Proteolytic digestion of bovine submaxillary mucin (BSM) and its impacts on adsorption and lubricity at a hydrophobic surface

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Abstract

Mucin is the major constituent of the mucous secretions that cover epithelial surfaces exposed to the environment in human/animals. The primary function of mucous gels is known to provide protection to epithelial surfaces from invasive microbes and physical insults by forming a lubricating layer. About 50 to 80% of the molecular weight of mucin is attributed from post-translational N- and O-linked glycosidic modifications. The ability of the glycosidic modifications to retain water at the epithelial surface facilitates lubrication. N- and C-terminal interactions and also entanglement of the glycosidic modifications lead to a viscoelastic material that offers excellent lubrication properties [1-4]. Since mucins are composed of various biochemical functional moieties, it is expected that biochemical treatments of mucins lead to alteration in the structure, conformation, capabilities to adsorb onto engineering material surfaces, and consequently lubricity. In this study, proteolytic digestion on bovine submaxillary mucin (BSM) and its impacts on the size, structure, surface adsorption, and lubricating properties were studied. Two proteases with distinctly different cleavage specificities, namely trypsin and pepsin, were employed. SDS-PAGE analysis using two staining methods showed that only the unglycosylated terminal regions of BSM were degraded by the proteases and the central, glycosylated regions remain nearly unaffected. Size exclusion chromatography (SEC) and dynamic light scattering (DLS) studies indicated that tryptic digestion mainly led to the reduction in size, whereas pepsin digestion rather led to the increase in size of BSM. Less complete cleavage of terminal peptides by pepsin and subsequent aggregation between BSMs were thought to be responsible for the increased size. Far-UV circular dichroism (CD) spectra of the protease-treated BSMs showed a slight change in the secondary structure owing to the removal of terminal domains, but the overall random coil structural conformation by the central glycosylated regions remained dominant and essentially unchanged. Surface adsorption properties as characterized by optical waveguide lightmode spectroscopy (OWLS) showed that tryptic and pepsin digestion of BSM resulted in a decrease and a slight increase in the adsorbed mass onto a hydrophobic surface (polydimethylsiloxane (PDMS)), respectively, compared to intact BSM. This is related to the partial preservation of peptide residues after pepsin digestion as confirmed by SEC and DLS studies. Despite a contrast in the adsorbed amount of the protease-treated BSMs onto the PDMS surface, both proteases substantially deteriorated the lubricating capabilities of BSM at the self-mated sliding interface of PDMS. The present study supports the notion that the terminal domains of BSM are critical to the adsorption and lubricating properties of BSM at hydrophobic interfaces.

References

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