Relative Binding Affinities of Recombinant Domain Mutants of the Human Polymeric Immunoglobulin Receptor (pIgR) for IgM.

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The Polymeric Immunoglobulin Receptor (pIgR) is the primary transport molecule of polymeric immunoglobulins (dimeric IgA and pentameric IgM) across epithelial cells. During this process pIgR binds (via the five homologous immunoglobulin-like domains of the ectodomain) to the polymeric immunoglobulins at the basolateral surface of epithelial cells. The complex of pIgR-antibody is internalised by endocytosis and sorted to the apical membrane surface. At the apical surface the receptor is cleaved at Arginine-585 and released bound to the polymeric immunoglobulin, now referred to as Secretory Component. This serves to protect the so-called secretory immunoglobulin against proteolytic cleavage. At the mucosal surface, these secretory immunoglobulins, principally IgA, act to prevent antigens, including microorganisms, from attaching to the mucosal surfaces and establishing infections (reviewed in 1). It is known that domain I is the primary domain involved in the interaction with polymeric immunoglobulins. pIgR domain I binding to IgA and IgM has been characterised by ELISA, and it has been stated that this domain is the major contributor to total molecule binding (2). The binding of all five ectodomains to IgM has now been characterised using evanescent wave biosensor analysis on Biacore. Recombinant human pIgR domain mutants were constructed by PCR amplification and cloning into bacterial expression vectors. The domain mutants were expressed in *Escherichia coli* BL21 (DE3). Mutants were refolded (*in vitro*) and purified to homogeneity and the binding was analysed using the BIAcore X system. Binding analysis was performed at varying flowrates and IgM concentrations and the association and dissociation binding rates and constants determined using Biaevaluation version 3.2. Primary analysis show a relationship between slower off rates (dissociation constants) and increasing number of domains.

1. Areoti B, Casanova J, Okamoto C, Cardone M, Pollack A, Tang K and Mostov K (1992) *Polymeric Immunoglobulin Receptor.* Int. Rev. Cytology 137 B: 157-168.

2. Bakos M, Widen SG and Goldblum RM (1994) *Expression and purification of biologically active domain I of the human pIgR.* Mol. Immunol. 31 (2): 165-168.