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Investigation of the Thermostability of Bovine Submaxillary Mucin (BSM) and Its Impact on Lubrication

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Abstract

Bovine Submaxillary Mucin (BSM) generates thin film layers via spontaneous adsorption onto hydrophobic surfaces such as Poly(dimethylsiloxane) (PDMS) and High Density Polyethylene (HDPE). A characteristic feature of mucin is its tribological- or lubricating properties. Circular dichroismspectroscopyrevealed that BSM is thermally stable over a wide range of temperatures (5–85 °C) in its conformation, and Pin-on-Disk tribometry at low speeds showed negligible influence on lubricating properties. Employing the Mini Traction Machine, BSM was found to retain comparable lubricating properties after heating to 80°C and subsequent cooling. Random coiled secondary- and lack of tertiary structure in BSM is believed to contribute to the heat tolerance observed with regards to its conformational and lubrication properties.

Selection and peer-review under responsibility of Asia-Pacific Chemical, Biological & Environmental Engineering Society.

Keywords:Bovine Submaxillary Mucin,Biolubrication,Surface Coating, Biotribology

1. Introduction

Mucin has previously been proposed as a source of biocompatible surface coatings [1-5]. The “slipperiness” of mucins makes themunique candidates for coating of polymeric biomaterials. Formation of mucin films on the surfaces increases the surface hydrophilicity of the materials, and in turn acts as a lubricant between contacting surfaces due to the facile retainment of water at the surface. A majority of mucin coating studies for biomaterials have been carried out on hydrophobic, polymeric surfaces [1-8]. The

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amphiphiliccharacteristics of mucin are known to cause it to spontaneously adsorb onto hydrophobic surfaces in aqueous solutions. The general understanding (Figure 1) of mucin is that it interacts with surfaces through the hydrophobic N- and C-terminals while the heavily glycosylated domains in the central region of the polypeptide protrude into the aqueous solution [9-10].

In this work, we have investigated the thermostability of Bovine Submaxillary Mucin (BSM) and its impact on the conformational changes and tribological properties. Circular dichroism spectroscopy was employed to monitor temperature-induced changes in the secondary and tertiary structure of the protein. The tribological properties of BSM at slow speeds were investigated using Pin-on-Disk tribometry while a Mini Traction Machine (MTM) was used to monitor changes in friction coefficient over a varying speed range and stepwise temperature increase.

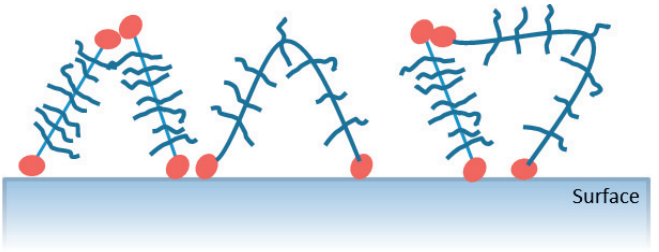

Figure 1.A schematic representation of the structure and conformation of mucin molecules adsorbed on a hydrophobic surface in an aqueous solution.

2. Materials and Methods

2.1. Bovine Submaxillary Mucin (BSM)

Bovine Submaxillary Mucin (BSM) was purchased from Sigma Aldrich (M3895-1G, type I-S, lot 039K7003V, St. Louis, MO) and dissolved in 10 mM phosphate buffer (pH 7.4) to a final concentration of 1 mg/mL.

2.2. Circular Dichroism (CD) Spectroscopy

Circular dichroism (CD) spectra of BSM dissolved in 10 mM phosphate buffer were obtained in a cylindrical quartz cuvette with 10 mm path length (Hellma GmbH & Co. KG, Müllheim, Germany) using Chirascan spectrophotometer (Applied Photophysics Ltd., Surrey, UK). Temperature was set using a CS/PCS Single Cell Peltier Temperature Controller (Applied Photophysics Ltd., Surrey, UK) and the temperature scan programmed using the Chirascan software. The far-UV spectra were recorded from 280 to 195 nm and near-UV spectra from 300 to 230 nm with step size of 2 nm and bandwidth of 1 nm. Temperature was set to 5°C in the beginning of the measurements and the sample was let to equilibrate in this temperature for 10 min. After this the temperature was increased with 1°C /min up to 85°C and again, down to 5°C. Spectra were recorded on every second temperature step. CD signal of the buffer background was subtracted from the data.

2.3. Pin-on-Disk(PoD) Tribometry

The lubricating behavior of BSM at low speed was investigated using a Pin-on-Disktribometer (CSM, Peseux, Switzerland). The approach used in this study relies on a loaded pin forming a contact with a disk. The sliding friction forces between them were measured at a controlled rotation speed of the disk at 5 mm/s,

while the pin remained stationary. The load on the pin was controlled by deadweight (5 N) while disk rotationwas enabled by a motor beneath the disk. Friction generated during sliding contacts was monitored by a strain gauge. Friction forces data were acquired over 20 rotations at a fixed radius of 5 mm and plotted for each rotation.The PDMS tribopair used in this study was cast by thorough mixing the base fluid and crosslinker of Sylgard 184 elastomer kit (Dow Corning, Midland, MI, USA) at a ratio of 10:1. Gentle vacuum was applied to remove any air bubbles generated during mixing. Disks were cast in a machined aluminum plate with flat wells designed to the dimensions (30 mm diameter × 5 mm thickness) of the tribometer. A 96 microwell plate (NUNCLON Delta Surface, Roskilde, Denmark) was used to cast the pin. The PDMS mixtures were then cured at 70°C overnight [11].BSM samples were heated in a water bath to 37- or 80°C and maintained at the desired temperature for 1 minute before cooling to room temperature. An additional sample was heated to 80°C for 30 minutes before cooling.

2.4. Mini Traction Machine (MTM)

A Mini Traction Machine (MTM, PCS Instruments, London, U.K.) was employed to characterize the lubricating properties of BSM during stepwise temperature increase and decrease in a mixed sliding/rolling contact regime. Unlike PoD, MTM provides a mixed sliding/rolling contact through independent speed control of the ball and disk. The slide/roll ratio (SRR) is defined as the percentage ratio between the difference of the mean of the ball velocity and the disk velocity. SRR=0% represents pure rolling contact while SRR=200% represents a pure sliding contact.

All measurements were performed with a fixed load (5N) and varying temperature. A HDPE ball and disk pair (Precision Plastic Ball Co, Franklin, IL) was used. SRR=10% was used in all measurements while speed was varied between 10 mm/s and 1000 mm/s.

3. Results and Discussion

3.1. Circular Dichroism Spectroscopy

CD spectroscopy measurements were carried out to monitor any secondary- and tertiary conformational changes of BSM that occurduring heating and subsequent cooling in the temperature range from 5°C to 85°C. The far-UV spectra (Figure 2A) revealed that the majority of the secondary structure of BSM appears to be random coils. As the vast majority of the polypeptide backbone is heavily glycosylated, the results confirm the current understanding of the overall structure of mucin [9-10]. If any secondary structure is present in the N- and C-terminal regions, the signal would be proportionately low compared to the signal from the rest of the structure. It was therefore not possible to determine whether there are any α-helices or β-sheets present in the terminal regions of the macromolecule. The temperature scan shows that the slight changes observed in CD spectra during heating, as probed byellipticity of anegativelocal minimum band (200 nm) and a positivelocalmaximum band (220 nm),disappears upon subsequent cooling, suggesting that heat induced conformational change of BSM is a reversible process in terms of conformational change (Figure 2B). However, whether refolding occurred or new random coil structures were formed was not possible to determine.The full near-UV spectrum (data not shown) was measured, and no structural signal was detected. Focusing on the lowest wavelengths of the near-UV spectrum revealed a negative band at 240 nm that could originate from disulfide linkages (Figure 2C) [12]. The band change was found to be fully reversible after heating to 85 °C (Figure 2D), and subsequent cooling to 5 °C.

3.2. Pin-on-Disk Tribometry

Lubrication in aqueous environments is one of the characteristic features of mucins. PoD was employedto investigate the lubricating properties of BSM after heat treatment at low speeds. The samples were heatedto physiological conditions at 37°Cor high temperature at 80°C, respectively, and equilibrated at the temperatures for 1 minute to emulate the conditions during the CD measurements. The samples were subsequently removed and allowed to cool to room temperature. To test the effect of extended exposure to heat, one sample was left at 80°C for 30 minutes before cooling. The measurements(Figure 3) showed that heating does not impact the lubrication properties of BSM as the sample 80 °C sample showed similar reduction of friction in comparison to PBS buffer only and the 37 °C sample. Prolonged exposure to heat did not increase the friction coefficient significantly either as no significant difference in friction coefficient was observed when comparing the samples heated to 80°C.This indicated that prolonged exposure to heat did not induce protein degradation or reduction of the molecular function of BSM

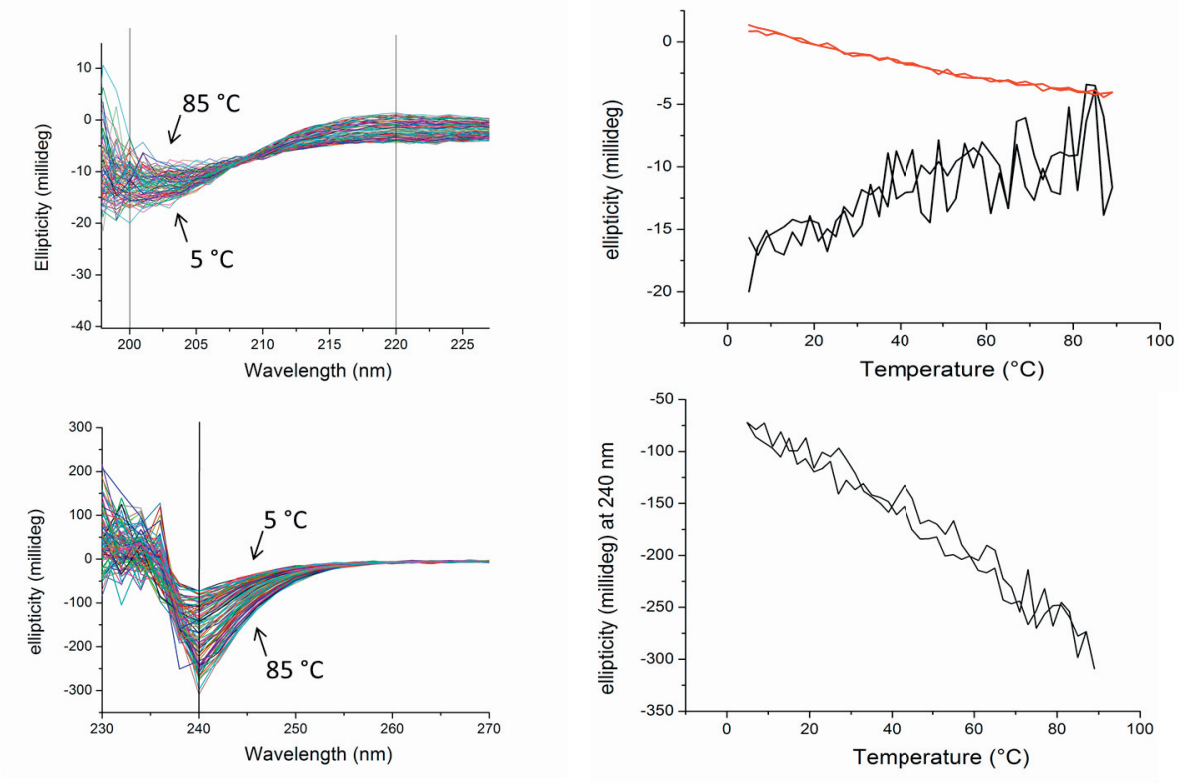

Figure 2. A. Far-UV spectra of BSM measured with 2C temperature step between 5°C-85°C and 85°C -5°C. B. Structural change of BSM monitored by change in CD signal at two wavelengths, 200 nm (black) and 220 nm (red) as a function of temperature. C. Low wavelength range near-UV spectra of BSM measured with 2 °Ctemperature steps between 5°C-85°C and 85°C -5°C. D. Structural change of BSM monitored by change in CD signal at 240 nm as a function of temperature.

3.3. Mini Traction Machine

The ability of BSM to lubricate over a range of temperatures was investigated by heating at intervals between 5°C and 80°C. Unlike PoD, the temperature was held constant during friction measurement. Upon reaching 80°C, the BSM solution was allowed to cool and the lubrication properties were monitored at the

same steps going down to 5°C (Figure 4). Common for all measurements in Figure 4 was that at low speeds the coefficient of friction was higher and subsequently decreased as rolling speed was increased. During temperature decrease, the friction coefficients measured were similar to those measured during temperature increase. This indicatesthat the lubricating properties of BSM were intactduring the process of heating and subsequent cooling. This was in agreement with the results obtained for both CD and PoD.

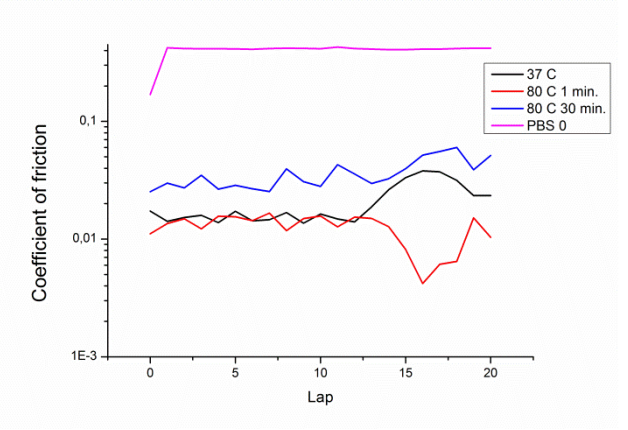

Figure 3. Coefficient of friction of BSM solutionplotted as a function of laps measured by Pin-on-Disk tribometer between PDMS surfaces. The load was 5 N with a sliding speed of 5 mm/s for 20 laps corresponding to a distance of 0.63 m.

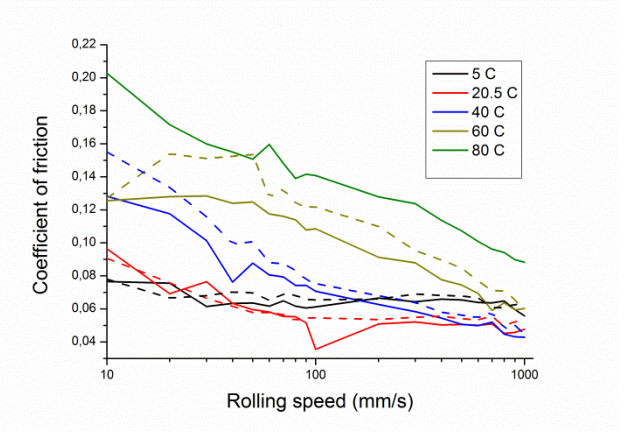

Figure 4. Coefficient of friction of BSM sample during stepwise temperatureincrease and decrease plotted against rolling speed. Measurements during increasing temperature steps are indicated by solid lines while decreasing temperature steps are dashed.

4. Conclusions

In this work, the thermostability of BSM was investigated. CD spectroscopy indicated BSM to have an overall randomly coiled secondary structure while no clear tertiary structure was seen. When heated up to 85 °C, BSM structure was found to undergo minor changes. These changes were fully reversible when the temperature was lowered again. BSM is able to retain its lubricating properties at low speeds after heating to

high temperature and cooling to room temperature. This was shown by Pin-on-Disk tribometry. Finally, MTM results showed that the lubricity of BSM is not reduced after heating at high speeds. Friction coefficient values obtained at specific temperatures were similar for measurements before and after heating to 80°C. The data presented here indicate that the overall lack of secondary- and tertiary structure in BSMare likely to contribute to the heat tolerance observed with regards to its lubrication properties.

Acknowledgements

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