ROLE OF ELECTROSTATIC INTERACTIONS IN THE LUBRICATION OF ARTICULAR CARTILAGE BY RECOMBINANT LUBRICIN

*Gleghorn, J P; **Jones, A R C; **Flannery, C R; +*Bonassar L J
*Cornell University, Ithaca, NY
**Wyeth Research, Cambridge, MA
+LB244@cornell.edu

INTRODUCTION

Lubricin/PRG4/SZP, a glycoprotein found in synovial fluid, has been shown to lubricate articular cartilage in boundary lubrication; however the mechanism of lubrication is not fully understood. A decrease in the friction coefficient (μ) of artificial bearings has been demonstrated with purified lubricin [1], showing the molecule can lubricate when in solution. When lubricin is coated on surfaces and not in solution, lubrication of cartilage is likewise observed [2]. Additionally, an unexpected increase in the equilibrium friction coefficient (μ_{eq}) has been reported at high doses of recombinant human lubricin (rh-lubricin) in a cartilage on glass system in boundary lubrication [3]. It is unclear how lubricin lubricates tissue and artificial surfaces, and if the mechanism is the same for both soluable and bound lubricin. Previous studies have established protocols for the removal of lubricin from the surface of cartilage by changes in the ionic environment [4]. The objective of this study was to investigate the relative contributions of soluble and bound fractions of lubricin by measuring μ_{eq} at multiple doses in a range of ionic strength solutions.

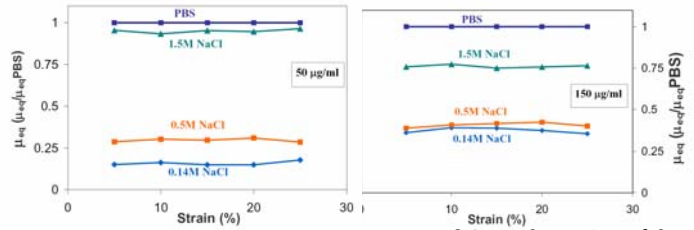
METHODS

Sample Preparation: Full thickness bovine osteochondral plugs 6mm in diameter were harvested from the patellofemoral groove of freshly slaughtered 1-3 day old calves. The cartilage was carefully cut with a scalpel from the bone producing a 2mm thick cartilage disc with the flat articular surface intact. Lubricin bound to the tissue was removed using a protocol described previously [4] in which the explants were incubated in 1.5M PBS for 10 minutes and then equilibrated in 138mM PBS for 1 hour.

Friction Testing: The μ_{eq} of cartilage discs was quantified using an established friction testing system [3]. Briefly, the instrument imposed a series of axial strains ranging from 5-25% on the cartilage cylinders while oscillating the tissue against a piece of glass at an entraining velocity of 0.32mm/sec to produce boundary lubrication. The equilibrium friction coefficient (μ_{eq}) of the system was calculated by measuring the normal and frictional shear loads with a custom biaxial load cell when the tissue has reached an equilibrium normal stress from the applied normal strain. Recombinant human lubricin at 50 μg/ml and 150 μg/ml was utilized as a lubricant, with the ionic strength adjusted by the addition of NaCl. The resulting lubricin baths of 0.14M, 0.5M, and 1.5M NaCl were utilized to test effect of electrostatic interactions on the ability of lubricin to lubricate cartilage in different ionic environments. In order to compare effects across the two rh-lubricin concentrations used, the data was normalized to the μ_{eq} of cartilage-glass using PBS as the lubricant.

RESULTS

For all conditions tested, μ_{eq} was independent of applied axial strain, confirming that μ_{eq} reflects the behavior of the tissue in boundary mode lubrication (Figs 1 and 2). Changes in ionic strength of the lubricin solution significantly altered the lubricating properties of lubricin at both applied doses. In 0.14 M NaCl, application of 50 μg/ml lubricin decreased μ_{eq} by 85% (Fig 1), with application 150 μg/ml lubricin decreased by only 68% ($p<0.05$). The lubricating effect of 50 μg/ml lubricin was completely removed by testing in 1.5M NaCl, but only partially inhibited by testing in 0.5M NaCl (Fig 1). In constrast, at a concentration of 150 μg/ml, lubricin retained some lubricating action, lowering μ_{eq} by 25%, but was not as effective as in 0.14M NaCl. Delivery of 150 μg/ml lubricin in 0.5M NaCl did not affect the lubricating ability of lubricin, lowering μ_{eq} by 65%, similar to that observed in 0.14M NaCl.



DISCUSSION

This study demonstrates that the ability of recombinant lubricin to lubricate cartilage is dependent on the ionic environment, suggesting that electrostatic interactions play a role in lubricin-mediated boundary lubrication of cartilage. Electrostatic interactions are known to regulate the binding of lubricin to cartilage [4] and may also influence aggregation of the molecule in solution. Both binding and aggregation of lubricin might be expected to affect the ability of the molecule to lubricate cartilage.

Previous studies have demonstrated that recombinant lubricin lowers μ_{eq} in a dose dependent manner, with maximal efficacy observed at a dose of 50 μg/ml [3]. At doses above 50 μg/ml, μ_{eq} increases, suggesting that the mechanism of lubrication may be different at these higher doses. In the current study, the lubricating effect of 50 μg/ml was completely abolished in 1.5M NaCl (Fig 1), a condition that removes bound lubricin from cartilage [4]. These data suggest that at doses at or below 50 μg/ml, the lubricating action is derived from lubricin that is bound to cartilage. In the presence of 0.5 M NaCl, there was a small, but significant loss of lubrication. Since this ionic strength does not remove lubricin from cartilage, the increase in μ_{eq} observed in this case may be related to other phenomena such as aggregation at the tissue surface.

At 150 μg/ml, the presence of 1.5 M NaCl inhibited the lubricating ability of lubricin only partially (Fig 2). This suggests that at this higher dose, lubricin acts via a mechanism that does not require binding to the cartilage surface. This is consistent with previous studies of lubrication of synthetic bearings, in which the concentration of purified lubricin required to lower μ_{eq} were approximately 200 μg/ml [1].

The data from the present study collectively point to two distinct mechanisms by which lubricin lubricates. The first mechanism involves lubricin that is bound to the surface of the tissue and the second involves lubricin in solution. Based on data from the current study and previous work [3], it appears that lubricin in solution does not lubricate as well as lubricin bound to the cartilage surface, based on observations that μ_{eq} at 50 μg/ml was approximately 50% that at 150 μg/ml.

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