Mono-PEGylation of denatured lysozyme and its *in-vitro* refolding

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Covalent modification of a protein with polyethylene glycol (PEG) has become one of the most widely used and well established drug enhancement strategies in the biopharmaceutical industry.[1] The general benefits enjoyed by PEGylation, such as prolonged serum half-lives or reduced immunogenicity *in-vivo*, are well known.[2] Usually, the PEGylation process is performed with purified proteins. After PEGylation, the desired PEGylate is recovered by a multi-step purification process.[3] The aim of this research is to demonstrate the feasibility of an integrated process of PEGylation and *in-vitro* refolding from inclusion body protein. We investigated whether a protein could be mono-PEGylated under a denaturing condition and also the PEGylated proteins could be refolded correctly. Using lysozyme as a model protein, we performed PEGylation based on reductive alkylation, in which mPEG-aldehyde of 5, 10, 20 kD as conjugated to the N-terminus of lysozyme. We used IEX and GPC chromatography in order to purify mono-PEGylated lysozyme. Then we unfolded mono-PEGylated lysozyme by adding urea and DTT, and also refolded it by dilution. As a result, we found that lysozyme was PEGylated in the presence of 8 M urea. Furthermore we found that the PEG molecule covalently attached to lysozyme showed little influence on its *in-vitro* refolding yield. This study suggested a possibility of IB protein PEGylation and subsequent refolding with a higher yield.

References

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