INVESTIGATING THE ROLE OF THE ECM IN CARDIO PROTECTION: PRODUCTION AND CHARACTERIZATION OF A BIOACTIVE RECOMBINANT AGRIN

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Mature cardiomyocytes (CM) are unable to proliferate, therefore injury to the myocardium results in permanent damage. However, in 2017 the extracellular matrix protein agrin was found to successfully induce myocardial repair in rodent models of cardiac infarction, futher confirmed in porcine models in 2019. It was proposed that agrin increases CM proliferation by interacting with the cell surface receptor αDystroglycan (αDG). To better understand this interaction, the following downstream processes and agrin's regenerative capacity we have produced and purified a recombinant, bioactive form of agrin which we have named, DBAF (dystroglycan binding agrin fragment), which contains only the domains that interact with αDG. DBAF has been characterised using a variety of biochemical and biophysical methods including solid phase binding, and small angle x ray scattering (SAXS). We have found that DBAF is stable upon both chemical and physical denaturation. SAXS analysis has shown that DBAF is compact, and futher compacted in the presence of calcium (Ca^{2+}). Solid phase binding indicated DBAF binds tightly to αDG in a Ca^{2+} dependant manner. Based our molecular model predicting the Ca^{2+} binding site we produced and purified two single mutants and a double mutant of specific residues within the predicted Ca^{2+} binding site. Solid phase experiments revealed that αDG binding is strongly reduced in the mutants, confirming the involvement of such residues in coordinating Ca^{2+} and SAXS experiments futher showed that Ca^{2+} has an effect in compacting the protein.

Keywords: Agrin, cardiomyocyte, regeneration, recombinant agrin, Ca2+