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Influences on the stability of collagen triple-helix

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ABSTRACT

Collagen forms a triple helix structure which consists of three tightly coiled polyproline II-type (PPII) strands with the repeating amino acid motif Xaa-Yaa-Gly, where Xaa and Yaa are often proline (Pro) and hydroxproline (Hyp), respectively. The thermal and mechanical stability of the collagen triple-helix is a complex balance between several factors, e.g. sterical influences of substituents, stereoelectronic effects, or interstrand interactions. On the basis of computational studies, we have analyzed the influence of these effects and demonstrate that even comparatively small effects, like the stereoelectronic gauche-effect, and intramolecular hydrogen-bonding can have an impact on the triple-helix stability. In addition, our results suggest that hydrogen bond-like interactions perpendicular to the helix axis increase the stiffness of collagen. Our studies provide a deeper insight into the structure-directing factors in collagenous materials and will help to improve the synthesis of collagen-like biomaterials.

1. Introduction

One approach for novel developments and technological innovation is to draw inspiration from nature. Bio-based and bio-inspired materials are becoming increasingly interesting for industrial applications, not only because of their unique features, but also due to economic reasons and the need of sustainable development.

Collagen is considered as one of the most useful and promising biomaterials [1]. The importance of collagen is based on its widespread occurrence as structural protein representing the most abundant mammalian protein [2]. It makes up one third of all proteins in the human body and is an essential part of connective tissue. The skin, for instance, consists to more than 50% of collagen being responsible for skin strength and elasticity. Like other bio-based materials it has several superior features such as excellent biocompatibility, weak antigenicity, and biodegradability rendering it an excellent scaffold for biomedical applications, in particular, in cosmetic surgery in the context of regenerating lost or damaged tissue, but also as drug delivery system [2]. Although collagen extracted from animals is readily available for medical purposes, it would be highly desirable to have a synthetic source in order to avoid possible complications including immunogenicity and transmission of infective diseases from animal to human [2,3]. The synthesis and design of collagen-like materials is, however, a challenging task and requires a comprehensive understanding of the collagen structure and the factors influencing its stability [3]. Due to its exceptional mechanical characteristics, collagen is, however, also interesting for nanotechnological applications, for example, as a mechanomutable material or as template for de novo material design [4,5]. Despite numerous experimental and computational studies, e.g. Refs. [3–11] and references therein, it is still not fully understood which factors are determining for the extraordinary structural and mechanical properties of collagen. It is important to understand the complex balance between effects like sterical influences of substituents, stereoelectronic effects, or interstrand interactions at the atomistic level for designing innovative products and materials with distinct properties.

For rational product design and product enhancement, a detailed understanding at the atomistic and molecular level is indispensable. In particular, the knowledge of structural properties is of utmost importance, since the function and other molecular properties are driven by the molecular structure. Molecular modeling is a versatile tool for providing critical insights into molecular structure, processes, and properties. A whole hierarchy of in silico methodologies offers the possibility to address problems at different length and time scales.

In the following, we will present a strategy for studying structural and mechanical properties at the atomistic level of a collagen-like triple-helix using quantum mechanical and force field-based methods. In the first part, we will analyze systematically the conformational preferences of hydroxyproline which represents the major building block of collagen and discuss its impact on the collagen triple-helix stability. In the second part, we will explore the influence of the hydroxyl group on the elastic properties of the collagen triple helix. Our studies provide a deeper insight into the structure-directing and stability-influencing factors in collagenous materials at the atomistic level, while our modeling

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strategy can represent a useful approach for guiding synthesis of collagen-like biomaterials by predicting material properties.

2. Computational details

The computational studies were performed using Scienomics MAPS software suite [12]. Model structures were created and pre-processed using the building tools of MAPS. The software tool Confab [13] has been used as stand-alone version for performing conformational searches.

2.1. Ab initio calculations

Four initial model structures of hydroxyproline (Hyp) isomers differing in the proline ring pucker and the configuration at C4 (also denoted as Cγ) have been created. The N-terminal side has been capped with an acetyl moiety (CH_3CO—) and the C=O group with —OCH_3 forming a methyl ester at the C-terminal side, which has been proven to provide valuable model structures for studying conformational preferences of proline derivatives incorporated in polyproline or collagen-like helical structures [14–16]. For each model structure a conformational search has been performed using the software tool Confab [13]. Confab uses a systematic approach to explore the conformational space and Ebejer et al. [17] have demonstrated its ability to generated a diverse set of low energy conformers. The conformers obtained from the conformational search were geometry optimized at BLYP-D3/def2-TZVP [18–21] level applying Grimme's empirical D3 dispersion correction [22].

2.2. Molecular dynamics simulation

Molecular dynamics (MD) simulations were performed using the LAMMPS [23]. As structural basis served a collagen-like model structure obtained from the RCBS database [24] with the PDB-ID 1V6Q [25]. The structure was saturated with hydrogen atoms. The AMBER force-field was assigned [26,27] and 50 water molecules have been added to the unit cell using MAPS Amorphous Builder. In order to investigate the influence of the hydroxy groups on the elastic properties, a second structure in which the hydroxy groups have been replaced by hydrogen was prepared in a similar way. Since the unit cell dimensions in a and c direction were rather short, a $2 \times 1 \times 2$ super cell was built for each model allowing to use a reasonably large cutoff of 10 Å for calculating non-bonded interactions, while long-range interactions were treated using a Ewald/n summation. The system was equilibrated at a temperature of 298 K and a pressure of 1 Atm. A 100 ps NPT simulation using the multiple time step algorithm as implemented in LAMMPS was followed by a 50 ps NPT simulation using a single time step of 1 fs. The final structure was used for simulating the elastic deformation. Two separate types of in silico experiments have been carried out: (1) A continuously tensile stress was applied separately in *x*, *y*, and *z* direction in a range from 1000 Atm to 5000 Atm. (2) The cell size was continuously increased separately in *x*, *y*, and *z* direction up to 5% of the original size. For the analysis of the stress simulations, the mean values of the number of hydrogen bonds within the collagen helix was calculated based on the hydrogen bond analysis as implemented in MAPS.

3. Results and discussion

Collagen forms a triple helical structure which consists of three tightly coiled polyproline II-type (PPII) strands with the repeating amino acid motif Xaa-Yaa-Gly [3], where Xaa and Yaa are often proline (Pro) and hydroxproline (Hyp), respectively [3]. There is experimental evidence that Hyp in Yaa position has a stabilizing influence, whereas Hyp at Xaa position destabilizes the collagen triple-helix [3,6,7]. Furthermore, the configuration in Hyp is critical for the stability of collagenous materials [3,8,9]. A stabilizing effect is only observed when the hydroxy group is in *4R* configuration, while the *4S* diastereomer leads to a destabilization [3,16]. One hypothesis discussed in literature is that the stabilization of collagen is due to water mediated hydrogen bonding [10,11,28,29]. Raines et al. [3] replaced Hyp in collagen-like model peptides through fluoroproline (Flp) that is usually reluctant to form hydrogen bonds and observed similar trends in the collagen stability like for Hyp which implies that effects other than hydrogen bonding have to play a role in stabilizing the triple-helix. Experimental and theoretical studies [3,15,16] indicate that stereoelectronic effects dominate the conformation of substituted proline model compounds. In the following, we will present a work flow for analyzing the conformational preferences of hydroxyproline and discuss the implications of stereoelectronic effects as structure directing element.

Table 1
Energy differences between lowest energy isomers of Ac-Hyp-OMe in kJ/mol. A negative sign of $\Delta E_{\text{trans-cis}}$ indicates that the *trans* isomer is preferred. For the *4R* stereoisomer, the positive energy difference $\Delta E_{\text{endo-exo}}$ indicates a preference for the exo ring pucker, i.e. for the gauche isomer. For the *4S* stereoisomer the negative $\Delta E_{\text{endo-exo}}$ implies a preference for the endo ring conformation and thus also for the gauche isomer.

Stereoisomer	$\Delta E_{\text{endo-exo}}$ (kJ/mol)	$\Delta E_{\text{trans-cis}}$ (kJ/mol)
4R	2.6	−5.2
4S	−21.7	−8.5

3.1. Conformational preferences of hydroxyproline

For analyzing the conformational preferences of Hyp, model structures for each possible combination of configuration at C4 (*4R*/*4S*) and ring conformation (exo/endo) were created and a systematic conformational search was performed using the Confab software tool [13]. Each conformer was geometry optimized at DFT level including dispersion corrections (for further details, see Computational details section). Energy differences between the energetically most favored exo and endo isomers and *trans* and *cis* isomers, respectively, are listed in Table 1.

The relative energies indicate a clear preference of the *trans* conformation of the amide group which is in line with previous studies on related compounds [14–16]. For both diastereomers (*4R* and *4S*), a gauche conformation between the hydroxyl and the amide group is energetically favored. For the *4S* isomer, however, the preference is considerably stronger which can be explained by a structural analysis of the optimized structures.

The optimized geometries of *4R*-Ac-Hyp-OMe and *4S*-Ac-Hyp-OMe are illustrated in Fig. 1.

The energetically most favored *4R* stereoisomer shows an exo conformation of the pyrrolidin ring and a trans conformation of the amide bond. In the *4S* isomer, a trans conformation of the amide bond and an endo conformation of the ring is observed. This means that in both isomers, the hydroxy group at C4 and the amide group adopt a gauche conformation and that the proline ring conformation is governed by the gauche-effect [30]. A further stabilizing effect comes through the *trans* conformation of the amide bond. Fig. 2 illustrates that the angle between the oxygen of the acetyl group and the carbonyl group of the ester is 100° which is near an idealized so-called Buergi–Dunitz angle [31] that is known to allow for stabilizing n–π^* interactions [3,15,16].

There is evidence that the gauche-effect per-organizes the peptide backbone torsional angles in such a way that n–π^* interactions are possible [3]. It should be noted that in *4R* with endo conformation and in 4S with exo conformation the angle is also near 100°.