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MEETING REPORT

Carbohydrate Recognition Proteins

University College, Dublin, Ireland, 13 September 1995

The colloquium on carbohydrate binding proteins was organized by Maureen Taylor (Oxford) and held on 13 September 1995 during the Biochemical Society meetings at University College, Dublin. Interestingly, selectins were mentioned only peripherally indicating that, despite frenetic activity in the selectin area recently, they are not the only show in town so far as mammalian lectins are concerned. In the opening talk Bernard Hoflack (Heidelberg) asked "Why two mannose-6-phosphate receptors?". Homozygous null mutant mice lacking the large or small M6P-receptor are viable and fertile although increased levels of lysosomal enzymes are secreted into body fluids. Using appropriate mouse crosses Hoflack has generated fibroblast cell-lines that express neither, one or both receptors. In general cells lacking either receptor secrete lysosomal enzymes at a level about half that shown by the double null mutants. Over-expression of either receptor in the double mutant does not restore normal lysosomal function fully indicating that certain isotypes of many lysosomal enzymes prefer one receptor over the other. Hoflack answered his question by glycosylation differences: the large receptor prefers diphosphorylated lysosomal enzymes whereas the small receptor selects monophosphorylated glycoforms. These glycosylation patterns appear to different extents on particular lysosomal enzymes: for example re-expression of the small M6P-receptor in double null mutants corrects the secretion of cathepsin D less well than re-expression of the large receptor. Hoflack suggested that the down-regulation of one M6P-receptor in normal cells may lead to the secretion of those lysosomal enzymes that are decorated in a regulated way with the preferred carbohydrate ligand. Such regulation may be important in the preferential secretion of a particular set of lysosomal enzymes, for example by macrophages or osteoclasts during tissue remodelling. Nancy Dahms (Milwaukee) described her studies on the two-site binding of diphosphorylated ligands by the large M6P-receptor. Earlier work had implicated arginine residues in M6P binding. Dahms has now located a critical arginine (R435) in domain 3 and an R435–A435 mutation in a recombinant protein containing domains 1–3 reduces M6P-binding significantly. Sequence homology identified a second critical arginine (R1334) in domain 9 and double mutations (R435-A, R1334-A) inactivated the intact receptor. Transfection of L-cells which lack endogenous receptor with wild-type receptor, single-site mutants or the double site mutant shows that the single-site derivatives are almost as efficient as wild-type receptor in targeting and retention of lysosomal hexosaminidase B. This result could suggest that single site binding is sufficient for most purposes in lysosomal enzyme targeting or that assembly e.g. dimerization of the single site mutant receptors occurs in transfected cells overexpressing them, perhaps reconstituting an active double site binding complex. An unanswered and interesting question concerns the function of domains 4–6 in the large M6P receptor. Are these present simply for appropriate spacing of the two M6P-binding sites or do specific as yet unidentified ligands bind in this region?

The next two talks concerned galectins, formerly called S-lectins, a family of relatively small galactose-binding proteins. Francoise Poirier (Paris) has shown, by *in situ* hybridization and antibody staining, interesting patterns of expression of different galectins in mouse embryos. Overlapping expression of galectins 1 and 3 occurs in trophectoderm of implanting blastocysts and of galectins 3, 5 and 7 in the skin of late embryos. By constrast galectins 1, 3, 5 and 6 show up at specific sites, namely myotomes, notochord, erythrocytes and gut epithelium respectively. Perhaps most interesting is the complementary expression of galectins in the forming skeleton: galectin 1 and 5 in bones and galectin 3 in cartilage. In the developing somites galectin 1 is an early marker of muscle differentiation, appearing before MyoD. Poirier has generated homozygous null mice deficient in either galectin 1 or 3. The mice are so far viable and fertile.

Implantation is normal, which may not be unexpected given coexpression of these galectins in blastocysts. Whether functional redundancy suffices to carry through the null mice into a carefree old age remains to be seen. The phenotype of double mutant mice, which are being bred, may throw more light on the problem. Colin Hughes (London) continued with the roles of galectin 3. *In vitro* experiments show that galectin 3 can weaken or promote cellular adhesions, for example of human breast carcinomas. Cell lines established from infiltrating ductal tumours, representing an early stage of malignancy, express low or moderate levels of galectin 3. However, exogenously added lectin binds to the cells and promotes their migration through a Matrigel barrier. By contrast, breast tumour cell lines established from ascites or pleural effusions, representing late stage malignancy, express very high levels of galectin 3 mainly at intercellular contacts and are non-invasive in the Matrigel assay. A link between galectin 3 and cell motility is suggested by its induced surface expression on epithelial cells in remodelling tissues, for example on type 1 pneumocytes repopulating the alveolar surface in injured lung, and in inflammatory phagocytes. Cell-type specific surface receptors for galectin 3 include in macrophages the integrin CD11b/CD18 and the heavy chain of CD98, a putative calcium channel protein.

Kurt Drickamer (Oxford) discussed his studies on the calcium-dependent lectins, namely the asialo-glycoprotein receptor ASG-R and the mannose-binding protein MBP which, unlike the galectins, show blatant rather than subtle differences in carbohydrate-binding specificities. The carbohydrate-recognition domains (CRD) of these lectins are significantly homologous in sequence, and contain conserved residues that are critical in creating the CRD fold, which also forms two binding sites for Ca atoms. Fine specificity of carbohydrate binding by the CRDs comes from additional protein-sugar interactions. Introduction of short peptide segments from the ASG-R sequence into the MBP sequence, for example replacing an EPN triplet by QPD, induces galactose binding specificity in a still predominantly MBP background structure. Intriguing differences in carbohydrate-binding specificity are seen in the ASG-R in liver, where GalNAc-terminated glycans are preferred over Gal-terminated structures, and macrophages where little or no preference is shown. By domain swapping and site-directed mutagenesis Drickamer has found that a single asparagine residue inserted into the macrophage receptor increases GalNAc preference substantially.

Paul Crocker (Oxford) reported on the sialoadhesin family, sialic acid-binding proteins containing a homologous V-set Ig domain and variable numbers of C2-set domains. The prototype sialoadhesin is macrophage restricted and may play roles in myeloid cell differentiation and clearance of aged neutrophiles. CD33 is more widely expressed in myeloid cells whereas CD22 and MAG are found only in B cells and myelinating glia respectively. By using enzymatically remodelled erythrocytes Crocker finds significant differences in sialic acid binding among family members. Sialoadhesin and CD33 bind α2-3 sialyl O- and N-glycans whereas murine CD22 binds α2-6 sialyl N-glycans, especially the N-glycolyl form of sialic acid. This binding specificity is related functionally to cell-type regulated glycosylation. For example, in bone marrow mixed cell populations, sialoadhesin picks out only myeloid cells. For sialic acid binding, the V-set domain of sialoadhesin is both necessary and sufficient for sugar binding and mutation of one residue abolished binding. Since this residue is conserved in all family members, further sugar-protein interactions must exist to confer specificity and these remain to be identified. The function of the extraordinary number of C2-set domains (17 in sialoadhesin) also needs to be clarified. Interestingly, transfection of COS cells with CD 33 constructs failed to generate cells with the ability to bind HL60 target cells unless the COS transfectants were first treated with sialidase. This implies the existence of cis-interactions between CD33 and α2$\rightarrow$3 terminated glycoproteins (perhaps even CD33 itself?) in the plane of the plasma membrane, raising the possibility that similar interactions regulated by sialylation may play a role in modulating trans-binding functions mediated by the sialoadhesins.

The final talk by Karel Bezouska (Prague) concerned the C-type lectins encoded in the natural killer NK gene complex. These comprise several multigene families, localized on chromosome 6 in rodents and the syntenic chromosome 12 in humans, that function as activating or inhibiting receptors of NK cells. The rat NKR-P1 lectin binds to a very wide range of oligosaccharides including blood-group antigens, ganglio-series glycolipids and glycosamino-glycan fragments. The activation of NK cells requires the induction and surface expression of NKR-P1, binding to suitable carbohydrate determinants present on surface molecules of the target cell, signal transduction and NK cell activation. Exposure of NKR-P1 positive cells to GM2-bearing liposomes (GM2 is an excellent receptor for NKR-P1) induces a slow, transient calcium flux which may be related to the signalling mechanism.

Overall the colloquium conveyed the ever-widening interest in the mammalian lectins and showed again the importance of these molecules in research aimed at understanding sugar-protein interactions at a molecular level.

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