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Understanding lipid recognition by protein-mimicking cyclic peptides

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ABSTRACT

This paper describes our investigation of the structural determinants of a designed cyclic peptide (cLac, cyclic peptide mimicking lactadherin) (Zheng, H.; Wang, F.; Wang, Q.; Gao, J. *J. Am. Chem. Soc.* **2011**, *133*, 15280–15283) for phosphatidylserine (PS) recognition. A highly efficient strategy that takes advantage of the native chemical ligation (NCL) chemistry has been developed for the synthesis and labeling of cyclic peptides in general. Ala scanning of the cLac peptide revealed a sophisticated model for PS binding, in which the peptide scaffold assembles multiple polar residues to balance the desolvation and electrostatic interactions (salt bridge and hydrogen bonding) to achieve lipid selectivity. The results suggest that cLac effectively mimics the membrane binding mechanism of the parent protein lactadherin.

1. Introduction

As a defining feature of a living cell, the plasma membrane serves as a gate for material exchange between the cell and its environment. Of equal importance, it also mediates numerous signaling events from outside stimuli to internal response. It is becoming increasingly clear that membrane lipids are not mere bystanders in material exchange and signaling. Instead, they play active roles by directly functioning as receptors or by recruiting relevant proteins and controlling the spatial organization of these protein molecules.[2] Therefore, the spatiotemporal distribution of lipids underlies essentially all functions of the membrane. Recent mass spectrometry analysis of the lipidome (the collective population of lipids) suggests over 1000 distinct membrane lipids in a mammalian cell.[3] Given the complexity of the lipidome, it is highly desirable to have affinity ligands for individual lipids so that their spatiotemporal distribution can be tracked and correlated to biological phenotypes.

We have undertaken a program to develop affinity ligands for membrane lipids. Inspired by peptide natural products such as cinnamycin (Fig. 1), which specifically binds phosphatidylethanolamine (PE),[4,5] we hypothesized that properly designed cyclic peptides could serve as effective receptors of membrane lipids.[6] Indeed, our previous work shows that cyclic peptides that mimic the lipid-binding protein lactadherin display selective binding to phosphatidylserine (PS).[1] PS is known to exclusively reside in the inner leaflet of healthy cells and its externalization accompanies important processes including cell apoptosis and blood coagulation. By tracking the surface exposed PS, a lactadherin mimicking peptide (cLac) with fluorophore labeling effectively stains apoptotic cells as the parent protein does. In this contribution, we report a structure–activity study of the cLac peptide in terms of lipid binding. Importantly, we also describe a highly efficient strategy for cyclic peptide synthesis and labeling that is based on the native chemical ligation chemistry.

2. Cyclic peptide synthesis

Most previous reports on cyclic peptide synthesis utilized a side chain-immobilized Asp or Glu residue on resin (Fig. 2a).[7,8] Selective deprotection of the main chain COOH group after peptide synthesis enables peptide cyclization with common amide bond formation conditions (e.g., the use of PyBOP). While this method applies to various peptide lengths and sequences, side products are often seen because of epimerization of the C-terminal residue, incomplete cyclization, and formation of peptide dimers. We envisioned that native chemical ligation (NCL) chemistry,[9] which takes advantage of the orthogonal reactivity of an N-terminal cysteine and a C-terminal thioester, could afford a more efficient protocol for cyclic peptide synthesis. Indeed, the pioneering work by Benkovic and associates showed that cyclic peptides could be generated in bacterial cells with intein-mediated formation of thioesters.[10] A similar strategy has later been adopted by Kritzer et al. to create cyclic

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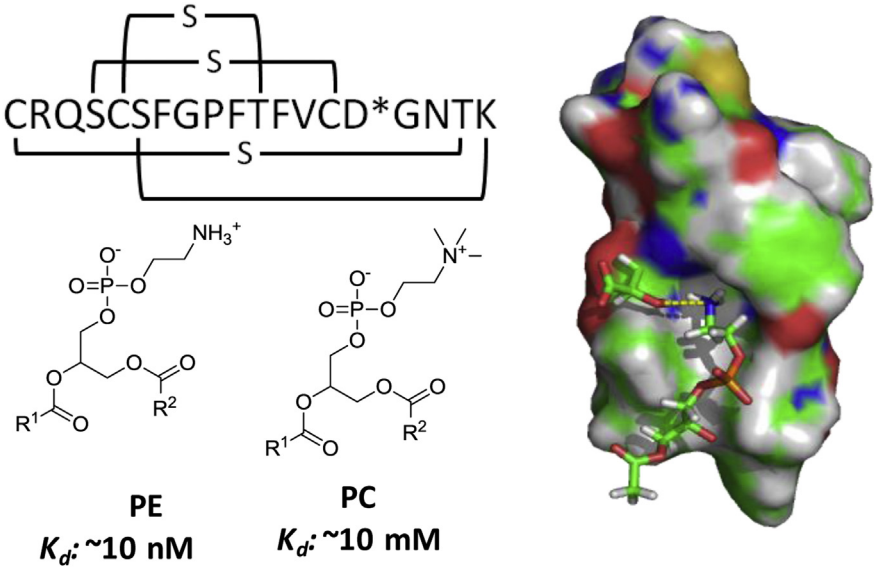

Fig. 1. Illustration of the peptide natural product cinnamycin, which is known to specifically recognize phosphoethanolamine PE. The cartoon representation of the lyso-PE–cinnamycin complex was generated from the PDB file 2DDE, with the peptide showing its surface potential (blue: positive; red: negative) and lysoPE shown as sticks (C: green; O: red; N: blue; P: orange; H: white). The apparent K_d data were extracted from Ref. 5.

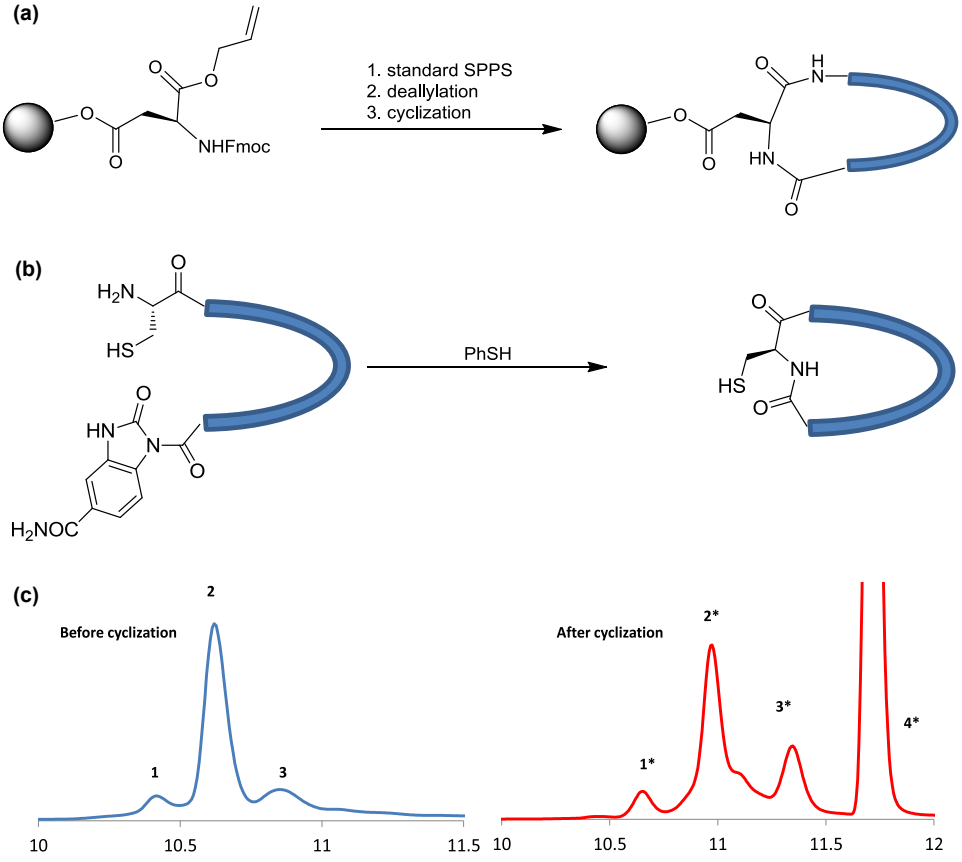

Fig. 2. Illustration of peptide cyclization strategies. (a) On resin cyclization with side chain-immobilized Asp; (b) peptide cyclization with native chemical ligation; (c) exemplary HPLC traces of a cLac peptide before (left) and after (right) the cyclization reaction. 1 and 1*: unidentifiable impurities; 2: the linear precursor of cLac-wt; 2*: cLac-wt; 3: the Arg truncation product of 2 from solid phase peptide synthesis; 3*: the Arg truncation product of 2*; 4*: 4-mercaptophenylacetic acid. Both linear precursors (2 and 3) cyclized cleanly into the desired peptide macrocycles (2* and 3*).

peptides in yeast cells.[11] Importantly, NCL-cyclized peptides carry a free Cys side chain, which provides a handle for peptide labeling with various thiol-reactive fluorophores that are commercially available.

The synthesis of peptide thioesters via the Fmoc chemistry had been difficult until recently. Elegant work from the Dawson lab showed that solid supports functionalized with 3,4-diaminobenzoic acid (named as Dawson Dbz resin) allows facile