Poly(ethylene glycol)-modified Proteins: Solution and Interfacial Behavior, and Unconventional Applications in BioProcessing and Drug Delivery

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The covalent attachment of poly(ethylene glycol) (PEG) polymer chains, or "PEGylation," improves the efficacy of protein drugs by extending their half-lives in the circulation without adversely affecting biological binding activity: the PEG chains are thought to hinder recognition by proteases, inhibitors and antibodies through steric interactions and to retard renal clearance through increased molecular size. These effects are typically attributed to a protective "shroud" of PEG wrapped around the protein, however the morphology of PEG-protein conjugates has not been definitively characterized. Enthalpic biophysical measurements and entropic thermodynamic arguments suggest that the shroud morphology is less likely than a "dumbbell" morphology where the PEG component is a relatively unperturbed random coil adjacent to the globular protein, at least for conjugates with a single attached PEG chain. Further, the adsorption behavior of PEG-protein conjugates is not clear as PEG itself is surface-active, yet PEGylation of surfaces tends to reduce non-specific protein adsorption. We sought to more fully understand the solution and adsorptive behavior of PEG-protein conjugates and then to use this understanding to explore new applications of protein PEGylation in bioprocessing and drug delivery.

While our dynamic light scattering measurements are consistent with the dumbbell model for mono-PEGylated proteins, we have used small angle neutron scattering with proteins conjugated with per-deuterated PEG chains to show that that the dumbbell model is correct. We have translated these findings into the development of new, high selectivity protein affinity chromatography media for use in bioseparations. By PEGylating the immobilized protein affinity ligand outside of the target binding site, we aim to discourage the non-specific binding of contaminant species via the steric mass of the random coil PEG chain without decreasing target binding. We find selectivity enhancements for IgG-class antibodies of greater than 50% for Protein A affinity chromatography media modified with 5 kDa and 20 kDa PEG chains relative to the un-modified media, without loss of antibody binding affinity. Increased contaminant rejection by Protein A media has important implications for simplifying downstream processing operations for monoclonal antibody production and for extending the operating lifetime of this expensive class of bioseparations media.

Adsorption isotherms for PEG-protein conjugates on both hydrophilic and hydrophobic surfaces show reduced extents of adsorption and unusual transitions indicative of molecular reorientations. We have probed adsorbed conjugates using atomic force microscopy and neutron reflectivity to reveal a transition from an orientation where both protein and conjugate interact with the surface to one where the protein interacts with the surface and the PEG chain extends into solution as surface concentration increases. We have exploited PEGylation to reduce denaturing adsorptive interactions between proteins and interfaces that limit the successful delivery of protein drugs from poly(lactide-co-glycolide) (PLG) microsphere delivery systems. Oil/water interfaces are present during the generation of protein-loaded PLG microspheres by the double emulsion technique and solid/water interfaces are present as the microspheres erode during delivery. The depressed isotherms of conjugates reduce the extent of adsorption at denaturing interfaces and the attached PEG random coils serve as steric diluents at interfaces. While PEGylation with 20 kDa PEG has little effect on protein behavior at ethyl acetate/water interfaces, at PLG/water interfaces we find decreased extents of adsorption, increased reversibility of adsorption and decreased tendency to aggregate. These results have translated to 50+% and 100+% improvements in active protein release for monoPEGylated and diPEGylated ribonuclease A, respectively.