

P015 Peptide inhibition of heparin-RANTES interactions
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Chemokine-GAG interactions play a key role in immune responses and stimulate leukocyte migration across the endothelium. The main structural domain for chemokine binding is characterised by the BBXB motif where B represents a basic amino acid. One of the best understood chemokines is CCL5 (RANTES); Lortat-Jacob's group (Vivès *et al.*, 2004) have shown that CCL5 binding to heparin is through the high affinity ⁴⁴RKNR⁴⁷ motif, but also a second lower affinity site, ⁵⁵KKWVR⁵⁹. The aim of this work was to create a series of peptides that contained either the individual binding sites or both in combination that would compete with CCL5 binding. We have developed a competitive binding assay for CCL5 using I¹²⁵-iodinated CCL5. Competitive binding is measured by a reduction of labelled CCL5 bound to heparin. A peptide containing both binding sites effectively competed with CCL5. Our dose response data generated a sigmoid curve, characteristic of single site competition. A scrambled version of this peptide was generated to preclude a simple charge effect; this peptide did not inhibit CCL5 binding. Peptides containing either of the binding sites alone was insufficient to compete with CCL5. We hypothesise that the second lower affinity binding site is required to stabilise binding of the principle BBXB motif of CCL5 to GAGs.