PRG4 EXCHANGE BETWEEN ARTICULAR CARTILAGE SURFACE AND SYNOVIAL FLUID

INTRODUCTION: The boundary lubrication function of articular cartilage is mediated in part by proteoglycan 4 (PRG4) molecules. PRG4 is secreted into synovial fluid (SF) by chondrocytes in the superficial zone of articular cartilage, synoviocytes, and meniscal cells. To provide boundary lubrication, these molecules must be adsorbed to the articular cartilage surface, where they have indeed been immunolocalized previously. Cartilage-on-cartilage sliding can modulate the surface PRG4 content in a manner dependent upon the bathing fluid, suggesting that articular surface-bound PRG4 molecules exchange with those in the bath. The mechanism by which PRG4 binds to the articular surface is unknown, but may be through covalent or non-covalent interactions with hyaluronan (HA) and lipids, or disulfide mediated self-aggregation. Thus we hypothesized that PRG4 binding at the articular surface depends on PRG4 concentrations of both the surrounding fluid ([PRG4]surf) and the articular surface itself ([PRG4]cart), and on PRG4 interactions with other molecules at the surface. The objectives of this study were to determine i) the effect of PRG4 exchange between articular cartilage surface and synovial fluid, ii) dissociation of native PRG4 from a bath. PRG4 preparation. Cartilage disks were incubated in DMEM, 10 ng/ml TGF-β3, 0.01% BSA, 25 µg/ml ascorbate (1 ml/(10^6 cells·day)) for 12 days. From the conditioned medium, PRG4 was purified by DEAE anion exchange chromatography and concentrated with a 100kDa MWCO (YM-100) filter. PRG4 purity was >90% as determined by SDS-PAGE. PRG4 quantification. The concentration of PRG4 per area of articular surface, [PRG4]cart was determined by guanidine extraction and ELISA with mAb 3-A-4, a gift from Dr. Bruce Caterson. (i) Association. Cartilage disks (n=4) with (S, 0-400µm depth) or without (M, 400-800µm) an intact articular surface were incubated for 72h at 4°C in PBS with added PRG4 (either purified or in SF) to generate solutions with PRG4 concentrations, [PRG4]surf of, 5, 50, 150, or 450 µg/ml. Upon termination, disks were washed 3x5 min in PBS, and [PRG4]cart was quantified. Other disks were incubated in either PBS or SF with [PRG4]surf of 100 µg/ml, and [PRG4]cart was visualized at the articular surface by en bloc immunohistochemistry (IHC) with mAb 3-A-4 and peroxidase-based detection, using isotype-matched IgG as a negative control. (ii) Dissociation/repletion. In some S cartilage disks (n=12), the initial (CTRL) [PRG4]cart was quantified immediately after harvest. Other S or M disks (n=4) were incubated for 24h in PBS, 2M NaCl, 1% SDS after wipe with 10% SDS swab, 10mM dithiothreitol + 55mM iodoacetamide (DTT+IAA), or 10U/ml Streptomyces Hyaluronidase (HAase) at 25ºC; or 72h in PBS with vigorous nutation (PBS+). All disks were then washed (6x10 min in PBS), incubated further with either PBS ([PRG4]surf of 0 µg/ml) or SF ([PRG4]surf of 122 µg/ml), washed again, and analyzed for [PRG4]cart. Repleted PRG4 was calculated as [PRG4]surf of SF-post-treated disks minus that of PBS-post-treated disks. Statistics. ANOVA was used to determine effects of cartilage zone, [PRG4]surf, and carrier fluid for association experiments; and pre-treatment for dissociation/repletion experiments.

RESULTS: Association. PRG4 associated strongly with S disks. [PRG4]cart was higher overall (p<0.001) in S (0.90±0.10 µg/cm²) than in M (0.17±0.05 µg/cm²) disks, in agreement with intense and specific IHC staining for PRG4 in S disks compared to M disks (Fig. 1A). PRG4 content in S disks (Fig. 1B) was similarly high under all bathing conditions, whether PRG4 in bath was purified or from SF. Dissociation/repletion. PRG4 was depleted from S disks by certain treatments, and repleted fully by subsequent bathing in SF. CTRL S disks had [PRG4]cart of 0.59±0.09 µg/cm², which was lowered after 24h treatment with HAase (by 99%) or SDS (by 72%), or 72h with PBS+ (by 52%) (Fig. 2). After subsequent bathing in SF, [PRG4]cart returned to CTRL levels for all groups. M disks treated similarly as controls contained little PRG4 (0.12±0.06 µg/cm²) after extraction, with little (0.12±0.06 µg/cm²) repletion by SF.

DISCUSSION: Taken together, these results suggest that PRG4 at the articular surface is normally bound quite strongly, but can exchange with the PRG4 in SF under certain conditions. The finding that [PRG4]cart of S cartilage remains high and does not depend on [PRG4]surf after static incubation for 72h (Fig. 1) suggests that native PRG4 does not readily exchange with that in the surrounding fluid (Fig. 3A). The finding that native PRG4 can be removed (Fig. 2,3B) and repleted with PRG4 from the bath (Fig. 2,3C) suggests a mechanism by which PRG4 molecules, secreted into the SF by many tissues surrounding the joint cavity, help to maintain lubrication function at the articular surface. Given the near-complete removal of native PRG4 by HAase and subsequent repletion by SF (which also contains HA), HA may facilitate PRG4 binding to the articular surface.


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Figure 1. Cartilage PRG4 immunostaining (A) and S disk content (B) after 72h in baths with PRG4, purified (pur) or from SF. Mean±SEM, n=4.

Figure 2. PRG4 of cartilage S disks after dissociation (m) and SF-mediated repletion (c). Mean±SEM, n=4-12. *p<0.057, **p<0.01.

Figure 3. Model of PRG4 exchange between articular cartilage and synovial fluid. Native PRG4 does not readily exchange with PRG4 in solution (A), but can, under certain chemical and mechanical conditions, be removed (B), and repleted (C).