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Determination of Polydispersity of a Human Colonic Mucus Glycoprotein Using Rate-Zonal Centrifugation and Laser Light-Scattering

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Abstract: The colonic epithelium is coated by a visco-elastic gel, mucus that lubricates and protects the mucosa from the external environment. Mucus glycoproteins (mucins, MUCs) are major macromolecular components with MUC2 representing the major gel-forming secretory mucin. Previously we purified MUC2 from a cell culture environment with either three rounds of isopicnic centrifugation or gel filtration chromatography followed by a single round of centrifugation. In the present work, using rate-zonal centrifugation in a gradient of guanidinium chloride we fractionated this purified MUC2 and its subunits. Analyses of the fractions showed that the unreduced MUC2 molecules are quite large and polydisperse in molecular mass. Reduction of disulphide bonds cleaved the large polydisperse molecules into smaller and more uniformly sedimenting species. Individual fractions from both the unreduced and reduced MUC2 molecules were then subjected to laser light-scattering performed as absolute intensity measurements to obtain average molecular mass (M_r) and radius of gyration (R_G). The M_r for the unreduced and reduced MUC2 was 30×10^6 and 16×10^6 and R_G was in the range 290-309 nm and 200-260 nm, respectively. We concluded that colonic MUC2 mucin can be described as polydisperse and the combination of rate-zonal centrifugation and laser light-scattering techniques could certainly be applied as an effective method to determine their polydispersity.

Key Words: Polydispersity, colonic mucins, rate-zonal centrifugation, light-scattering

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