Engineering site-specific bioconjugation to a biosurfactant for improved stability in biological applications

Hossam H. Tayeb,[1] Marina. Stienecker,[1] Anton P. J. Middelberg[1] and Frank Sainsbury[1*]

The University of Queensland, Australian institute for Bioengineering and Nanotechnology, St Lucia, QLD 4072, Australia

The option to tune the interfacial properties of nanomaterials is a significant step towards future sophisticated biological applications. Modification of proteins by chemical bioconjugation of functional moieties such as polyethylene glycol (PEG) is a crucial aspect of biologic drug delivery.[1] Similar functionalisation of nanoparticles has the potential to improve their biocompatibility and efficacy when bioconjugation is controlled and compatible with both characterisation and application. DAMP4, a surface active protein, is remarkably stable[2] and, therefore, potentially amenable to modification via a wide range of bioconjugation chemistries. DAMP4 facilitates the surface modification of peptide-stabilised emulsions,[3] which offer high solubility of hydrophobic drugs, biocompatibility and tunable cell receptor targeting.[4] Here we engineered DAMP4 to substitute one or two lysine residues with cysteines for site-specific PEGylation via malemide reactions. We biosynthesized mutated DAMP4 variants in *E. coli* and purified them using multistep-column chromatography. Circular dichroism spectroscopy was used to confirm the solution structure and stability under different thermal conditions for all variants. The interfacial activity was also measured for all proteins at the oil-in-water interface. The efficiency of PEGylation was assessed by SDS-PAGE and showed >95% bioconjugation. The function of bioconjugates was characterised following the integration of these proteins onto emulsions, where size and zeta potential of the oil droplets indicated the presence of conjugated PEG polymers. Finally, each PEGylated protein was applied with different PEG densities onto the TNEs interface to examine their biological stability against isotonic solutions in the presence and absence of the serum proteins to obtain insight for their future pharmaceutical applications.

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