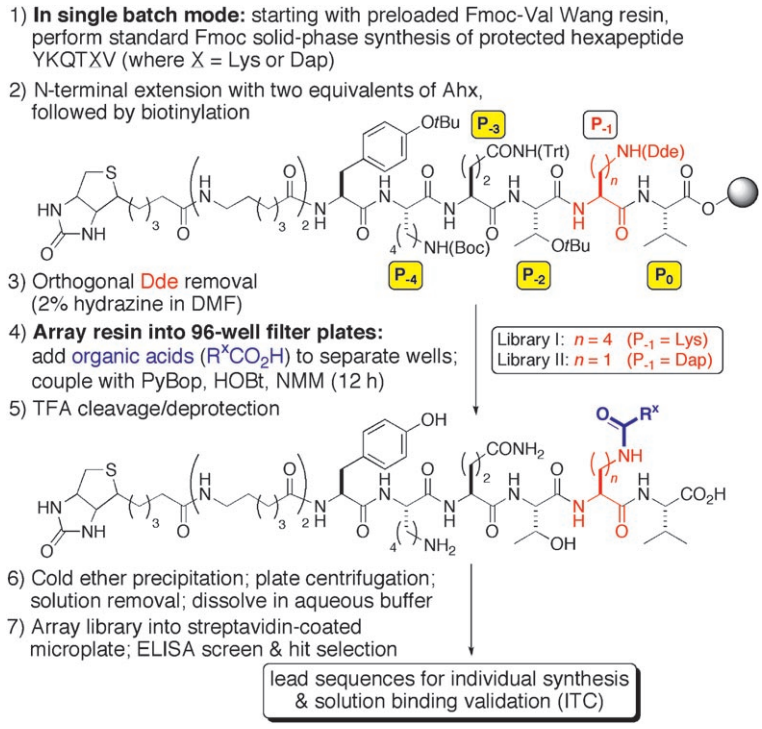
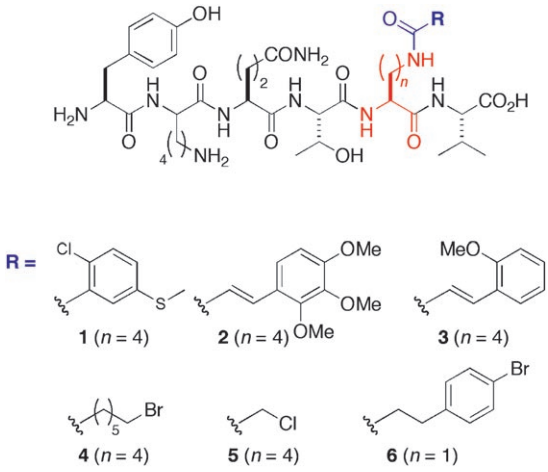


Scheme 1. Synthesis and screening of C-terminal-displayed organic-modified libraries for PSD-95 PDZ3.



Scheme 2. Organic-modified peptides for PSD-95 PDZ3 based on selected sequences from screening Libraries I (ligands **1**–**5**) and II (ligand **6**).

These two ligands, deriving from different libraries, may thus represent convergence towards a discrete region of the protein surface in which favorable binding interactions can occur. Similarly, the slightly lowered affinity pair of compounds **2** and **3** may also reflect a salutary outcome for aromatic positioning in a more distal location.

On further examination of ligand **1**, the K_d of 0.55 μM equates to a full order of magnitude improvement in affinity over that of YKQTKV. While the addition of the chlorinated aromatic ring might implicate a hydrophobic effect with an energetically favorable release of water, this is not necessarily in accord with the observation that the change in free energy is attributable solely to enthalpy ($\Delta\Delta H = 1.8$ kcal mol^{-1}). This suggests the formation of discrete, specific molecular interactions with the protein surface, attributable to the added organic acid moiety.

The metamorphosis of YKQTKV into **1** successfully evades the annoying phenomenon of enthalpy–entropy compensation,[9] in which structural modification of a ligand yields energetic gains made in binding enthalpy that are offset by a comparable unfavorable loss in binding entropy (or vice versa). While **1** does experience a small decrease in entropy, it subtracts little from the large ΔH value, which is almost fully transferred to ΔG.

To probe the nature of the interaction between ligand **1** and PDZ3, we turned to protein NMR and conducted HSQC chemical shift perturbation experiments.[10] Separate titrations of ^{15}N isotopically labeled PDZ3 with **1** and with unmodified YKQTKV revealed that while the binding of both generated chemical shifts from residues within the established P_{-1} site, ligand **1** also evoked *additional* responses from regions just beyond the demonstrated binding pocket (Figure 2). This was accompanied by similar unique shifts further removed from the P_{-1} site. In both cases, such shifts may connote direct residue interactions, as well as those that are propagated through conformational motions. In either event, this provides preliminary support to our conjecture that such organic-modified ligands can access protein-binding modes that a native peptide cannot.

Table 1. Thermodynamic binding parameters of organic-modified peptide ligands and PDZ3 of PSD-95.[a]

Ligand	K_d [μM]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	$T\Delta S$ [kcal mol^{-1}]
YKQTKV	6.3 (±1.1)	−7.1 (±0.1)	−4.1 (±0.1)	3.0 (±0.1)
1	0.55 (±0.1)	−8.6 (±0.1)	−5.9 (±0.1)	2.7 (±0.1)
2	1.5 (±0.1)	−7.9 (±0.1)	−4.4 (±0.1)	3.5 (±0.1)
3	1.4 (±0.1)	−8.0 (±0.1)	−4.5 (±0.1)	3.5 (±0.1)
4	2.2 (±0.6)	−7.7 (±0.2)	−3.6 (±0.1)	4.1 (±0.1)
5	2.0 (±0.2)	−7.8 (±0.1)	−4.1 (±0.1)	3.7 (±0.1)
6	0.33 (±0.1)	−8.9 (±0.1)	−5.3 (±0.1)	3.6 (±0.1)

[a] Values are each the arithmetic mean of at least two independent experiments (error shown beside each value reflects the range).

In summary, our design strategy as executed has led to potent compounds directed towards a PDZ domain of prime neurobiological importance. The success achieved here lays the foundation for future expansions of this work; in addition to 1) increasing the number of organic acids used, these include 2) placement of acylation sites at P_{-3} and beyond, thus allowing for iterative additions for multiple organic modifications on a single backbone, 3) the use of other amino side chain donor residues of variable length (such as ornithine), to

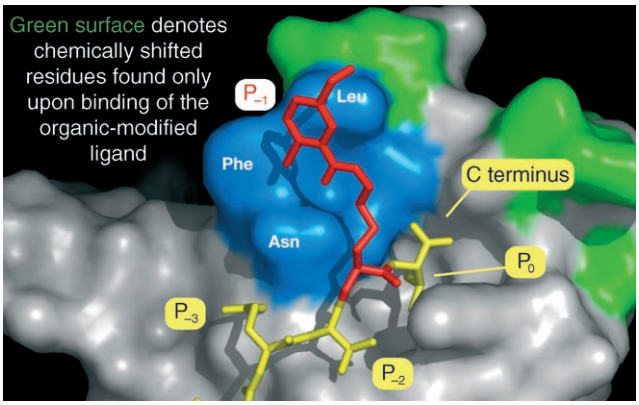


Figure 2. Model of the side chain of **1** (red) supplanting that of Trp in the KKETWV-PDZ3 structure. Positioning of the side chain of **1** is not energetically minimized, and is intended only to portray the potential extent of occupancy. Differential HSQC chemical shifts upon binding that are unique to **1** are mapped onto residues of the PDZ3 structure (green).

explore the molecular recognition space of the protein surface further, and, of particular significance, 4) application to other PDZ domains. Considering the scarcity of small binding ligands for these proteins, this last point is especially attractive, since many PDZ domains adhere to a common modular binding formula that can be rapidly exploited by the extensible approach presented here.

Detailed procedures for conducting the synthetic, assay, and HSQC experiments are provided in the Supporting Information.

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Keywords: calorimetry · combinatorial chemistry · NMR spectroscopy · peptides · protein domains

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