

Lepur A, Salomonsson E, Nilsson UJ, Leffler H (2012). Ligand induced galectin-3 protein self-association. *J Biol Chem.* Jun 22;287(26):21751-6. Epub 2012 May 1.

Many functions of galectin-3 entail binding of its carbohydrate recognition site to glycans of a glycoprotein, resulting in cross-linking thought to be mediated by its N-terminal noncarbohydrate-binding domain. Here we studied interaction of galectin-3 with the model glycoprotein asialofetuin (ASF), using a fluorescence anisotropy assay to measure the concentration of free galectin carbohydrate recognition sites in solution. Surprisingly, in the presence of ASF, this remained low even at high galectin-3 concentrations, showing that many more galectin-3 molecules were engaged than expected due to the about nine known glycan-based binding sites per ASF molecule. This suggests that after ASF-induced nucleation, galectin-3 associates with itself by the carbohydrate recognition site binding to another galectin-3 molecule, possibly forming oligomers. We named this type-C self-association to distinguish it from the previously proposed models (type-N) where galectin-3 molecules bind to each other through the N-terminal domain, and all carbohydrate recognition sites are available for binding glycans. Both types of self-association can result in precipitates, as measured here by turbidimetry and dynamic light scattering. Type-C self-association and precipitation occurred even with a galectin-3 mutant (R186S) that bound poorly to ASF but required much higher concentration ( $\sim 50 \mu\text{M}$ ) as compared with wild type ( $\sim 1 \mu\text{M}$ ). ASF also induced weaker type-C self-association of galectin-3 lacking its N-terminal domains, but as expected, no precipitation. Neither a monovalent nor a divalent N-acetyl-D-lactosamine-containing glycan induced type-C self-association, even if the latter gave precipitates with high concentrations of galectin-3 ( $> \sim 50 \mu\text{M}$ ) in agreement with published results and perhaps due to type-N self-association.