

P008 The molecular basis of inter- α -inhibitor heavy chain transfer onto hyaluronan

**W. Tongsoongnoen¹, M.S. Rugg², A.C. Willis²,
E. Fries³, C.M. Milner¹, A.J. Day¹**

¹Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, ²Department of Biochemistry, University of Oxford. ³Department of Medical Biochemistry and Microbiology, Uppsala University.

The inflammation-associated protein TSG-6 can form covalent complexes with the heavy chains of inter- α -inhibitor (i.e. HC1 and HC2) and these (TSG-6-HC1 and TSG-6-HC2) can act as intermediates in the covalent transfer of HC onto hyaluronan. Hyaluronan decorated in this way (termed HC-HA), which forms in RA and OA synovial fluids, is more aggregated having altered mechanical and cell-binding properties compared to the unmodified polysaccharide.

Using single site mutants of recombinant full-length TSG-6 we have investigated the molecular basis of TSG-6-HC and HC-HA complex formation. Analysis of folded mutants has revealed that the hyaluronan-binding site present within the TSG-6-HC complex, necessary for hyaluronan transfer, is distinct from that previously characterised in the isolated Link module. Thus, it seems likely that a composite binding/transfer site is formed between part of the Link module hyaluronan-binding surface and another region of the TSG-6-HC complex (e.g. potentially within the HC).

Mg²⁺/Mn²⁺ are essential for the formation of both TSG-6-HC and HC-HA. We have identified the Mg²⁺ ion-binding site within the CUB module of TSG-6 and shown that this is involved in the formation of TSG-6-HC complexes.