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SHORT COMMUNICATION

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Sodium dodecyl sulfate agarose gel electropheresis and electroelution of high molecular weight human salivary mucin

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Abstract The aim of this study was to test the applicability of sodium dodecyl sulfate (SDS) agarose gel electrophoresis, electroelution and electrophoretic filtration as methods of separation, detection, and purification of high molecular weight human salivary mucin. SDS agarose gel electrophoresis of whole saliva and mucin prepared by density gradient ultracentrifugation revealed bands with molecular weights in excess of 450 kDa and between 1.6×10^6 and 2×10^6 Da. Electroelution of material from gel and subsequent electrophoresis resulted in highly purified high molecular weight material. Electrophoretic filtration, a method of collecting the material remaining from whole saliva in the slot after penetration of low molecular weight constituents into SDS polyacrylamide gel, failed to produce pure high molecular weight material. It is concluded that SDS agarose gel electrophoresis and electroelution are suitable methods for studying high molecular weight salivary mucin glycoproteins.

Keywords Saliva · Mucin · Electrophoresis · Electroelution · Agarose

Introduction

High molecular weight (MW) salivary mucins (Smuc) have been attributed several important roles in the oral environment in health and disease which are related, in particular, to tissue coating and interactions with the oral flora [12]. MW of mucins may be estimated by means of none-lectrophoretic techniques such as, for instance, light scattering and has been shown to range between one and a few million daltons [1]. However, for various reasons and purposes, routine laboratory use of electrophoretic techniques is more convenient and preferable. Handling of glycoproteins with the MW in excess of 10^6 Da and especially their purification are, however, difficult and usually require multiple step procedures without the certainty of producing adequately pure material. Standard techniques such as, for instance, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) do not easily lend themselves to studying Smuc because electrophoretic mobility of molecules with an MW exceeding 800 kDa is inhibited by the insufficiently large pore size of polyacrylamide gels [9]. In experimental work on high-MW Smuc, these difficulties are usually contravened by using material prepared under dissociative and reducing conditions. This approach may help obtain pure monomeric moieties but renders them unsuitable for functional studies, which require the preparation of intact molecules [14].

Agarose gels have a pore size of up to 150 nm and may be used in SDS agarose gel electrophoresis (SDS-AGE), facilitating mobility and separation of molecules with an MW of several million daltons [11]. Lack of commercially available suitable MW markers is a potential shortcoming of this method, which, however, might be compensated by application of purpose-made MW markers containing well-characterized high-MW molecules, such as, for instance, von Willebrand factor (vWF). This protein with the monomer MW of 450 kDa forms oligomers, which may be revealed using SDS-AGE [4]. In addition, calibration of vWF MW standards may be aided using commercially available bovine submaxillary mucin (Bmuc) with a known MW of 400 kDa. Furthermore, SDS-AGE might lend itself as a convenient method of preparative isolation of high-MW Smuc by means of excision and electroelution of the corresponding bands after electrophoretic separation of the material of interest.

Ultracentrifugation and gel filtration chromatography are common preparative techniques for high-MW Smuc [2, 5, 8, 13, 14]. The considerably higher density of highly glycosylated Smuc facilitates its separation from other proteins during centrifugation without differentiation on the basis of MW. Application of gel filtration chromatography, which separates molecule on the basis of their size,

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is difficult because of column blockage frequently caused by high viscosity and stickiness of Smuc. Electrophoretic filtration by means of SDS-PAGE [10] might potentially serve as a convenient method of isolation of Smuc taking advantage of its very high MW. While preventing migration into polyacrylamide gel, this feature of Smuc might facilitate its collection from gel surface in the slot, which would act as molecular filter after electrophoretic migration of all low-MW salivary constituents into the gel.

The aim of the present work was to test the applicability of SDS-AGE for separation and detection of high-MW human Smuc. To assist this aim, a combination of purpose-made MW markers was used with the intention of increasing the range of detectable MW. In addition, electroelution and electrophoretic filtration were tested as potential novel means of isolation and purification of Smuc.

Materials and methods

Dialysis membrane tubes were obtained from VWR International (Darmstadt, Germany). All chemicals were of reagent grade and, if not indicated otherwise, were purchased from Sigma (Deisenhofen, Germany). Bmuc (MW 400 kDa) and vWF were bought from Sigma and Roche Diagnostics (Mannheim, Germany), respectively. Seakem HGT(P), a low-endosmosis agarose, was obtained from FMC BioProducts (Rockland, ME, USA).

Unstimulated whole human saliva (WHS) was collected in the presence of protease inhibitors and sodium azide, clarified by centrifugation, and subjected to CsCl density gradient ultracentrifugation. The material from fractions with the density between 1.52 and 1.40 g/cm^3 corresponding to mucins [13] was recovered and desalted by centrifugal filtration (maxi centrifuge filter 100 kDa MW cutoff, Nalge Nunc International, Rochester, NY, USA).

The buffer (40 mM Tris–HCl, 0.1% SDS, 0.1% EDTA, pH 7.4) used for gel preparation and as electrode buffer was prepared as described elsewhere [6] and supplemented with 4 M urea. For the 3% agarose gels as used in the present work, a 4-M urea concentration was chosen on the basis of preliminary trials which had shown that lower urea content inhibited the resolution of vWF, while higher concentration prevented agarose solution from gelling. The gel thickness was approximately 3 mm, and electrophoresis was carried out in a horizontal gel chamber (Easy-Cast, AGS, Heidelberg, Germany), which was cooled to 4°C. Constant current mode (30 mA) was applied in order to avoid heating and melting of the gel. Approximately 20 μg of each protein sample was applied in the running buffer, which was supplemented with 25% glycerol. The duration of a typical run was 3 h. Detection of proteins was carried out by Coomassie staining. The periodic acid-Schiff method [3] was used after electroblotting onto nitrocellulose membrane (Perbio, Bonn, Germany) to confirm the glycoprotein nature of the corresponding bands (not shown).

For electroelution of high-MW material obtained from SDS-AGE of clarified WHS, a purpose-made comb was used during gel preparation, producing a single slot of the size of 60×5×2 mm. After a 3-h run, a narrow slice of the SDS-AGE gel was subjected separately to Coomassie staining for detection of the band of interest, which was then excised from the gel and subjected for 3 h to electroelution in a dialysis membrane tube. Subsequently eluted material was concentrated by centrifugal filtration.

Electrophoretic filtration [10] of clarified WHS was accomplished in a 7.5% polyacrylamide gel under standard SDS-PAGE conditions [7], without the use of mercaptoethanol. Gels of 5-mm thickness were prepared as above. After a 3-h duration, SDS-PAGE was stopped, and the polarity of the power supply was inverted for 30 min. The content of the slot was removed; the slot was repeatedly rinsed with buffer and pooled material concentrated by centrifugal filtration. Samples of material obtained from electroelution and electrophoretic filtration and vWF and Bmuc were subsequently applied to SDS-AGE.

Results and discussion

Figure 1 shows a typical gel obtained from SDS-AGE of Bmuc, vWF, Smuc, and WHS. There was a satisfactory resolution of the multimeric composition of vWF as shown by the several bands in lane 2, representing the 450-kDa monomer and its oligomers increasing in size in increments of the MW of the monomer [4]. In lane 1, the distinct band of Bmuc of 400 kDa served as an additional marker for the identification of the vWF monomer. In the absence of commercially available appropriate MW markers, both vWF and Bmuc were used in the present study as purpose-made MW markers in order to ensure a reliable recognition of bands with an MW exceeding 10^6 Da.

Lane 4 shows the SDS-AGE separation of two components within Smuc as obtained from density gradient centrifugation. Compared with the vWF standard, the MW of the band with the lowest electrophoretic mobility represents Smuc constituents with an approximate MW of

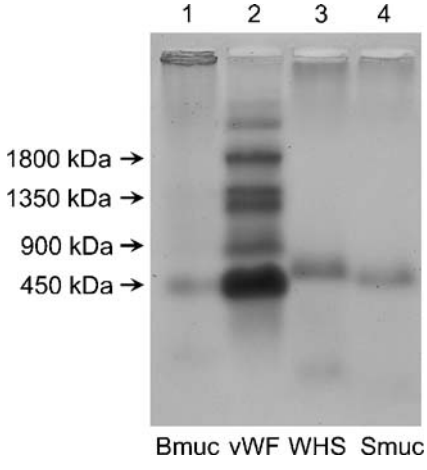

Fig. 1 SDS agarose gel electrophoresis and Coomassie staining of bovine submaxillary mucin (Bmuc, *lane 1*), von Willebrand factor (vWF, *lane 2*), whole human saliva (WHS, *lane 3*), and high-MW human salivary mucin as prepared by density gradient centrifugation (Smuc, *lane 4*)

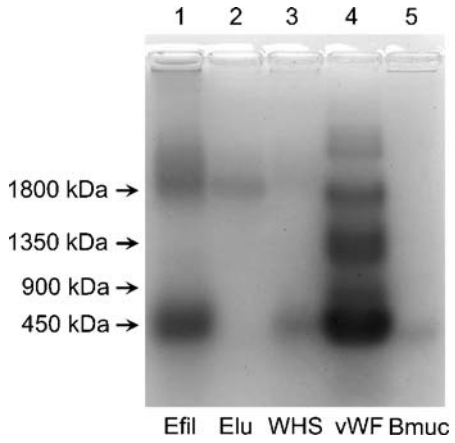

Fig. 2 SDS agarose gel electrophoresis and Coomassie staining of bovine submaxillary mucin (Bmuc, *lane 5*), von Willebrand factor (vWF, *lane 4*), whole human saliva (WHS, *lane 3*), material recovered by electroelution of the high-MW band of SDS agarose electrophoresis of whole saliva (Elu, *lane 2*), and material recovered by electrophoretic filtration of whole saliva on SDS polyacrylamide gel (Efil, *lane 1*)

2×10^6 Da. According to its electrophoretic mobility, the apparent MW of the second band is in excess of 450 kDa. WHS (lane 3) contains two bands identical to those of Smuc and additional material with lower MW, which, however, cannot be estimated by means of the MW markers used.

The effect of SDS in electrophoretic protein separation is based on the masking of the individual protein charge, which is done by covering the entire molecule with a layer of negatively charged alkylsulfate molecules. Nonglycosylated and scarcely glycosylated proteins exhibit a marked relationship between their MW and electrophoretic mobility. On the other hand, sugar moieties of glycoproteins bind less SDS than the polypeptide, which may cause different electrophoretic mobility compared with that of nonglycosylated proteins [9]. Therefore, only highly glycosylated proteins such as Bmuc and vWF were used in this work to prevent an inappropriate comparison between the apparent MW of glycosylated proteins and that of nonglycosylated proteins.

Figure 2 shows the SDS-AGE separation of WHS and the material obtained from electroelution and electrophoretic filtration. The material recovered by electroelution of the high-MW band of SDS-AGE of WHS is in lane 2. There was only one broad band with the apparent MW ranging between 1.6×10^6 and 2×10^6 Da, confirming previous assumptions of the very high MW of Smuc [12]. As shown by the presence of two broad bands in lane 1, which contains material obtained by means of electrophoretic filtration of WHS, there was no definite separation of low-MW salivary constituents from high-MW Smuc during SDS-PAGE of WHS, which was accomplished as part of electrophoretic filtration. The most likely explanation of this finding is the occurrence of the so-called heterotypic complexing between Smuc and other salivary constituents [12], preventing penetration of low-MW constituents of WHS into the gel during SDS-PAGE. Apparently these low-MW constituents were retained in the SDS-PAGE slot as parts of high-MW aggregates and were subsequently separated by SDS-AGE (Fig. 2, lane 1). On the basis of this finding, electrophoretic filtration appears unsuitable as a method of isolation and purification of unreduced Smuc.

It may be concluded from these observations that SDS-AGE and electroelution are suitable techniques for direct separation and visualization of unreduced high-MW Smuc. These methods provide a novel approach to MW determination and purification of Smuc and possibly other glycoproteins with very high MW.

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