142ac Molecular Studies of Proteins Encapsulated in Hydrogels

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The project envisions the investigation of hydrogels as the means to encapsulate antibodies, enzymes, heme-proteins or others biomolecules which could serve as drug delivery carriers, scaffolds for tissue growth, among many other applications. Hemoglobins from Lucina pectinata and myoglobin form horse skeletal muscle were utilized as model proteins. Crystallization protocols for hemoglobin I, II, II, from Lucina pectinata and several mutants were developed utilizing the vapor diffusion hanging drop technique. Preliminary results were obtained utilizing 30% w/v poly(ethylene glycol) 4000 in 0.1M tri-sodium citrate dihydrate at pH 5.6 and 0.2M ammonium acetate; and, 0.8M potassium sodium tartrate tetrahydrate in 0.1M HEPES-Na at pH 7.5. Protein encapsulation was performed by imbibition using various polymer morphologies including neutral, anionic, and cationic hydrogels. For this purpose, hydrogel networks composed of poly(ethylene glycol) monomethyl ether monomethacrylate (molecular weight 200, 400 and 1000) crosslinked with poly(ethylene glycol) dimethacrylate (molecular weight of 200, 600, 1000), methacrylic acid crosslinked with poly(ethylene glycol) dimethacrylate (molecular weight of 200, 600, 1000), and dimethyl amino ethyl methacrylate crosslinked with poly(ethylene glycol) with the same molecular weights as previously described, were synthesized by free radical solution polymerization. Polymer solutions were cast between two microscope slides separated by Teflon® spacers and cut into disks of approximately 1cm diameter. Washed polymer disks were placed in a protein solution for various hours. Methacrylic acid based hydrogels incorporated the highest amount of protein when compared to poly(ethylene glycol) or dimethyl amino ethyl methacrylate polymers. Measurements of the correlation length of the networks revealed that methacrylic acid hydrogels possessed the highest correlation length (15.66-24.38nm) when compared to poly(ethylene glycol) containing matrices (0.28-0.62nm) or the dimethyl amino ethyl methacrylate (1.31-1.66nm). The myoglobin hydrodynamic radius was reported to be approximately 2.05nm. These results indicated that the neutral and the cationic hydrogels may have not incorporated significant amounts of proteins due to significantly lower pore sizes when compared to the hydrodynamic radius of the protein. Release experiments showed that solute transport mechanism in anionic hydrogels is a combination of Fickian diffusion and chain relaxation. Molecular simulations utilizing coarse grain models were utilized to simulate the hydrogel networks and an all atom model was employed to simulate the behavior of the protein in aqueous solutions. The program NAMD was employed to simulate the protein in aqueous solution. Preliminary results indicated that at room temperature hemoglobin I possessed a high flexibility and its hydrodynamic radius significantly increased. Methacrylic acid network simulations were performed with GROMACS and ACDLAB ChemSketch. Without water the network collapsed as can be experimentally observed.