Design of a Synthetic Receptor for the Calmodulin-Binding Domain of Calcineurin

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In recent years, considerable progress has been made in the area of design and analysis of α-helical coiled coils.[1] Systematic studies of synthetic peptides have elucidated the features required for the construction of two-, three-, and four-stranded coiled coils with either parallel or antiparallel topologies.[2] The three-dimensional structures of many designed coiled coils and helical bundles have been solved at high resolution.[3] These highly symmetrical structures may serve as starting points for engineering functional molecules with more complex and intricate geometries. As a first step in this direction, we have designed a helical hairpin peptide that binds specifically to the CaM-binding domain[4] of calcineurin, a phosphatase involved in a number of signal transduction schemes.[5]

Our design of the synthetic receptor (SR1) arose from a thorough analysis of the thermodynamics of assembly[2g,h] and the three-dimensional structure[3e] of a coiled coil peptide, coil-Ser. This peptide assembles in aqueous solution in a noncooperative process in which two random coil monomers associate into an α-helical dimer. This helical dimer then binds a third helix, resulting in the formation of an antiparallel three-helix bundle.[2h] This finding suggests that a covalently stabilized helical dimer might form a receptor for a third helical peptide. As an initial target, we attempted to design a receptor for the α-helical, CaM-binding domains of calcineurin. CaM recognizes a basic amphiphilic α-helix as a conserved structural feature found in most of its target enzymes.[6] By altering its conformation, CaM is able to adjust to the surface topographies of a wide variety of basic amphiphilic helices, independent of their amino acid sequences.[6b] The goal of the present work was to design a receptor that would be less flexible and hence more specific than CaM. We attempted to design a receptor that would be specific for the CaM-binding domain of calcineurin,[7] and that would not bind to the corresponding domain from smooth muscle-MLCK.[8] Although the overall charge and physicochemical properties of the two domains are similar, their surface topographies differ providing opportunities for differential recognition. The hydrophobic residues in calcineurin are primarily aliphatic and line one side of the helix.[7] By comparison, the apolar surface in MLCK includes larger, aromatic hydrophobes, which lie in two distinct patches on the helix.[8]

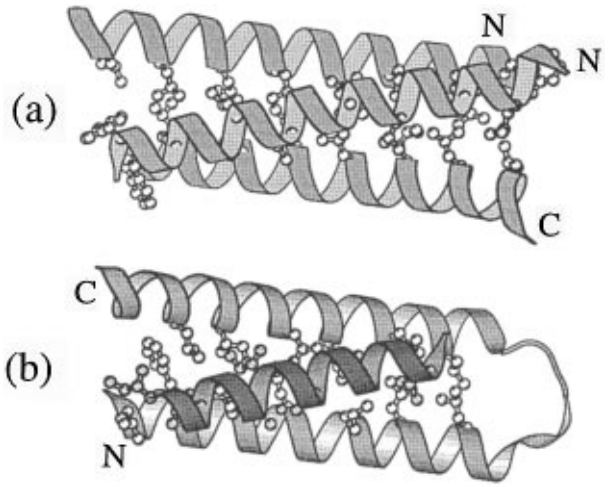

Figure 1. Design of a calmodulin mimetic from the trimeric structure of coil-Ser. The sequence of calcineurin 396 to 414 was aligned with that of coil-Ser such that the hydrophobic residues of the calcineurin peptide would replace the apolar Leu side chains of coil-Ser at all but one location (Lys_{399}). These changes were then introduced into the structure of one of the helices of coil-Ser, and the other two helices were re-designed to bind $CN_{396-414}$. The figure illustrates the ribbon representations (generated with Molscript[15]) of (a) the coil-Ser trimer, as determined by X-ray crystallography,[3e] and (b) the corresponding model of the SR1−$CN_{396-414}$ complex.

The design of SR1 progressed in several distinct steps involving interactive computer graphics and an automated side chain repacking algorithm. The sequence of the CaM-binding domain of calcineurin was first "threaded" onto the backbone[9] of one of the three helices in the coil-Ser trimer, such that there would be an optimal correspondence between the hydrophobic residues of the two structures (Figure 1). Next, an antiparallel loop[10] was constructed between the remaining antiparallel pair of coil-Ser helices. Polar residues were then introduced at partially exposed positions of the helical hairpin in geometries

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(4) Abbreviations used: CaM, calmodulin; SR, synthetic receptor; MLCK, myosin light chain kinase; CN, calcineurin; CD, circular dichroism; MOPS, 4-morpholinepropanesulfonic acid.

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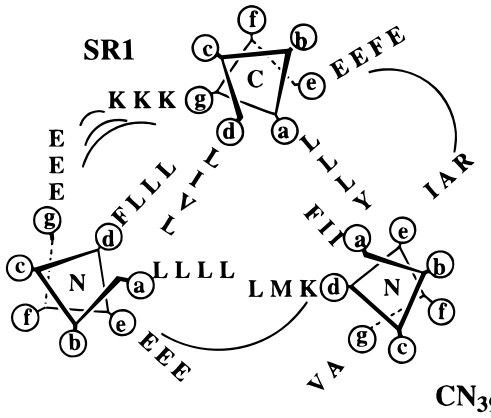

Position	g abcdefg abcdefg abcdefg abcdefg
CoilSer	E WEALEKK LAALESK LQALEKK LEALEHG
$CN_{396-414}$	IRNKIRA IGKMARV FSVLR
$SR1_{1-26}$	E LEAFEKE LAALESE LQALEKE LEAL
$SR1_{32-60}$	E YAALEKK LAAVFSK LQAIEKK LEALEHG
$SR1_{27-31}$ (Loop)	*GGNPD*

Figure 2. Sequences of coil-Ser, $CN_{396-414}$, and SR1 as well as a helical wheel diagram of the SR1−$CN_{396-414}$ complex.

that would allow hydrogen-bonding and electrostatic interactions with the basic side chains of the docked peptide. Finally, a genetic algorithm[11] was used to design a new set of hydrophobic side chains in the helical hairpin, such that its apolar surface would be complementary to that of the calcineurin peptide. The resulting sequence of the SR1 receptor and a helical wheel representation is reported in Figure 2.

The synthetic receptor was prepared by standard solid phase methods.[12] Its binding to the CaM-binding domain of calcineurin ($CN_{394-414}$)[7a] and its secondary structure were evaluated with methods that previously had been developed to investigate CaM−target peptide complexes.[13] The CD spectrum of the receptor shows a double minimum at 208 and 222 nm, indicative of α-helical structure. The mean residue ellipticity at 222 nm ($[\theta]_{222} = -26\,200$ deg cm^2 dmol^{-1}) is slightly less than the value obtained for coil-Ser ($[\theta]_{222} = -31\,700$ deg cm^2 dmol^{-1}), which has been shown by X-ray crystallography to exist as a three-helix bundle.[3e] Thus, the CD spectrum of SR1 is consistent with the designed helix−loop−helix motif, which was modeled with approximately 90% of the residues in an α-helical conformation and 10% in a loop conformation.

In the absence of the SR1, the spectrum of $CN_{396-414}$ is consistent with a predominantly random coil conformation, lacking appreciable defined secondary structure. Addition of a single equivalent of SR1 to $CN_{396-414}$ leads to a large increase in the magnitude of the ellipticity (relative to the individual components) indicative of the formation of a complex. The magnitude of the ellipticity observed in the difference spectrum ($[\theta_{222}] = -16\,200$ deg cm^2 dmol^{-1} and $[\theta_{208}] = -16\,700$ deg cm^2 dmol^{-1}) corresponds to approximately 3−4 turns of helix,[14] suggesting that $CN_{396-414}$ becomes largely helical upon binding. Titration of SR1 with $CN_{396-414}$ indicates that a 1:1 complex is formed with an apparent association constant of 1×10^6 M^{-1} (Figure 3). The association constant decreased from 1×10^6 to 4×10^5 M^{-1} as the salt concentration was varied from 0.05 to 0.2 M. This suggests that both electrostatic as well as hydrophobic interactions contribute to the stability of the complex. Importantly, the specificity of SR1 for $CN_{396-414}$ was demonstrated in experiments in which a peptide spanning the CaM-binding domain of smooth muscle-MLCK (residues 494−513)[8a] was titrated into SR1. No increase in ellipticity was observed in this case (Figure 3).

The stoichiometry of the interaction of SR1 was further probed by sedimentation equilibrium ultracentrifugation. In the absence of $CN_{396-414}$, SR1 exists as a dimer in solution. However, upon addition of a single equivalent of $CN_{396-414}$, sedimentation equilibrium indicates that the SR1 dimer dissociates and binds one molecule of $CN_{396-414}$, resulting in a 1:1 complex ($M_{app} \approx 10\,000$). This change in association state of the receptor might be exploited to allow fluorescence-based detection of peptide binding.

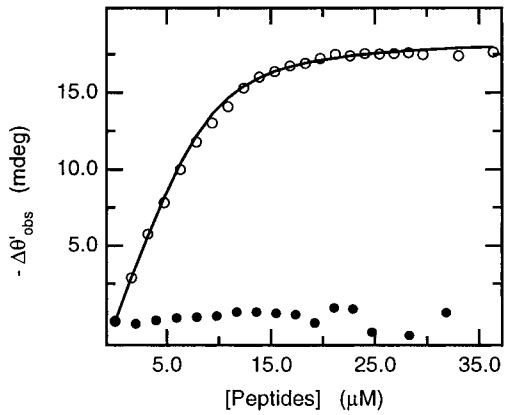

Figure 3. CD titration of SR1 with $CN_{396-414}$, open circles, and $MLCK_{494-513}$, closed circles. The background-corrected ellipticities[13] ($\Delta\theta'_{obs}$, see Supporting Information) of $CN_{396-414}$ and $MLCK_{494-513}$ are plotted *versus* the peptide concentrations. To a 9 μM solution of SR1 (10 mM MOPS, pH 7.0, 50 mM NaCl, 25 °C) successive aliquots of $CN_{396-414}$ or $MLCK_{494-513}$ were added and the ellipticity at 222 nm recorded. The apparent association constant (1×10^6 M^{-1}) and the stoichiometry (1.09 ± 0.08) were calculated by fitting the data to a binding isotherm (Supporting Information). The smooth curve through the data for $CN_{396-414}$ shows a theoretical fit generated by using these constants. The computed association constant represents a lower limit for the true association constant because the binding requires the energetically unfavorable dissociation of the SR1 dimer.

This work provides an important step in the design of synthetic receptors for a variety of peptides and also furthers our understanding of molecular recognition. Extending this work using templates other than coiled coils, it should be possible to build binding sites for a variety of other structural motifs.

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Supporting Information Available: Curve fitting of the CD titration and CD spectra of $CN_{396-414}$ (4 pages). See any current masthead page for ordering and Internet access instructions.

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