The binding of interleukin 2 to heparin revealed by a novel ELISA method.

Saloua Najjam 1, Roslyn V. Gibbs 2, Myrtle Y. Gordon 3 and Christopher C. Rider 1.

1Division of Biochemistry, Royal Holloway College, Egham, Surrey, 2School of Pharmacy and Biomedical Sciences, University of Portsmouth, Hampshire, and 3Department of Haematology, Royal Postgraduate Medical School, London, UK.

An increasing number of polypeptide growth factors and cytokines are now known to bind to heparin and heparan sulphate glycosaminoglycans. Well studied examples are members of the chemokine and fibroblast growth factor families. However other growth factors including pro-inflammatory and haematopoietic cytokines also bind to heparin [1-4]. Such interaction with glycosaminoglycans of the extracellular matrix and cell surface may be important in protecting cytokines against degradation, and in localising them close to their sites of secretion.

We have developed a novel ELISA approach for the investigation of heparin-cytokine interactions. For this heparin is covalently bound via its reducing terminus to a protein carrier, bovine serum albumin (BSA), using sodium cyanoborohydride. This coupling method is intended to ensure that the heparin chains remain accessible, for subsequent engagement in protein-binding interactions.

Antithrombin III (AT III) and fibroblast growth factor-2 (basic-FGF), both proteins with well characterised heparin-binding properties, bind to the heparin-BSA complex in our ELISA. In both cases, binding is a dose dependent and saturable over the 0-30ng range. Free heparin competes with binding of both proteins to the complex, with an EC50 value of around 500ng/ml. With AT III, we have found that an AT III-binding heparin fraction separated by affinity chromatography on immobilised AT III, is a strong competitor in our ELISA. Conversely the low affinity heparin fraction is a poor competitor. Thus our ELISA shows the anticipated specificity in heparin-protein interactions.

We have now shown that recombinant human interleukin 2, rIL-2, binds to the heparin complex (see Fig 1). The binding is dose dependent, but over a range of 0-100ng does not reach saturation. As with AT III and FGF-2, free heparin displaces rIL-2 from the complex, but with rIL-2 the EC50 for inhibition of binding is an order of magnitude higher, at around 7μg/ml. Thus it would appear that the affinity of IL-2 for heparin is lower than that of AT III and FGF-2.

By using various polysaccharides to compete with the immobilised heparin-BSA complex for binding to rIL-2, we are able to examine the specificity of the interaction. Thus far we have found that chondroitin sulphate fails to compete, whereas fucoidin competes strongly. These results therefore confirm and extend previous affinity chromatography studies which showed that rIL-2 binds fucoidin and heparin but not chondroitin sulphate [3].

This binding of IL-2 to heparin-like glycosaminoglycans is likely to maintain high concentrations of the cytokine at local tissue sites of release, thus enabling a paracrine mode of immune activation in vivo. Such localisation should be considered in the therapeutic exploitation of recombinant IL-2 in order to minimise the toxic effects which occur on systemic administration.

We gratefully acknowledge the financial support of the Leukaemia Research Fund.