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Molecular mechanisms of aqueous boundary lubrication by mucinous glycoproteins

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ABSTRACT

Mucins have long been recognized as instrumental to biolubrication but the molecular details of their lubrication mechanisms have only been explored relatively recently. The glycoprotein PRG4, also known as lubricin, shares many features with mucins and appears to lubricate through similar mechanisms. A number of studies have contributed to a more in-depth understanding of mucin adsorption and layer formation on surfaces and the mechanisms by which these layers lubricate. Although mucinous glycoproteins differ in their aggregation properties, their adsorption behaviors on surfaces, and in their ability to reduce friction, they share important similarities favorable for lubrication. They are highly hydrated, they adsorb strongly to a broad range of surfaces, and the layers they form are both sterically and electrostatically repulsive, all attributes thought to contribute to boundary lubrication. They also hydrophilize hydrophobic surfaces, promoting the formation of aqueous fluid films that can lower friction at already relatively low sliding speeds. In this paper we briefly review current knowledge of mucin adsorption and lubrication, with a focus on recent advances.

1. Introduction

Efficient lubrication of moving internal and external surfaces of living organisms is vital to minimize wear and loss of energy in locomotion, and for the efficient processing of foods in the mouth and gastrointestinal tract [1••,2]. This is accomplished to a large extent by highly hydrated, amphiphilic, glycoproteins that coat hydrophobic surfaces, rendering them hydrophilic and electrostatically and sterically repulsive. These properties at interfaces are favorable for both boundary lubrication and aqueous fluid film lubrication. These glycoproteins occur as mucins in mucus and in tears and as mucinous glycoproteins known collectively as PRG4 in synovial fluid and on the cartilage surface.

While recent reviews provide excellent summaries of mucin structure, function [6•] and rheology [7], and while it is clear that lubrication is an important aspect of mucinous glycoprotein function, we are not aware of a review specifically addressing some of the underlying mechanisms of boundary lubrication by these macromolecules. In this review paper we consider a number of studies that have contributed to the current understanding of mucin adsorption and how it affects friction and wear.

A better understanding of mechanisms of boundary lubrication by mucinous glycoproteins has applications in diverse areas. It is expected to inform the design of biocompatible low friction and/or non-fouling coatings for contact lenses and biomedical devices (e.g., endoscopes and catheters) and the formulation of artificial saliva, tear fluids, and lubricants for the treatment of osteoarthritis. It has much to contribute to the understanding of the structure–function relationship of mucinous glycoproteins in the biological context. For example, improved understanding of themechanisms by which these biomacromolecules govern friction and wear at articular joint interfaces may provide new insights into the mechanical contributions to joint degeneration in osteoarthritis, a debilitating disease which affects over 40 million people in the Unites States alone [8]. Finally, a better understanding of the aqueous lubrication mechanisms by mucins may lead to advances in technical lubrication settings that are not suited for the use of oil-based lubricants.

2. Background

2.1. Mucin structure

Mucins are large (0.5–20 MDa), highly glycosylated proteins [4,9] whose biophysical properties are due primarily to the highly hydrated oligosaccharide side-chains that make up 50%–90% of the molecular weight. These oligosaccharide side-chains are about 5–15 monomers long and are O-linked to the serine and threonine residues of the protein core with a sufficiently high density that they are forced, by steric interactions, to stretch away from the central protein core in a "bottlebrush" configuration (Fig. 1a). These oligosaccharide side-chains are most commonly composed of N-acetyl glucosamine, N-

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acetyl galactosamine, galactose, fucose, and sialic acid [4], and other carbohydrate residues have also been detected [10•]. Sialic acid residues are negatively charged and, along with sulfate groups, generally give mucins a net negative charge. Globular regions at the amino and carboxy terminals, and sometimes interspersed within the protein, tend to be hydrophobic. Because they contain hydrophilic (glycosylated) and hydrophobic (unglycosylated) domains (*i.e.*, an ampholytic, block-copolymer structure), mucins are able to adhere strongly to a wide range of surfaces by hydrogen bonding, hydrophobic interactions, and by electrostatic interactions [6•,11,12]. This ability to bind to a range of substrate surface chemistries has been shown to be important for the function of mucins as boundary lubricants and wear protectants.

Mucins adopt an extended linear conformation due to steric repulsion between hydrated carbohydrate groups and between carbohydrates and the peptide backbone [13]. High local osmotic pressure, generated by counterions drawn to negatively charged moieties associated with the sugars, further contributes to the extended conformation. Mucin conformation can be observed by atomic force microscopy (AFM); for example, ocular mucins have an extended conformation with a persistence length of about 36 nm and contour lengths ranging from 200 nm to 1500 nm (Fig. 1c) [14]. Nonetheless, mucins tend to have a sufficient flexibility to form loops and hairpin turns [14,15]. Similarly, electron microscopy of epithelial mucins (cervical, gastric, and bronchial) revealed a linear conformation with some loops and hairpin turns and contour lengths ranging from 100 nm to 5000 nm [15].

Most mucins occur naturally in a sufficiently high concentration so that they can aggregate to form viscous gels by means of molecular entanglement and hydrophobic interactions [6•]. This has been studied by solution rheology, where a recent study on purified porcine gastric mucin identified a concentration-dependent viscosity (η) transition from a dilute regime ($c \sim \eta^{1/2}$) to an entangled regime ($c \sim \eta^{3/2}$) [10•]. Furthermore, the globular regions of mucins often contain cysteine groups that are involved in inter- and intramolecular disulfide bonding [6•]. While membrane-bound mucins tend to be monomeric [16], secreted mucins, which play a more prominent role in lubrication, are typically homo-oligomers of mucin subunits arranged into flexible linear chains (Fig. 1b) [4].

A number of proteins, though not classified as mucins, also contain heavily glycosylated mucinous domains which endow them with molecular properties similar to those of mucins [17]. In the context of lubrication, two important mucinous glycoproteins are lubricin and surface zone protein (SZP) [18,19], products of the gene proteoglycan 4. Lubricin (Mw ~228 kDa) is expressed by synoviocytes [20] and has a concentration of about 200 μg/ml in synovial fluid [21]. It is post-translationally modified with β(1–3) Gal-GalNAc incompletely capped with sialic acid [22]. Superficial zone protein [23] (Mw~345 kDa) is specifically synthesized by chondrocytes in the superficial zone of articular cartilage [24]. Here we refer to lubricin and SZP collectively as PRG4, unless specifically indicated. Like mucins, PRG4 has globular amino and carboxy terminal regions separated by a large, heavily glycosylated central domain. Carbohydrates make up approximately 50% of the total molecular weight [5] The semiflexible, extended conformation is similar to that of mucins, with a contour length of approximately 220 nm [19,25••] and with some propensity for kinks in the central region (Fig. 1d) [19]. The globular terminal regions of PRG4 are, however, smaller than those of mucins. Also, while mucins are typically negatively charged, the overall charge on PRG4 is slightly positive at physiological pH due to a large

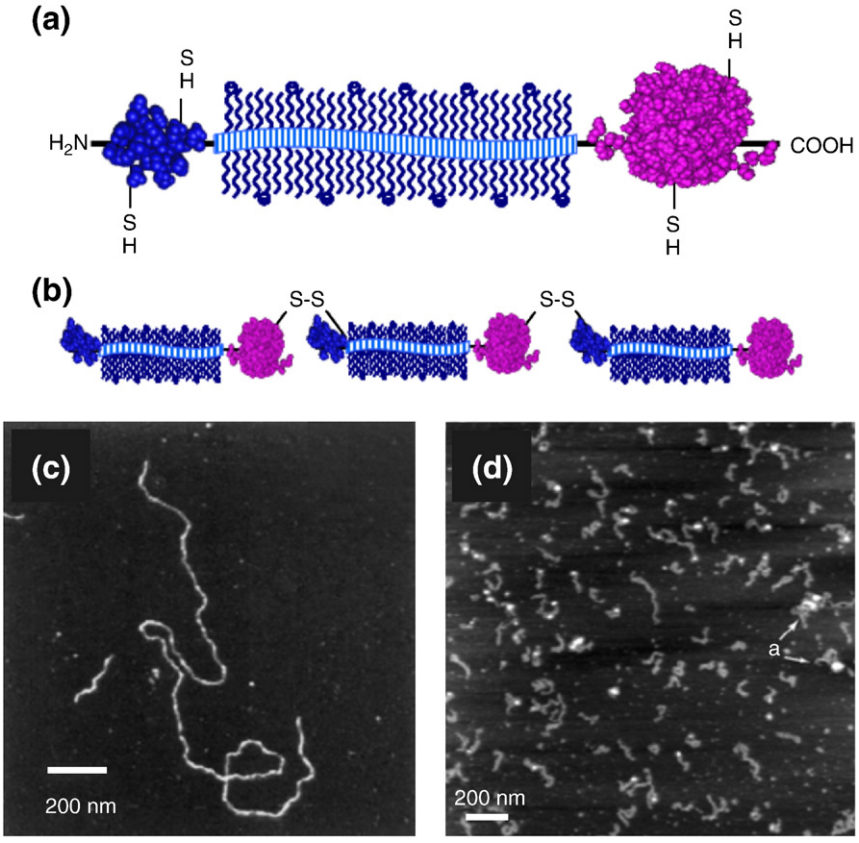

Fig. 1. Structure of (a) a typical mucin subunit showing a central linear glycosylated domain flanked by globular domains and (b) a mucin molecule composed of subunits linked linearly by disulfide bonds. AFM high resolution images showing (c) isolated occular mucin and (d) PRG4. Fig. 1c and d adapted with permission from [45] and [28•], respectively.