Photoswitches **Very Important Paper**

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Controlling Biological Activity with Light: Diarylethene-Containing Cyclic Peptidomimetics**

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Abstract: *Photobiological processes in nature are usually triggered by nonpeptidic chromophores or by modified side chains. A system is presented in which the polypeptide backbone itself can be conformationally switched by light. An amino acid analogue was designed and synthesized based on a reversibly photoisomerizable diarylethene scaffold. This analogue was incorporated into the cyclic backbone of the antimicrobial peptide gramicidin S at several sites. The biological activity of the resulting peptidomimetics could then be effectively controlled by ultraviolet/visible light within strictly defined spatial and temporal limits.*

Molecules with structures and properties that can be reversibly controlled by light (photoswitches) have attracted considerable interest in different branches of science. Amongst the known photoswitches,[1] two classes are prominently represented, namely *cis–trans* interconvertible azobenzenes (Figure 1 A) and diarylethenes that undergo reversible photoisomerization between "open" and "closed" forms (Figure 1 B). These photoswitches have found use in molecular electronics and various other applications.[2] One of the most exciting concepts in this field involves the incorporation of photoswitching units into biomolecules, in particular peptidomimetics, in order to control their biological activity by light.[3]

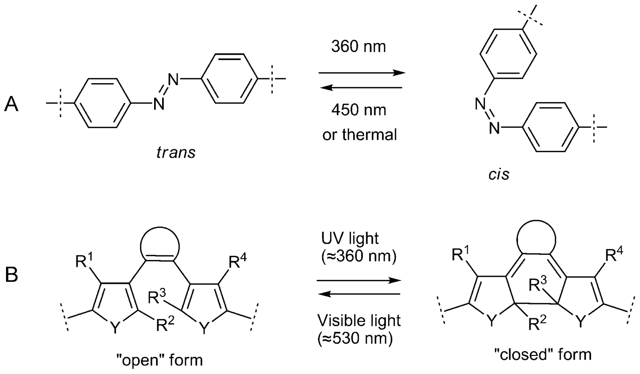

Figure 1. Two classes of molecular photoswitching fragments: azobenzenes (A) and diarylethenes (B). R^1, R^4 = H, alkyl, or aryl; R^2, R^3 = alkyl; Y = S, O or N.

In nature, light-driven conformational changes in proteins play a fundamental role in mediating photobiological processes.[4] They are usually triggered by nonpeptidic chromophores or by modified side chains,[5] yet there are no examples in which the polypeptide backbone itself undergoes direct photoconversion. To bridge this gap, photoswitchable amino acid analogues have been designed and incorporated into peptides. Most of the reported peptidomimetics are azobenzene derivatives.[6] Light-triggered< changes in their conformation,[7] binding affinities,[8] aggregation properties,[9] and folding[10] have been be demonstrated. However, reports on the use of diarylethene-based photoswitches for the same purposes are still scarce[11] despite the fact that the diarylethene moiety offers advantages in terms of the thermostability of both isomers, and high fatigue resistance.[12] This fact is attributable to the relatively modest light-induced structural changes in the diarylethene fragment. Since small changes can usually be accommodated by a flexible peptidomimetic backbone, the pronounced structural rearrangements that would be necessary to affect biological activity might not be readily attained. Nevertheless, it has been possible to photoregulate biological activity through the use of diarylethene derivatives. By exploiting the differences in molecular flexibility between the "open" and "closed" forms, diarylethene-based two-pronged enzyme inhibitors have been designed and successfully applied.[11a,b]

We and others[11d] have hypothesized that photoisomerization of the diarylethene unit could lead to significant

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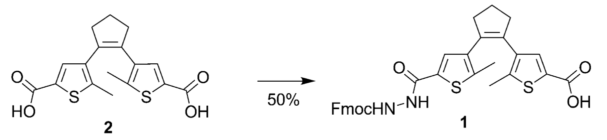
perturbations in the context of cyclic molecules, including peptidomimetics. The ring size, flexibility, and strain will depend on the isomeric state of the photoswitchable unit. This dependence could result in appreciably different overall conformations and, consequently, biological activities for the two isomers.

Herein, we report the design, synthesis, and application of an amino acid analogue that contains a diarylethene scaffold. It can be reversibly photoisomerized under mild conditions by irradiation with ultraviolet/visible light. This photosensitive amino acid analogue was incorporated into the backbone of the cyclic antimicrobial peptide gramicidin S (GS),[13] the structure and biological activity of which could thus be successfully controlled by light.

Our diarylethene-based amino acid analogue **1** differs from a recently reported analogue[11d] in that we replaced the aminomethyl group with an electron-withdrawing isostere, namely a carboxylic acid hydrazide moiety. Any direct attachment of an electron-donating amino or aminomethyl group to the heterocyclic moiety of the photoswitch might give an oxidizable and potentially air-sensitive molecule.[14] By coupling the known dicarboxylic acid **2** with Fmoc–$NHNH_2$, the protected diarylethene-based photoswitchable amino acid analogue **1** was obtained in a single step (Scheme 1), ready for use in standard solid-phase peptide synthesis.



Scheme 1. Synthesis of the photoswitching building block. Conditions: Fmoc–hydrazine, DIC, DIPEA, DMF, 8 h. Fmoc = 9-fluorenylmethyloxycarbonyl, DIC = 1,3-diisopropylcarbodiimide, DIPEA = *N*,*N*-diisopropylethylamine, DMF = *N*,*N*-dimethylformamide.

The sequence of GS [cyclo(^{D}FPVOL^{D}FPVOL), O = ornithine, ^{D}F = D-phenylalanine] has a symmetrical antiparallel β-pleated conformation connected by two type-II′ β turns.[15] This natural antibiotic has a highly amphiphilic structure and is thereby able to permeabilize membranes and kill bacteria.[16] We selected three nonpolar dipeptide units (L^{D}F, ^{D}FP, and PV) to be replaced with our photoswitch (Sw). The incorporation of **1** thus yielded three different peptidomimetics GS-Sw(LF), GS-Sw(FP), and GS-Sw(PV) (Figure 2). It was anticipated that their structure and shape would be reversibly altered upon irradiation with UV and visible light. In particular, it was anticipated that the strained "closed" diarylethene scaffold might no longer support the amphiphilic side-chain pattern required for biological activity.[16c,d]

Indeed, molecular dynamics (MD) simulations revealed major conformational differences between the different photoforms of the three photoswitchable GS analogues. The "open" forms were found to resemble GS more closely than the "closed" forms in terms of several parameters that reflect the overall distribution of hydrophobic and hydrophilic patches on the molecules (Figure 3).

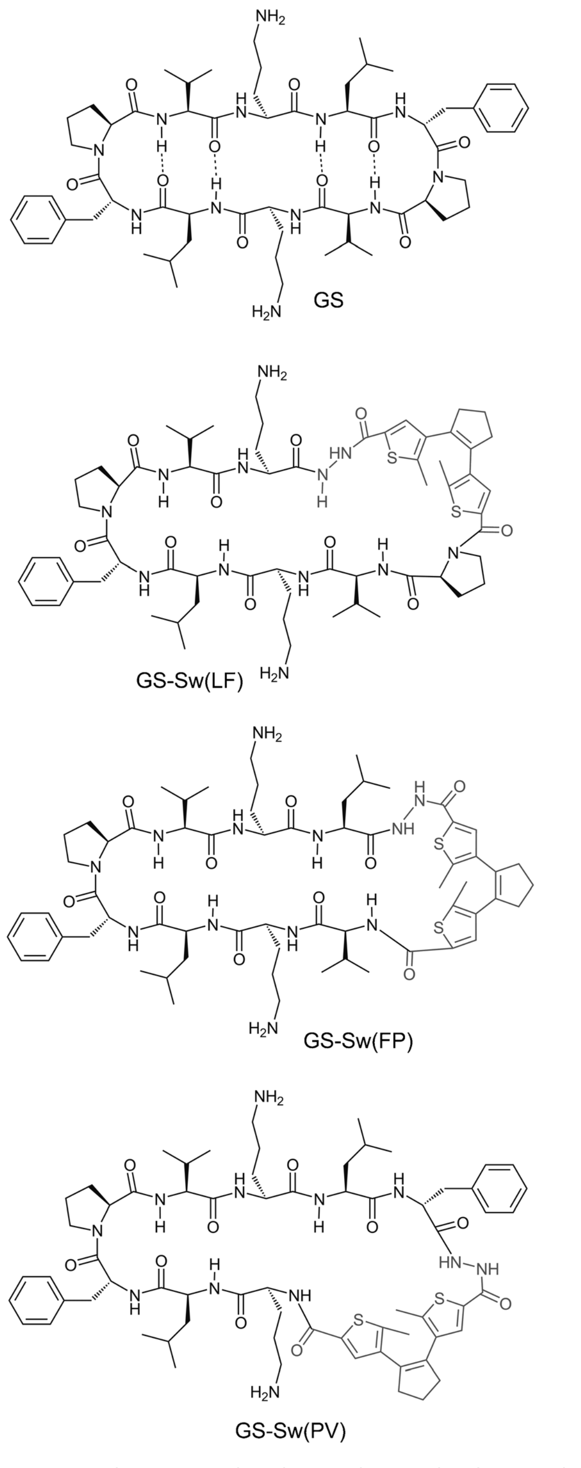


Figure 2. Gramicidin S (GS) and its three analogues; the photoswitchable peptidomimetics GS-Sw(LF), GS-Sw(FP), and GS-Sw(PV).

We calculated the hydrophobic moment as a measure of the distribution of polar and nonpolar surface patches for all of the GS analogues (see the Supporting Information). The largest hydrophobic moment (8.9, corresponding to the highest amphiphilicity), was found for the unmodified GS peptide. Peptidomimetics in the "open" form had slightly reduced values [e.g., 7.8 for GS-Sw(FP)], but they were even

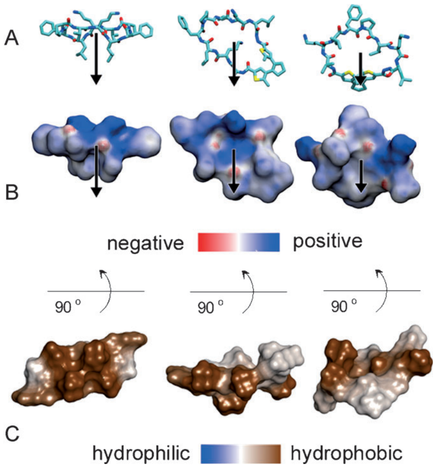


Figure 3. Representative snapshots from 600 ns MD simulations of GS (left) and its analogue GS-Sw(FP) in the "open" (center) and "closed" (right) forms. A) The amphiphilic molecules are aligned such that their calculated hydrophobic moment vectors are viewed from the side, and the arrows indicate the relative length of the vectors. B) The solvent-accessible surfaces of the molecules (aligned as in the top row), depicting the electrostatic surface potentials from continuum solvent calculations. C) Kyte–Doolittle hydrophobicities, viewed from the perspective of the membrane plane.

Table 1: In vitro biological activities of GS and its photoswitchable analogues.

Cells	GS	GS-Sw(LF) open	GS-Sw(LF) closed	GS-Sw(FP) open	GS-Sw(FP) closed	GS-Sw(PV) open	GS-Sw(PV) closed
			Antimicrobial activity [μg mL^{-1}][a]				
S. aureus	2	8	128	4	32	4	16
S. epidermidis	2	16	128	8	64	4	32
S. xylosus	1	8	128	8	32	4	32
			Hemolytic activity [μg mL^{-1}][b]				
Erythrocytes	12	47	⩾128	6.5	72	6	58

[a] measured as MIC; [b] measured as 50% lysis of human erythrocytes compared to the action of 0.1% Triton X-100.

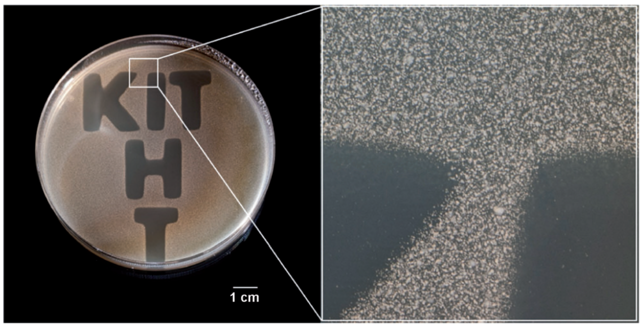


Figure 4. A demonstration of the differences in the antimicrobial activities of the "closed" (background) and "open" (within the lettering) forms of GS-Sw(FP) against *S. xylosus*.

lower for the "closed" analogues [e.g., 6.3 for GS-Sw(FP)]. In addition, we calculated the solvation free energies, which also showed the "closed" GS analogues to be less amphiphilic than the "open" ones.

All three peptidomimetics [GS-Sw(LF), GS-Sw(FP), and GS-Sw(PV)] were synthesized by using standard Fmoc solid-phase peptide synthesis methods.[17] The products were photochromic and changed color reversibly upon irradiation of the solutions with UV ($\lambda = 256$ nm) or visible (530 nm) light. The photoswitchable fragment could be "opened" and "closed" reversibly by irradiation within 20–50 min, just as reported for other diarylethene derivatives.[12] The "open" and "closed" forms had distinctly different HPLC retention times, thus enabling their preparative separation.

Gratifyingly, antimicrobial assays with various Gram-positive bacteria clearly showed that the minimal inhibitory concentrations (MIC) of the three GS analogues can indeed be controlled by light. All three peptidomimetics exhibited antimicrobial activity in the "open" form and were almost as active as GS itself (Table 1). The analogues in the "closed" form were distinctly less active than those in the "open" form with all of the bacterial strains tested. Hemolytic side effects are a major problem associated with the use of GS.[18] This toxic activity followed the same trend as the antimicrobial activity, with the "closed" forms showing considerably reduced hemolytic activity (Table 1 and the Supporting Information).

Within a certain concentration range, the peptidomimetics suppressed bacterial growth in vitro in the "open" form, while being completely inactive in the "closed" form. This effect is visualized in Figure 4, which shows a plate in which bacteria were treated with GS-Sw(FP) in the "closed" form (8 μg mL^{-1}), illuminated with visible light through a mask, and then incubated to allow for bacterial growth. The resulting pattern of living versus dead bacteria shows a distinct boundary between the active and inactive forms of the peptidomimetic.

The MD simulations discussed above provide a clue to rationalize the molecular basis of the drastic differences in biological activity: photoswitching leads to changes in amphiphilicity, and the amphiphilicities of the more active "open" peptidomimetics resemble most closely that of the unmodified GS. This hypothesis is supported by the chromatographic retention behavior of the different photoforms in reversed phase HPLC, which is known to roughly reflect molecular amphiphilicity.[19] The retention times were found to be almost equal for the "open" forms and unmodified GS, but were significantly reduced for the "closed" forms (Table S1 in the Supporting Information). Furthermore, MALDI-TOF analysis of GS and its analogues in H_2O/D_2O mixtures showed that the "open" forms undergo hydrogen exchange similar to GS, whereas the "closed" forms differed. Finally, circular dichroism measurements also qualitatively support the structural similarity between GS and the "open" forms under certain environmental conditions (see the Supporting Information). The changes in amphiphilicity upon photoswitching must thus be attributed to distinct adjustments in the conformation and flexibility of the peptidomimetic backbone (see NMR spectroscopy in the Supporting Information), which are concomitantly transmitted to the relative positioning of the side chains.

In conclusion, we have designed a robust photoswitchable diarylethene-based amino acid analogue. When incorporated into the backbone of cyclic peptides, this amino acid analogue can lead to considerable changes in biological activity upon photoisomerization, as demonstrated herein by the modification of the natural antibiotic GS. Such molecules may have many potential application in medicine as "smart" therapeutics against localized microbial infections.[20]

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