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Computational study of polymorphic structures of α- and β- chitin and chitosan in aqueous solution

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ABSTRACT

Chitin is a natural biopolymer and the second most abundant after cellulose. This polysaccharide can be found in the biomass in different polymorphic forms. Chitosan is one of the most important derivatives obtained from the deacetylation of chitin. In this work, Molecular Dynamics simulations of chitin and chitosan nanoparticles enabled us to evaluate their different conformation and solubility properties. The Molecular Dynamics simulations show that the arrangement of the chains of chitin and chitosan significantly affects the structural behavior of these biopolymers in aqueous solution.

1. Introduction

Chitin is the second most abundant structural polysaccharide in nature after cellulose; however, it has twice the rate of replenishment [1]. This natural biopolymer can be presented in different structural forms, according to its biological function and its natural source; these forms are differentiated according to the arrangement of the carbohydrate chains. The α form has chains arranged alternately antiparallel; the β form has all chains in parallel and the γ-chitin has two chains in one direction with an additional inverted chain [2–4]. According to Atkins, the γ-chitin is a variant of the α family [5].

The α-chitin is more abundant and has higher thermodynamic stability, probably due to hydrogen interactions between chains; the β-chitin has a lower density, with weaker intermolecular interactions between the strands, while the γ-chitin was not considered as a subject of study in this work, as it is very rare [2,6].

Chitosan is the main derivative of chitin obtained from its complete or partial deacetylation. The degree of acetylation (GA) can be defined as the percentage of monomeric units of acetamido-2-deoxy-D-glucopyranose present in the polymer chain, wherein chitosan is considered soluble in aqueous solutions of organic or inorganic acids, or when the GA is less than about 50% [7–9].

The chitosan structure is shown schematically in Fig. 1, where "*n*" indicates the degree of polymerization units of *N*-acetylglucosamine (*N*-acetyl group) and glucosamine units (amino group) [10].

Both chitin and chitosan are of economic value, mainly due to their versatility and biological activities. These biopolymers have many advantages for application—they are biodegradable in nature and in living organisms, biocompatible and non-toxic [6], highly renewable and an economically viable raw material, have powerful healing properties [11] and are capable of interaction with environmental contaminants from many natural sources [12]. However, due to its insolubility in aqueous solution, chitin's potential for applicability is limited, while chitosan has shown to be a promising biomaterial in various sectors, with the possibility of a variety of derivatives for use in specific purposes [13]. Examples of the sectors of application include: blood anticoagulant, green chemistry, food, agriculture, biomedicine and pharmacy, antifungal, antibacterial, as a flocculant agent in organic compounds, heavy metals adsorbent in residual water and ion exchange properties [2,14–17].

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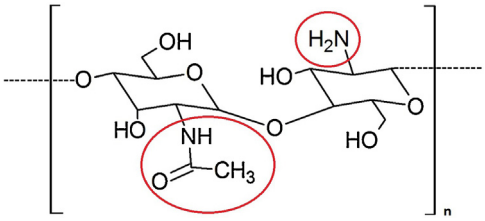

Fig. 1. Schematic representation of the chitosan polymer structure. Where "*n*" indicates the degree of polymerization [1].

In practically all these applications, the chitin/chitosan derivatives chemically or enzymatically modified can be used in different morphologies as fibers, films, hydrogels, membranes, microspheres and nanoparticles [13]. Due to this versatility, there is a necessity for more detailed studies on the behavior and polymorphic structures of these macromolecules. However, the major investigations into the behavior of nanoparticles of chitosan and chitin are limited to macroscopic evidence [18], leaving the characterization of these biopolymers incomplete, especially regarding their physicochemical properties and chemical behavior—which are essential parameters in the various industrial sectors previously mentioned.

In this regard, theoretical computational methods are a useful alternative in understanding the experimental properties, so that predictions can be made about the behavior of polymorphic forms of their filaments in aqueous solution and about the dynamics of the system [4,18,19].

Thus, in this study, a series of computer simulations using Molecular Dynamics (MD) was performed, to investigate the most significant differences between the α and β polymorphic forms of chitin and chitosan and to confirm the existence of a structural distinction between the polymorphic forms of chitosan and chitin, from which they are derived [6,20].

2. Computational methods

The initial structural framework to simulate the solvated chitin and chitosan chains was a modeled 10-mer polysaccharide chain, arranged in a 3×2 matrix in a linear conformation, in accordance with the X-Ray Crystallographic Databases [21]. The structures of the chains were modeled according to a two-fold helix geometry, with the dihedral angles around the glycosidic bond about $\varphi \sim -90^{\circ}$ and $\psi \sim +90^{\circ}$ [20]. The snapshots of the initial minimized structures of the chitin polymorphic forms are represented in Fig. 2.

A rectangular simulation box was built at 1.5 nm from the polymer chains. In the initial structure, the acetylation degree (DA) was 20% (the most easily obtained and found in experimental works [22]), with pH 6.5 (pK_a of amine groups = 6.3–7.2) [23]. In order to describe these conditions, two monomers were acetylated and, from the present amino groups, half were considered protonated and half deprotonated. This adopted sequence follows the same protocol modeled in our previous works, because this distribution of repeating groups in chitosan is obtained by homogeneous deacetylation and is more useful in commercial applications [8,20]. The chitosan chains were placed in a rectangular simulation box at a distance of 2.0 nm from the polysaccharides (the simulated chains are summarized in Table 1).

All the modeled systems were solvated by filling the box with SPC water model [24] molecules. The water molecules were also inserted into the available space inside the nanoparticles, forming hydrated crystals. Chloride ions were added to neutralize the ionic strength of the chitosan systems. Each system was energy-minimized using 10,000 steps of the steepest descent method [25].

After minimization, the solvent was equilibrated by performing 10 ps MD simulation at 50, 150, and 298 K, with the solute non-hydrogen atoms positionally restrained with a constant force of 1.0×10^3 kJ mol^{-1} nm^{-1}. Following the solvent equilibration, for the nanoparticles of chitin and chitosan respectively, a total of 40 ns and 35 ns MD simulations were performed in an isothermal-isobaric (NPT) ensemble using the leapfrog algorithm, with a 2 fs time step. The configurations were recorded every 1 ps for analysis. During the MD simulation, the translational and rotational motion of the center of mass was removed at every time step. The temperature was kept at 298 K by coupling solute and solvent separately to Berendsen thermostats with a relaxation time of 0.1 ps. The pressure was maintained at 1.0 bar by coupling to a Berendsen barostat [24] via semi-isotropic coordinate scaling, with a relaxation time of 10 ps and a compressibility of $4.5.10^{-5}$ bar^{-1}. Water stretching and bending motions were constrained by the LINCS algorithm [26]. A 1.4 nm cutoff was used for the short-range electrostatics and van der Waals interactions. Long-range electrostatics contributions were treated via the generalized reaction field [27] with $\epsilon = 66$. All simulations were carried out using the GROMACS 4.5.3 [26,28] program with GROMOS53a6 force field, which includes the parameter set for carbohydrates developed by Lins et al. [29]. The results were analyzed using the VMD [30] and Grace [31] programs.

3. Results and discussion

To further understand the behavior of the polymorphic forms of chitin and chitosan in aqueous solution, some parameters of the molecular dynamic simulations were analyzed to obtain an insight into the dynamic behavior of the systems. The results obtained are shown as follows:

According to the literature, the two polymorphic forms of chitin are insoluble in water and their molecular description can be difficult to characterize due to experimental limitations [7]. Due to our simulation setup, we were able to describe the atomistic explanation of how both structures behave in aqueous solution.

The analysis of the Molecular Dynamics trajectories of the α-chitin and β-chitin systems allowed us to verify that 27.5 and 10 ns, respectively, are necessary for the systems to reach energetic and structural equilibrium. Thus, after 28 ns simulation, the values for the mean total energy (Table 2a) and Root Mean Square Deviation of atom distances (RMSDist) were calculated and the low standard deviations from the mean values were displayed, reinforcing the system convergence.

The first structural parameter used to determine the behavior of polymorphic structures of chitin in aqueous solution was the Root Mean Square Deviation (RMSD) of the molecules during the simulation. It is clear from Table 2a that the average values of RMSD for the α- and β-chitin are similar, but the higher standard deviation displayed for the β-chitin suggests greater mobility of this polymorphic structure compared with the α-chitin. The rigidity of the α-form can also be confirmed by the Root Mean Square Fluctuation (RMSF) of the nanoparticle, which displayed a lower value for the α-chitin, indicating less flexibility of this macromolecular fiber. Another important parameter for evaluating the behavior of the chains is Lateral Diffusion. It can be obtained by calculating the Mean Square Displacement (MSD), plotting an MSD curve as a function of time. This is calculated by registering, at each time-frame, the position of each particle and square of the distance [32]. In this case, Einstein's relation for Brownian motion study is used [33]. Therefore, the higher value of lateral diffusion for the β-chitin structure reinforces the initial hypothesis. On the other hand, the same comparison made for the chitosan displayed the opposite behavior, and this difference is directly related to the difference in the degree of acetylation between the chitin (100%) and chitosan (20%). A detailed discussion of the differences between the polymorphic structures of chitosan will be carried out later in this work.