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Augmented reversible photoswitching of drug-target interaction through "surface borrowing"

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ABSTRACT

Reversibly switching bioactive molecules by light could allow spatiotemporal control of their biological functions, but designing such drug molecules with large functional differences between two light-switchable states remains difficult. We extend the concept of "borrowing protein" to "borrowing surface" for the design of photo-switchable molecules. By using an azobenzene conjugated cyclosporin derivative as a model system we have demonstrated that the enhanced steric hindrance resulting from this "borrowing surface" design can cause augmentation of the functional difference between the *cis* and *trans* conformers of this light-switchable compound. Interestingly, not only near UV light of 366 nm but also visible light of 430 nm or 525 nm can induce efficient photoswitching of the interactions between the light-responsive ligand and target protein. Additionally, by using molecular modeling and docking techniques we obtained structural insights into the switchable protein-ligand interaction, illustrating the effect of steric hindrance associated with the borrowing surface design.

1. Introduction

Reversibly photoswitching drug molecules represent a very attractive avenue to achieve spatiotemporal control of their pharmacological properties [1–5]. The light-induced structural changes could be highly selective and orthogonal to other biochemical processes. The aromatic ring structures of photo-switchable moieties, such as azobenzene and spiropyran, are essential for their responsiveness to light of relatively long wavelength, which is not absorbed by most biomolecules, such as proteins and DNA [1]. However, to introduce such photo-switchable group into drug molecules is often limited by the structure-activity relationship between the drug and target, resulting in either abolished protein-ligand interaction, or only minor differences between the two conformers [4]. Therefore, minimizing the loss of activity upon chemical modification while augmenting the functional difference between two reversibly switchable conformers is essential for designing such light-responsive compounds.

We are interested in regulating immune cell function with light-switchable derivatives of the immunosuppressive drug cyclosporin A (CsA) [4,6]. The immunosuppressive mechanism and structure-activity relationship of CsA are well-understood [7,8]: CsA binds to its target, the peptidyl prolyl *cis*/*trans* isomerase (PPIase) cyclophilin 18 (hCyp18), and the resulting hCyp18/CsA complex inhibits the immunologically important protein phosphatase calcineurin (CaN). The inhibition of CaN and downstream NFAT (nuclear factor of activated T cells) pathway, which is, amongst others, responsible for the production of cytokines, causes immunosuppression. Though CaN inhibition is associated with the hCyp18/CsA/CaN tertiary complex formation, neither CsA nor hCyp18 alone causes any detectable inhibition of CaN. This gain-of-function mechanism of CsA has made it possible to build a light-switchable system on distinct biological scales and hierarchical levels [9–11]. Recently, we have designed a "borrowing protein" system to augment the light switchable effect of photo-responsive CsA derivatives, and allow tuning of the immune response reversibly with light irradiation (Fig. 1A). After irradiation at 366 nm, CsA-AB-Biotin in *trans* can be switched to the *cis* conformer (>90%) [4]. The irradiated sample will be referred as *cis*, though a small amount of *trans* conformer still remains at the photo-equilibrium, as the cis conformer weakly absorbs at 366

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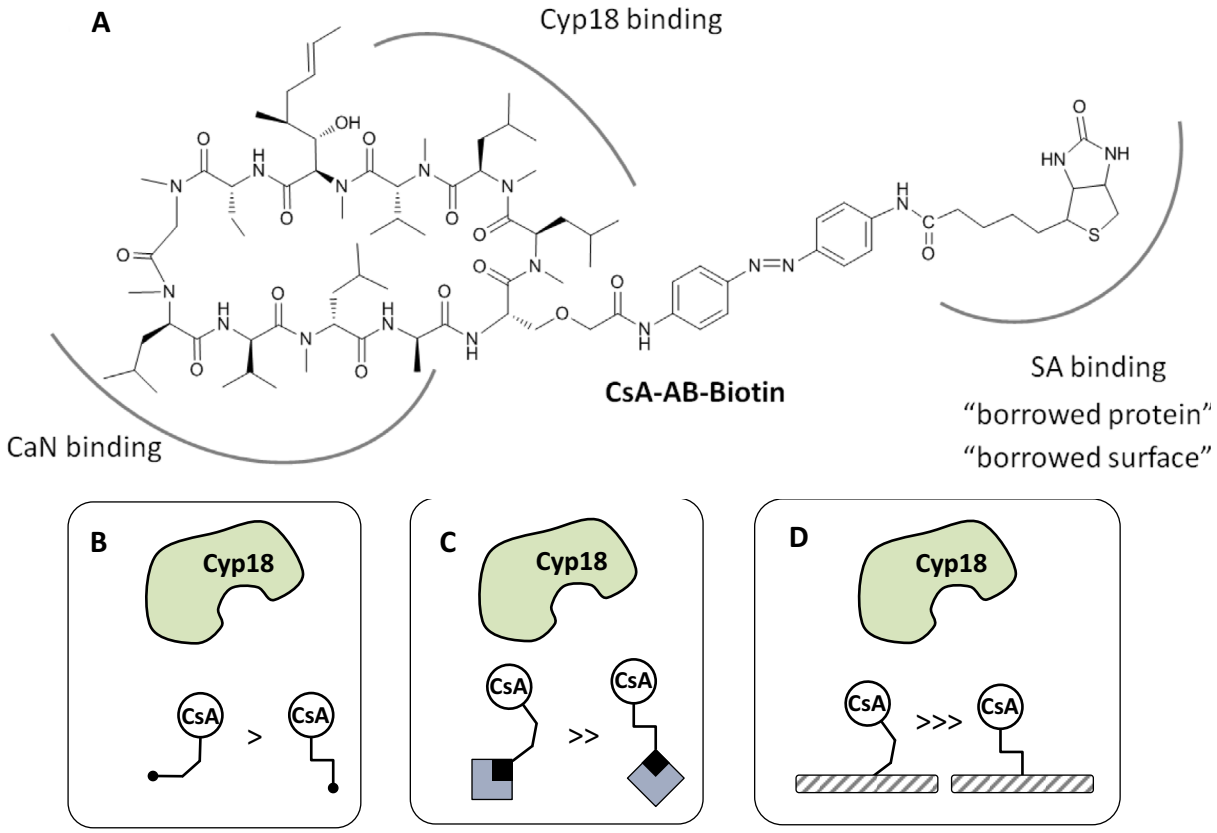

Fig. 1. Photo-switchable CsA derivative. Structure of photo-switchable cyclosporine derivative CsA-AB-Biotin in *trans* conformation and its structure-activity relationship (A). Schematic illustration of complex formation between hCyp18 and non-irradiated (*trans* conformation) or irradiated (*cis* conformation) CsA-AB-Biotin. Because of the increasing steric effect, the "borrowing surface" approach (D) could lead to further augmentation of the functional difference between the *cis* and *trans* conformers (B), as compared with the "borrowing protein" approach (C).

nm. The *cis* to *trans* thermal isomerization of CsA-AB-Biotin is slow. The half time of *cis* exceeds 3 h at 25 °C and a first-order rate constant $k_{cis\ to\ trans}$ is 0.3 h at 37 °C [4]. Therefore, we were able to carry out photoswitching experiments on cells without constantly irradiating the cells. In this report we extend the concept of "borrowing protein" to "borrowing surface" for the design of photo-switchable drugs. Additionally we could demonstrate that visible light is also able to photo-isomerize the compound. Moreover, we applied molecular modeling and docking to illustrate the structural basis of the photo-switching process in a multi-component complex and to explain the different pharmacological profiles of both conformers.

2. Materials and methods

2.1. Reagents

If not otherwise indicated, all chemicals were obtained from Sigma-Aldrich (Deisenhofen, Germany). CsA-AB-Biotin was synthesized according to previous reports [3,4]. Immobilized Glutathione (GSH), Streptavidin (SA) Sepharose, and ECL reagent were purchased from GE Healthcare (Freiburg, Germany) and all peptide substrates from BACHEM (Bubendorf, Switzerland). GST-hCyp18 and His_6-hCyp18 were expressed in *E. coli* and purified as described in [12]. In order to isolate hCaN Aα the preparation protocol of Mondragon et al. was followed [13]. The anti-hCyp18 antibody was from Cell Signaling (Frankfurt am Main, Germany) and the anti-hCaN A α was an in-house polyclonal antibody produced in rabbit.

2.2. Methods

2.2.1. Co-precipitation experiments

GSH matrix (20 µl per sample) was saturated with GST-hCyp18 before the washed beads were incubated for 30 min with an excess of CsA-AB-Biotin. An additional washing step removed all free ligand. The samples were irradiated as indicated and SA was added. Finally, the washed beads were boiled in laemmli buffer and eluted proteins were separated in SDS-PAGE, western blotted and visualized by ECL (enhanced chemiluminescence) reaction. Alternatively, SA matrix was incubated directly with CsA-AB-Biotin before irradiation was performed. The amount of matrix-bound His_6-hCyp18 was analyzed in the same way as described above. The formation of quaternary CaN/hCyp18/CsA-AB-Biotin/SA complex was investigated using the same procedure with an additional CaN incubation step.

2.2.2. Determination of enzyme activities

The peptidyl prolyl *cis/trans* isomerase activity of hCyp18 was measured with a protease-coupled assay with minor modifications [14]. The experiments were carried out in HEPES buffer, pH 7.8, at 7 °C, using succinyl-Ala-Phe-Pro-Phe-4-nitroanilide as substrate and α-chymotrypsin as the auxiliary protease. The phosphatase activity of CaN in presence of CsA-AB-Biotin was determined in a scintillation assay with ^{33}P-labeled phosphocasein as substrate according to the protocol of Zhang et al. [4], otherwise the standard scintillation proximity assay with ^{33}P-labeled RII-phosphopeptide was used [15].