A SIMPLIFIED METHOD OF DETERMINING SYNOVIAL FLUID CHONDROITIN SULFATE CHAIN LENGTH

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Introduction: The concentration, isomer composition, and immunoreactivity of glycosaminoglycans (GAGs), such as chondroitin sulfate (CS), have been widely studied as biomarkers to monitor changes in the metabolism of joints. We have shown previously, by sequential use of gel filtration chromatography (Superose 6) and fluorophore-assisted carbohydrate electrophoresis (FACE), that CS chains in synovial fluid of injured joints are significantly longer than those in normal joints. FACE is a technique that requires specialized laboratory equipment and a high level of technical expertise. Additionally, FACE technique is much more expensive in terms of materials and time per sample. The aim of the present study was to determine whether dimethylmethylene blue (DMMB) analysis could be done instead of FACE to determine CS chain length in synovial fluid.

Methods: Synovial fluid was obtained from carpal joints of: (1) 8 normal Thoroughbred horses after 8 weeks rest, (2) the same horses after 9 months treadmill training, and (3) 7 Thoroughbred horses with osteochondral (OC) injury from racing. The protocol was approved by the Institutional Animal Care and Use Committee, Superose 6 chromatography and FACE analysis had previously been performed on these samples. Aliquots (250 µl) of the same synovial fluids were digested in proteinase K, filtered, and then eluted on a Superose 6 column. Column effluent was collected in 1 ml fractions. Fractions 1 through 13 were discarded; fractions 14 through 23 were collected and analyzed. Our previous work has shown that the sulfated GAG contained in these fractions is chondroitin sulfate. Fractions were vacuum dried and reconstituted in 100 µl of water. DMMB was performed by modification of the Blyscan® method (Biocolor, Accurate Chemical Supplies, Westbury, NY), adapted to a 384-well microplate technique. Blyscan® dye reagent (1 ml) was added to each sample, shaken for 30 min, then centrifuged (13,000 g) for 20 min. Supernatant was decanted with care not to disturb the precipitate. Blyscan® dissociation reagent was added (300 µl or 600 µl) and the sample vortexed. Standards and samples (100 µl aliquots) were pipetted into a 384-well microplate and absorbance was read at 650 nm. Data from each fraction of each sample was compared between the 2 assay methods by the Wilcoxon matched-pairs signed-ranks test, with P<0.05 considered significant.

Results: Sulfated GAG from synovial fluid was quantitated in µg/ml of fluid by DMMB, according to fragment size. Figure 1A shows synovial fluid CS content of the 3 groups as determined by DMMB. For comparison, Figure 1B shows total sulfated chondroitin disaccharide (Δdi6S and Δdi4S) for the same samples determined by FACE. For both methods, the CS peak occurs at fraction #18 in synovial fluid from joints with OC injury. Similarly, CS in synovial fluid from exercised joints peaks in fraction #19 with both assays. CS in synovial fluid from rested joints peaks in fraction #19 when determined by DMMB, but peaks in fraction #20 when measured by FACE. When µg/ml amounts of CS for each data point were compared between assays, results for exercised (n=80) and OC injury joints (n=70) were significantly different (P<0.0001), but results for rested joints (n=80) were not (P=0.92).

Conclusions: When used for quantitation of sulfated CS in synovial fluid (µg/ml), DMMB gave results that were significantly different than those from FACE. DMMB may be measuring other sGAGs in addition to CS. However, when performed after Superose 6 chromatography, DMMB assay appears to discriminate the longer CS chains found in synovial fluid from OC injured joints compared to the shorter chains seen in normal joints. Thus, for the specific purpose of determination of CS chain length, the more simple and less expensive DMMB assay may be a replacement for FACE assay. Use of a 384-well microplate allowed use of low volumes of DMMB reagents. This facilitated quantitation of the small amounts of CS in fractions collected from Superose 6 chromatography.


Figure 1. CS content (µg/ml±SD) in Superose 6 fractions (14-23) of synovial fluid from 8 normal Thoroughbred horses after 8 weeks rest (Rested), the same horses after 9 months treadmill training (Exercised), and 7 Thoroughbred horses with osteochondral (OC) injury from racing. A: DMMB assay of CS content. B: FACE assay of CS disaccharide (Δdi6S and Δdi4S) content. CS chain length decreases with increasing Superose 6 fraction number.

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