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Flexibility vs rigidity of amphipathic peptide conjugates when interacting with lipid bilayers

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ABSTRACT

For the first time, the photoisomerization of a diarylethene moiety (DAET) in peptide conjugates was used to probe the effects of molecular rigidity/flexibility on the structure and behavior of model peptides bound to lipid membranes. The DAET unit was incorporated into the backbones of linear peptide-based constructs, connecting two amphipathic sequences (derived from the β-stranded peptide $(KIGAKI)_3$ and/or the α-helical peptide BP100). A β-strand-DAET-α-helix and an α-helix-DAET-α-helix models were synthesized and studied in phospholipid membranes. Light-induced photoisomerization of the linker allowed the generation of two forms of each conjugate, which differed in the conformational mobility of the junction between the α-helical and/or the β-stranded part of these peptidomimetic molecules. A detailed study of their structural, orientational and conformational behavior, both in isotropic solution and in phospholipid model membranes, was carried out using circular dichroism and solid-state ^{19}F-NMR spectroscopy. The study showed that the rigid and flexible forms of the two conjugates had appreciably different structures only when embedded in an anisotropic lipid environment and only in the gel phase. The influence of the rigidity/flexibility of the studied conjugates on the lipid thermotropic phase transition was also investigated by differential scanning calorimetry. Both models were found to destabilize the lamellar gel phases.

1. Introduction

A subtle balance between conformational flexibility and rigidity is crucial in proteins to exert their specific functions with optimal efficiency, e.g. in molecular recognition and/or enzymatic catalysis [1–4]. This balance varies significantly across any large structural unit and may correlate with the particular functions of different molecular domains [5,6]. The role of molecular rigidity/flexibility in biochemical processes should not be underestimated, as it affects the kinetic and thermodynamic parameters of chemical transformations and the supramolecular interactions in a complex manner. For example, it has long been believed that the entropy of rigid ligand binding to receptors should always be favorable compared to the binding of conformationally flexible analogs [7,8]. While this might be true for simple supramolecular host-guest complexes [9,10], quantitative investigations of proteins interacting with flexible and rigid ligands in water showed that this concept should be revised. In fact, the entropy penalty for the binding of rigid ligands to protein targets can be substantially higher than for their closest flexible analogs [11–18]. This difference was attributed to the stronger freezing of protein molecular motion that occurs upon binding of a rigid ligand [19,20] compared to the case of binding a flexible ligand. It was also noted that changes in non-bonding interactions throughout entire protein-ligand complexes, including interactions with water molecules and counter-ions, should be considered [21]. Only recently have powerful experimental techniques, in particular NMR and sensitive calorimetry, started to reveal the structural and energetic details of protein-ligand binding [22,23]. The elucidation of such details is important for practical applications, especially in medicinal chemistry. The restriction of conformational mobility is one of the most general principles of drug design; there are numerous examples where rigidified molecules bind to biological targets more tightly and display higher efficacy and selectivity than their flexible analogs [24]. However, there are also many examples where this principle fails [25–28], hence a deeper understanding of the role of conformational flexibility/rigidity in the interaction of drug candidates with their biological targets is of great value.

Even less studied than for enzymes and receptors is the role of conformational flexibility/rigidity in the case of polypeptides

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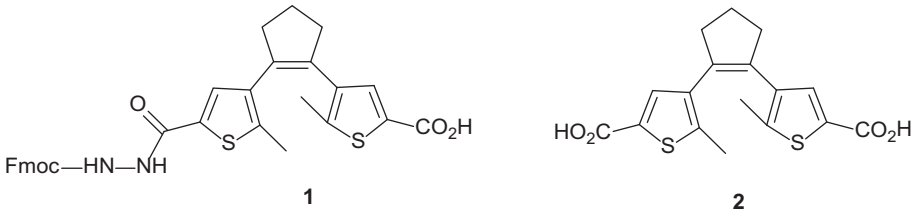

Fig. 1. Diarylethene (DAET) building blocks for SPPS: Fmoc-protected amino acid (**1**), and a dicarboxylic acid (**2**) used to prepare the model compounds studied in this work.

interacting with lipid bilayers. There are several reports in the literature indicating that protein flexibility is a key determining factor for important functions of membrane-active peptides. Bertocco et al. compared a fusion peptide GLFGAIAGFIEG-NHEt derived from the influenza virus hemagglutinin protein [29] with its conformationally restricted analog (containing three α-Me-valines at positions 2, 6 and 10 in place of the natural residues) and found that the restricted analog was less potent in promoting lipid mixing [30]. The authors concluded that the molecular flexibility of the fusion peptide and the resulting conformational plasticity were essential for the fusogenicity – the ability to destabilize the host membrane and facilitate transfection. An analogous study was carried out with the 22-mer antimicrobial peptide (AMP) piscidin 1; again, the rigid analog was shown to be less active [31]. In this case, a single replacement of a conformationally restricted proline by a flexible peptoid residue at the junction of two α-helical fragments resulted in a clear-cut enhancement of membranolytic activity. Other studies, however, demonstrated that more rigid AMPs might have stronger antimicrobial activities compared to their flexible counterparts [32]; and again there are also papers arguing that AMP activity may not change at all upon the purposeful rigidification of peptides [33]. Liu et al. systematically studied the "mechanical determinant" underlying the activity of amphipathic cationic AMPs, and established a "flexibility index" [34] that seems to be applicable to those AMPs that involve direct membrane damage. Furthermore, rigid and flexible molecules, when embedded in membranes, may affect the physical properties of lipid bilayers in a differential manner. A well-recognized example is the rigid molecule cholesterol, which causes a reduction in lipid chain conformational dynamics in the fluid phase, but leads to increased fluidity of the gel phase [35]. The influence of rigid/flexible peptides on membrane properties has also been studied, using various biophysical methods [36–38].

The studies cited above have prompted us to further address the role of conformational rigidity/flexibility in the membrane interactions of amphipathic peptides. We addressed these properties by designing linear model compounds, based on the two most common secondary structure elements (α-helix, β-strand), in which the junction between two formally independent peptide fragments would be either flexible or rigid. By using a molecular photoswitch as a cross-linking building block, a change in rigidity/flexibility can be achieved with a minimal difference in the number of atoms, the overall chemical bonding pattern and the chemical nature of the functional groups. In this paper, we describe the design of such model rigid/flexible peptide conjugates, and report the use of circular dichroism (CD) and solid-state ^{19}F-NMR to study their structural differences in model membranes. Their influence on the membrane properties was also addressed, using differential scanning calorimetry (DSC). We have not focused on any particular type of biologically active peptide, nor have we tried to generate a useful photoswitchable AMP. Nonetheless, our model molecules are of biological relevance in as far as they will help to elucidate the role of conformational rigidity/flexibility in real biological systems, like peptides interacting with biomembranes.

2. Materials and methods

2.1. Materials

All Fmoc-protected amino acids and reagents for peptide synthesis (DIPEA, diisopropylethylamine; HOBt, N-hydroxybenzotriazole; HBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazol; piperidine) were purchased from Iris Biotech (Marktredwitz, Germany) or Novabiochem (Nottingham, UK). The Fmoc-protected (*L*)-3-(trifluoromethyl)-bicyclo[1.1.1]pent-1-ylglycine (Bpg) was obtained from Enamine (Kyiv, Ukraine). Solvents for synthesis and purification were purchased from Biosolve (Valkenswaard, Netherlands) or Acros Organics (Geel, Belgium). Ultraviolet-grade chloroform and methanol for the sample preparation in biological and biophysical assays were obtained from VWR International (Bruchsal, Germany). Ultrapure laboratory grade Milli-Q water was used in all cases (prepared with an EMD Millipore system for water purification). The lipids were purchased either from Sigma-Aldrich (sodium dodecylsulfate, SDS) or from Avanti Polar Lipids (1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine, DMPC) and used without further purification. All other materials were of the highest purity available.

2.2. Synthesis of β/α-model peptides and their ^{19}F-labeled analogs

Standard Fmoc-based solid-phase peptide synthesis (SPPS) and commercially available reagents were used for the peptide synthesis. Leucine-preloaded Rink amide 4-methylbenzhydrylamine resin with a loading of 0.67 mmol/g (150 mg, 1 equiv) was used. Coupling of the amino acids was performed using the following molar ratios of the reagents: an Fmoc-amino acid (4 equiv), HOBt (4 equiv), HBTU (3.9 equiv), and DIPEA (8 equiv). A diarylethene-derived N-Fmoc-protected amino acid (Fig. 1, compound **1**) was prepared as described [39] and used as an individual SPPS building block to incorporate the photoswitching linker at an appropriate stage in the linear peptide sequence. The photoswitch was incorporated by coupling with **1** (1.5 equiv), HOBt (1.5 equiv), HBTU (1.45 equiv), and DIPEA (3 equiv). The coupling time in all cases was 40 min. N-Fmoc deprotection was carried out by treating the resin with 20% piperidine in dimethylformamide for 20 min. After completing the synthesis, the resin was washed with dichloromethane and dried under vacuum for 24 h. The peptides

Table 1
Composition of the DAET-linked peptide conjugate, representing the β/α-model, and list of its ^{19}F-labeled analogs.

Name	Sequence
β/α-model (unlabeled)	KIKIGAKI-**1**-KKLFKKILKYL-NH_2
β2I/α	K-*Bpg*-KIGAKI-**1**-KKLFKKILKYL-NH_2
β4I/α	KIK-*Bpg*-GAKI-**1**-KKLFKKILKYL-NH_2
β6A/α	KIKIG-*Bpg*-KI-**1**-KKLFKKILKYL-NH_2
β8I/α	KIKIGAK-*Bpg*-**1**-KKLFKKILKYL-NH_2
β/α3L	KIKIGAKI-**1**-KK-*Bpg*-FKKILKYL-NH_2
β/α4F	KIKIGAKI-**1**-KKL-*Bpg*-KKILKYL-NH_2
β/α7I	KIKIGAKI-**1**-KKLFKK-*Bpg*-LKYL-NH_2
β/α8L	KIKIGAKI-**1**-KKLFKKI-*Bpg*-KYL-NH_2
β/α10Y	KIKIGAKI-**1**-KKLFKKILK-*Bpg*-L-NH_2