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The effects of diatom pore-size on the structures and extensibilities of single mucilage molecules

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ABSTRACT

Diatoms secrete extracellular polymeric substances (EPS), or mucilage, around the cell wall that may serve to aid in motility and form a discrete layer that may help maintain thicker layers of EPS that have a greater role in adhesion. Mucilage molecules adhere to the diatom frustules, which are biosilica skeletons that develop from the diatom cell walls. Here, molecular dynamics methods were used to determine the characteristics of mucilage molecules as a function of pore size; notably 1,4-α-D-galacturonic acid, 1,4-β-glucuronic acid and 1,4-β-D-mannuronic acid. These uronic acids differ from each other in structure and extensibility as a function of their folding characteristics. Here, we find that when overlain upon a pore, mucilage molecules try to return to their native folded states but are restrained by their interactions with the silica surfaces. Furthermore, the extensibility of mucilage molecules over pore spaces affects the extent of mechanical energy required to straighten them. As such, different EPS molecules will affect sliding, friction and adhesion to subsequent layers of EPS in different ways. We conclude that higher EPS extensibility is homonymous with higher adhesive or frictive resistance since the molecules will be able to strain more before they reach the most extended (and thus rigid) conformation. The research herein is applicable to modern engineering as it yields insight into the biomimetic design of molecules and surfaces for improved adhesion or motility.

1. Introduction

Diatoms are mucilage-secreting unicellular microalgae with silicified cell walls (frustule). These microorganisms are often associated with biofouling problems [20] and have inherent mechanical protection from their glassy frustules. Frustules are made up of monosilicic acid, polymerised silicic acid and/or other organo-silica complexes [3]. Frustules are also ornamented with pores, the majority of which are typically between 3 and 50 nm in diameter [49], though pores below 10 nm long and 2–5 nm wide have also been reported in the literature [48]. Extracellular polymeric substances (EPS), or, mucilage, is secreted by the diatom, expressing itself through the pores, and this enables the diatoms a degree of initial motility [2] and/or initial settlement upon diverse substrates. Long-term biofouling *problematically* occurs on substrates such as pipes [22], maritime vessels [43] and membranes [38,47]. These materials often require extensive cleaning to remove biofilms formed by diatoms and other fouling micro-organisms, though in certain instances materials have to be replaced. Both cases result in unwanted economic losses. Nevertheless, there have been recent successful endeavours that utilise the combined biofouling and glass secreting characteristics of diatoms *beneficially* in advanced engineering materials [52]. Moreover, certain decorating organisms such as crabs *also benefit* from diatom biofouling by building up hierarchical architectures that they use to increase the effectiveness of carapace camouflage [42].

Mucilage molecules can be classified as either attached EPS or non-attached EPS [44]. Ford and Percival [23] reported that the predominant attached EPS carbohydrates in mucilage are β-1,3-linked glucoses with branches at Carbon-6. Mucilage consists primarily of polysaccharides, proteins, pyruvates, uronic acids, and SO_4^{2-} groups [6,23,44]. Monosaccharides of rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose have

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been identified in the diatom *A. caffeaeformis* [5], the most dominant of which are mannose, galactose and glucose [6]. Similar monosaccharidic presence has been reported by Ref. [44] as present in *Cylindrotheca closterium* and *Navicula salinarum*. In later research, Chiovitti and co-workers [14] reported the warm water extracted glucans (originating presumably from β-1-3 glucan chrysolaminaran) are predominantly intracellular. As such, it is unlikely that storage polysaccharides like β-1-3 glucan chrysolaminaran will form any large part of the initial mucilage layer, which has been described as essentially a marine gell aggregate [46]. More aged mucilage layers tend to be less sticky than fresh mucilage and contain longer molecules [50], which might in turn strengthen the mucilage layer by entanglement [46]. Though storage polysaccharides might leak to some extent through the diatom pores, they will likely be swiftly degraded by bacteria [27]. Moreover, the rate of any possible leaking will be an inverse function of the tortuosity of the path that the leaking molecules will have to take, which are in turn dependent upon the organisation of matter [1] across the diatom pore. Some of the more likely glycopolysaccharides involved in initial diatom attachment are galacturonic acid and glucuronic acid [39]. Attachment in the initial stages essentially sets up the diatom for motile gliding [36], which eventually becomes sessile attachment through the development of protuberant EPS pads, tubes and hold-fast like anchors secreted from the raphe [30,36]. Diatom gliding is also a means by which diatoms biofoul surfaces. Through gliding, the diatoms leave trails of attached secreted mucilage [28], which most likely detaches from attached underlying mucilage, or from the cell wall surface. AFM-based research by Higgins and co-workers [31] suggests that diatom settlement more commonly occurs on girdle bands, rather than on valves, which brings to light a seemingly pedantic settlement cycle in diatoms. The effectiveness of diatom settlement is most importantly a function of the surface of a material, the material characteristics and how it affects wetting [33]. In a study by Holland and co-workers [34] for example, diatoms were found to attach more strongly to PDMSe than to acid-washed glass.

Mucliage can merge with bacteria or other microorganisms to form biofilms made up predominantly of muco-polysacharrides [24], which in turn leads to biofouling-related problems such as substrate corrosion [10,21], the accumulation of contaminants such as fungi or protozoans [15], and the encouragement of macrofouling [9,16,42]. The attachment characteristics of diatom EPS are vital for successful colonisation and population growth [13]. Sessile attachment has been reported to arise from the immobile adhesion of a few diatoms, which then encourages the nucleation and ultimately adhesion of extended diatom colonies [37]. Mucilage molecules have distinct topographies and mechanical properties [29], which could suggest that EPS secretion is at some level, timed for either motility or adhesion. Adhesive mucilage has been found to be a biocomposite built into proteinous nanofibres that align in parallel [19]. A parallel arrangement such as this suggests a cellular level extrusion process takes place in the secretion of mucilage, however this concept has not as yet been documented, to the best of our knowledge.

The characteristics of attachment between diatom frustules and mucilage molecules are important in view of biofouling since they will affect the malleability or rigidity of the molecules, which will in turn affect the extensional properties of the molecules. Mucilage molecules with low extensibility and considerable rigidity are more likely to develop high stress intensities at interfaces, and may concurrently have reduced close-range contact with biofouling surfaces during the initial stages of settlement. Pore space is one feature of the frustule surface that we hypothesise affects molecular extensibility since molecules not in contact with the frustule surface are expected to try to return to their native folded states. To date, there have been no reports on the molecular mechanics of early-stage carbohydrate (EPS) secretions of diatoms and indeed, how these secretions affect motility and/or adhesion. Further, there have been no attempts to define the effects of pore space on the folding, or partial-folding characteristics of diatom EPS. In this paper, we use molecular dynamics methods to predict the effects of frustule pore sizes on the structures and extensibilities of single diatom mucilage molecules with an aim of elucidating fine balances between molecular attachment and molecular mobility.

2. Methods

Uronic acids falling under the category of *attached EPS* [44] included; 1,4-β-D-mannuronic acid, 1,4-α-D-galacturonic acid, and 1,4-β-glucoronic acid. These were constructed in Ascalaph Designer as single molecules. Each mucilage molecule was built up of 18 monomeric repeats. Silica sheets were also constructed in Ascalaph Designer in a 16 $\times$ 16 molecule array and *ab initio* simulations performed on all molecular structures to determine their partial atomic charges in preparation for molecular dynamics interaction simulations. The *ab initio* simulations, were conducted using the Firefly QC [25] package calculating electrostatic potential derived charges by MP2 (perturbation theory) alongside the 6-311+G(2d,p) basis set [18].

Molecular dynamics simulations were initially conducted on single mucilage molecules to ascertain their steady state folded structures. Following this, attachment simulations were conducted of single mucilage molecules upon two adjacent silica surfaces, separated by a distance to mimic the pore space. This separation and hence, pore size, was varied independently and in total three different nano-pore sizes were used for the simulation of four different mucilage molecules. Exact nano-pore sizes were determined using Discovery Studio Visualiser [7] as being 8.24 Å, 16.91 Å, and 30.04 Å smallest to largest, respectively. Single mucilage molecules were also simulated on complete silca sheets (with no pore space). In all porous model simulations, the entire silica sheets were given zero degrees of freedom and linear unfolded molecules positioned across the sheets with the centre of the molecule crossing over the nano-pore space and separated from the silica sheets by a distance of approximately 10 Å. For the complete sheet simulations (with no pore space) the molecules were placed lengthways across the centre of the sheet. A Monte Carlo method was used to energetically stabilise the molecule prior to molecular dynamics simulations. Molecular dynamics simulations were then conducted in vacuum using a Sheffield solvation model to perform the simulation under implicit water conditions [26]. An AMBER94 force field was used since it is focused on intermolecular and intramolecular interactions and is typically used for biomolecular modelling [12,51]. Modelling was conducted using a 5.5 fs time step and stopped at steady state. Post-simulation analyses were conducted using the Discovery Studio 4.1 Visualiser [7] to determine specific features including; angles, bends, distances, stretch, and twists.

3. Results and discussion

Mucilage molecules (EPS), more specifically; 1,4-β-D-mannuronic acid, 1,4-α-D-galacturonic acid and 1,4-β-D-glucuronic acid, were simulated under the conditions of implicit water to ascertain their folded structures at steady state. These formed α and β polysaccharides conforms. 1,4-β linkages are reported to often give rise to parallel molecular chains with intermolecular hydrogen bonding, resulting in crystalline polysaccharides [4,35,45]. In the models nevertheless, Fig. 1, β-glycosidic linkages occurring in specifically 1,4-β-D-mannuronic acid form β-type turns, which give