Supramolecular PEGylation as an Innovative Approach to Biopharmaceutical Formulation and Delivery

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Statement of Purpose: Biopharmaceuticals have transformed the practice of medicine, with many drugs coming to market in recent years in the form of peptides, proteins, and antibodies. These new classes of drugs have an array of attendant complications arising from poor stability that can lead to the active drug being converted into an inactive and/or potentially immunogenic form. Extensive efforts have been devoted to the development of excipients for use in biopharmaceutical formulation. An alternate approach to promote stability or modify pharmacological activity of protein drugs is through direct chemical modification with a prosthetic functional group such as poly(ethylene glycol) (PEG). Specifically, covalent PEGylation is known to increase protein solubility, limit access by proteolytic enzymes or opsonins, reduce glomerular filtration, and inhibit aggregation. Direct covalent modification, though, introduces complications from the need to isolate and purify the modified protein following labeling, potentially increasing immunogenicity or negatively impacting protein function and signaling. In contrast, supramolecular modification of therapeutic proteins affords a straightforward non-covalent route to improving protein stability and modulating activity or pharmacokinetics as a "designer" formulation excipient.[1]
Methods: Cucurbit[7]uril (CB[7]) moieties bearing a single PEG chain (CB[7]-PEG) strongly bind to aromatic amino acids such as the N-terminal B1-phenylalanine of insulin ($K_D \sim 1$ μM). Circular dichroism spectroscopy indicates this binding interaction does not alter the structure or activity of insulin, no matter the molecular weight of the PEG chain. Aggregation of insulin in formulation was determined using a transmittance assay whereby transmittance was measured at 540 nm, a wavelength at which insulin and CB[7]-PEG have negligible absorbance, in order to detect the formation of aggregates that scatter light over time. In vivo function of insulin formulations was evaluated using a mouse model of insulin-deficient diabetes, prepared using streptozotocin (STZ) to induce pancreatic β-cell death, whereby fasted mice were dosed with insulin formulations (1 IU/kg)) by subcutaneous injection.
Results: Supramolecular PEGylation of insulin with CB[7]-PEG imparts unprecedented stabilization to the protein in formulation. Defining aggregation time as the time upon which a 10% reduction in transmittance is observed, insulin aggregated following 13.6 ± 0.2 h (N=4) of agitation. Meanwhile, insulin formulated with CB[7]-PEG did not aggregate during the 100-hour kinetic study, nor over the next 100 days. Subsequently, in vitro insulin activity was measured for all insulin formulations through dose-response studies of AKT phosphorylation. Insulin formulated with CB[7]-PEG and aged for 100 days exhibited LogEC_{50} comparable to those for freshly dissolved insulin, while insulin aged for 100 days alone was completely inactive. Taken together with data for insulin aggregation, formulation with CB[7]-PEG was found to preserve both the stability and activity of insulin for over 100 days in stressed conditions. Following these studies, insulin formulations with and without CB[7]-PEG were administered subcutaneously in STZ diabetic mice. All insulin formulations reduced average blood glucose to a normoglycemic level (<200 mg/dL for a mouse). Animals that received either insulin alone became hyperglycemic again approximately 2 hours following administration; however, animals that received insulin formulated with CB[7]-PEG remained normoglycemic for longer time-frames that were a function of molecular weight of the PEG chain. Moreover, insulin formulated with CB[7]-PEG exhibited increased bioavailability (84% for insulin@CB[7]-PEG_{5k}; 60% for insulin alone), presumably on account of enhanced stability and reduced local uptake of insulin upon supramolecular PEGylation.
Conclusions: Supramolecular PEGylation of authentic therapeutic proteins provides a powerful approach to stabilization in formulation in vitro, as well as modulation of activity, bioavailability and pharmacokinetics in vivo.
References: [1] MJ Webber et al. Proc Natl Acad Sci. 2016; 113:14189-14194. [2] CL Maikawa et al. *submitted*.

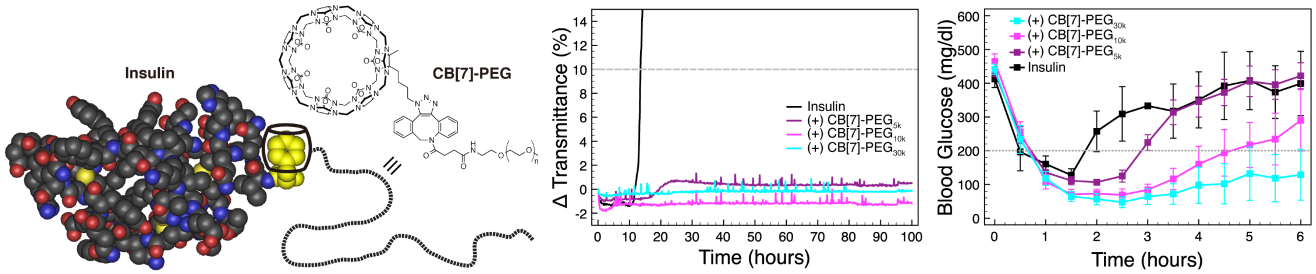

Figure 1: A designer excipient for supramolecular modification of proteins. A "designer" excipient comprising a conjugate of cucurbit[7]uril and poly(ethylene glycol) (CB[7]-PEG) binds specifically and strongly to aromatic amino acids, such as the N-terminal phenylalanine on insulin, via host-guest interactions. Monitoring of aggregation of insulin formulations under physiological conditions with continuous agitation over the course of 100 h, and extended for a total of 100 days, demonstrates no change in transmittance for insulin formulated with CB[7]-PEG. In vivo assessment of blood glucose levels in diabetic mice following administration of insulin (1 IU/kg) injected alone or formulated with or CB[7]-PEG of different molecular weights. Insulin administered in formulation with CB[7]-PEG demonstrated extended activity that was a function of molecular weight of the PEG chain (the dotted gray line indicates normoglycemia for a mouse).