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Functionalized foldamers: synthesis and characterization of a glycosylated β-peptide 3_{14}-helix conveying the T_N-antigen†

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Herein we describe the design, synthesis, and solution structure of a novel type of conjugate composed of a naturally occurring bio-active ligand bound to an artificial peptidomimetic backbone; in this first report on such *functionalized foldamers* we utilized a β-peptide as backbone and a GalNAc carbohydrate residue as ligand.

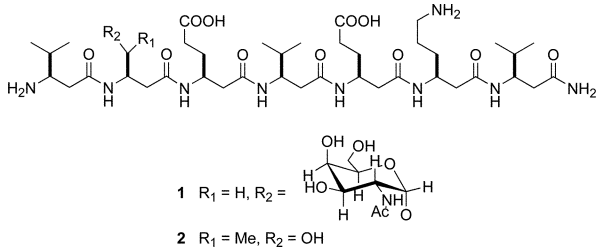
The *de-novo* design of functional peptides and proteins[1] has progressed tremendously in recent years, and has now also been extended to other polymers having a tendency to fold into compact periodic structures, so-called foldamers.[2] In fact, research on such unnatural oligomers serves an important role in this field, as it critically assesses our current level of understanding and tests the generality of the features that are responsible for folding and function.

β-Peptides, *i.e.* peptides composed of β-amino acids, are among the most thoroughly investigated foldamers to date.[3] Compared to α-peptides, β-peptides possess a higher degree of flexibility in their backbone structure, but nevertheless adopt well-defined secondary structures through the cooperative accrual of weak interactions. Several secondary structure elements, including various helices,[4a–d] sheets, and β-hairpins,[4e] have been reported for β-peptides, and the folding propensity has been shown to be critically dependant on the nature of the β-amino acids incorporated in the sequence.[4f] Peptides composed of β-amino acids have also been shown to display promising biological properties, *e.g.* antibacterial,[5a–e] somatostatin mimicking,[5f] disruption of protein–protein interactions;[5g] and are completely resistant to proteolytic[5h] and metabolic[5i] degradation.

The majority of proteins in Nature undergo post-translational modifications *via* linkages to carbohydrates, lipids, and phosporyl residues. These modifications are not only important for the biological function of the proteins, but also influence the structure of the underlying peptide backbone. To enhance our perception of *de-novo* protein design, we need to consider the influence of post-translational modifiers on the structure of the protein, especially when smaller secondary structure elements are in focus. Likewise, the study of functionalized foldamers, *i.e.* oligomers in which the unnatural backbone carries natural post-translational modifications, are expected to bring important information concerning the structural stability of such conjugates. Knowledge of this kind will also be essential for future development of such metabolically stable hybrid systems for biological applications.

To demonstrate the principle of functionalized foldamers we focused our initial attention on β-peptide **1**. β-Peptides composed exclusively of β^3-amino acids have an inherent propensity to fold into a 3_{14}-helix, although several exceptions are known.[3a,6]

The 3_{14}-helix is characterized by 14-membered $i \rightarrow i + 3$ N–H···O=C hydrogen bonded rings and has (*M*)-chirality when prepared from L-amino acids. The helical conformation may be further stabilized by electrostatic stabilization[7a–d] and by incorporating constrained cyclic β-amino acid residues.[7d] In the design of **1** we incorporated a β^3-hGlu in pos. 3 and a β^3-hOrn in pos. 6, with the anticipation that these would form a stabilizing salt-bridge interaction in the 3_{14}-helical conformation. β^3-hVal were chosen as hydrophobic residues, as branching at the side-chain is suggested to increase the folding propensity.[4f] All β^3-hVal residues were placed in an $i + 3$ arrangement, to allow hydrophobic interaction on one face of the helical rim. The β^3-hGlu at pos. 5 was incorporated for a possible hydrogen-bond formation with the carbohydrate residue at pos. 2.



1 R_1 = H, R_2 =

2 R_1 = Me, R_2 = OH

As an example of a common post-translational modification we chose *N*-acetylgalactosamine (GalNAc). In Nature, GalNac α-linked to the hydroxyl-function of serine or threonine residue, represent the simplest mucin structure, the T_N-antigen,[8] found to be over-represented on some human cancer-cell surfaces.

The protected glycosylated β-amino acid, Fmoc-β^3-hSer(α-D-GalNAc$(Ac)_3$)-OH, needed for the solid-phase peptide synthesis of **1** was prepared over 10 steps in 8% (!) overall yield analogous to the glycosylated β-amino acid.‡ The β-glycopeptide **1** was then assembled using Fmoc-solid phase peptide synthesis.‡

Circular dichroism (CD) spectroscopy provides characteristic signatures for the various helices reported for β-peptides.[3] The CD-spectrum of glycosylated β-peptide **1** is shown in Fig. 1. The spectrum in methanol shows a minimum, zero crossing, and a maximum at 215, 208, and 198 nm, respectively, corresponding to the formation of a left-handed 3_{14}-helix. The CD-spectrum of an unglycosylated analogue of **1**, *i.e.* β-peptide **2**, is also shown in Fig. 1. As seen from the intensity of the absorption, the incorporation of a GalNAc-carbohydrate residue on β-hSer in position 2 of the peptide leads to a slight destabilization of the 3_{14}-helical conformation in methanol solution. In water on the other hand, a dramatic decrease in the CD absorption is noted both for the glycosylated β-peptide **1** and the analogue **2**. This is not all that surprising, as water is known to destabilize the 3_{14}-helical conformation;[7a–c] however, the effect observed here appears to be larger than previously noted. Nevertheless, both peptides still give rise to the characteristic signature of the

† Electronic Supplementary Information (ESI) available: Experimental details, NMR spectra, list of NOE's, and details concerning the structure calculation. See http://www.rsc.org/suppdata/ob/b5/b503237g/

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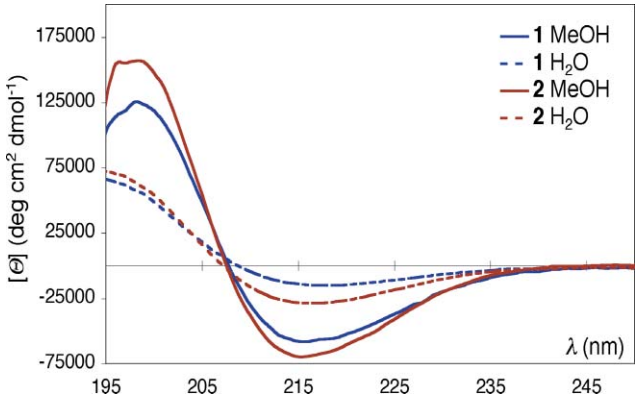

Fig. 1 Circular dichroism spectra of glycosylated β-peptide 1 (blue) and unglycosylated analogue 2 (red) in methanol (solid lines), and in aqueous phosphate buffer (dotted lines) at pH 6.9. All spectra were recorded at 0.1 mM conc. at 25.0 °C.

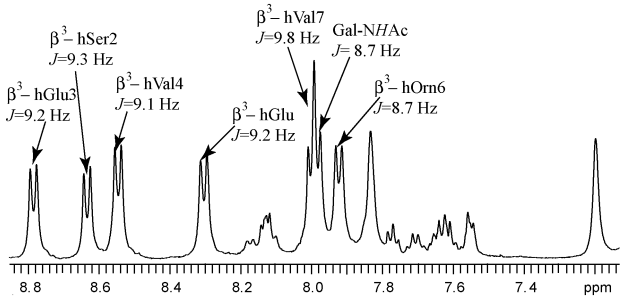

Fig. 2 Expansion of the amide region of the ^{1}H-NMR spectrum of glycosylated β-peptide **1** in MeOH. Signals from one major conformer are seen together with a number of weaker signals, indicating the presence of additional conformers.

3_{14}-helix, suggesting that both peptides, at least partially, sample this conformation also in aqueous solution.

In order to establish the proposed 3_{14}-helical conformation of the functionalized foldamer **1** in MeOH solution we next turned to NMR spectroscopy. Detailed analysis of the two-dimensional spectra allowed us to assign all resonances from the major conformer of **1**.‡ The spectrum shows good dispersion of the amide region, Fig. 2, indicating the presence of one main secondary structure. However, a number of weaker signals are also seen, suggesting that more than one conformer is present. In the ^{1}H-NMR spectra of unglycosylated β-peptide **2**, on the other hand, only one set of resonances is observed; this signifies a direct correlation between the NMR spectra and the stability of the 3_{14}-helix as determined from the intensity of the CD absorption at 215 nm.

A more detailed analysis of the NMR signals originating from the major conformer of **1** reveals that all ^3J(NH–C(β)H) coupling constants are large (8.7–9.5 Hz), in agreement with an antiperiplanar orientation of these protons. Furthermore, the ROESY spectra shows several non-sequential NOEs between $NH_i \rightarrow$ H–C(β)$_{i+2}$ and $NH_i \rightarrow$ H–C(β)$_{i+3}$, which are distinct for the 3_{14}-helical conformation. Only a few weak NOEs between protons of the carbohydrate and the peptide moieties could be observed; *e.g.* the γ-protons of β^3-hOrn at pos. 6 and the anomeric β-proton on the GalNAc residue. This is not unexpected, as most signals in this case are hidden under the suppressed solvent signal; moreover, NOEs between the side-chains of β-peptides are generally seldom observed.

A total of 40 NOEs were extracted and used as restraints in a Monte-Carlo conformational sampling. Fig. 3 depicts the superposition of 10 low-energy structures resulting from this calculation.‡ Two main conformational families, of comparable energies, were found, and may be considered representative for the folded structure of **1** in MeOH solution. As seen in Fig. 3a/b, the glycosylated β-peptide **1** forms a 3_{14}-helix in MeOH solution. The structures of the two conformational families found differ mainly at the *C*-terminal side of the helix, Fig. 3c. The difference between the two families is caused by the interactions of β^3-hOrn in pos. 6; in one family (blue) this residue has the designed interaction with β^3-hGlu in pos. 3, while the other family (green) is characterized by an interaction between β^3-hOrn and the GalNAc moiety at pos. 2, leading to an "opening" of the helix at the *C*-terminus.

The amide region of the NMR spectra of **1** in water is characterized by broad unresolved resonances, suggesting the presence of several interconverting conformations of **1** in water; in agreement with the CD spectroscopic measurements. Further design, such as incorporation of structural restrains,[7e] and/or other helix stabilizing modifications, recently proposed by Schepartz,[7c,d] will be needed to increase the helical stability of these functionalized foldamers in aqueous solution.

In summary, we have shown that a short β-peptide 3_{14}-helix can tolerate the introduction of an *O*-linked carbohydrate residue, at least in alcoholic solvents. Apart from being of fundamental interest for our understanding of the factors responsible for folding of β-peptides, this study demonstrates for the first time how foldamers can be used as carriers for naturally occurring post-translational modifications. The functionalized foldamers described herein could function as metabolically stable carriers for carbohydrate epitopes, and thus be of interest to the development of carbohydrate-based vaccines. Further use of such conjugates in the area of biomolecular recognition, *e.g.* modification of protein–protein interactions,[9] are also to be anticipated. However, before such applications can be realized, we need to refine our design of these conjugates so that the folded conformation remains the major conformation also in an aqueous environment. Work towards this goal is in progress.

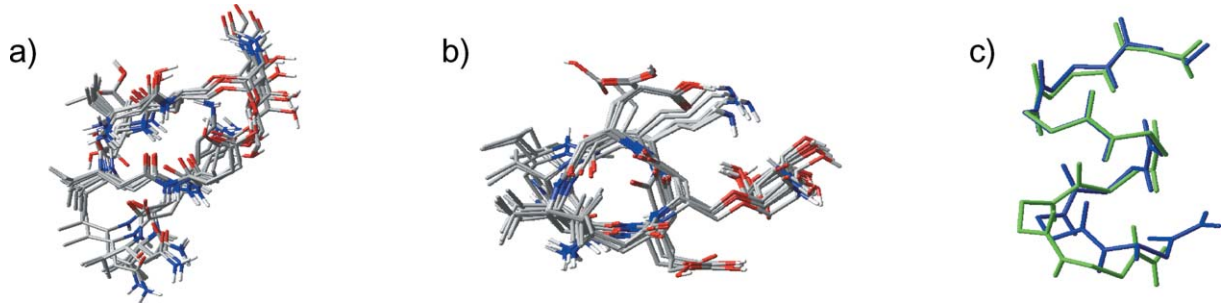

Fig. 3 NMR structures of glycosylated β-peptide **1** as a bundle of 10 low-energy structures calculated from a restrained Monte-Carlo conformational search: a) side-view; b) top-view; c) superposition of the backbones of the two conformational families found. (Non-polar hydrogen atoms have been omitted for clarity.)

Acknowledgements

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‡ See supporting information for details.

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