Fusion of a recombinant antibody fragment with a homo-amino-acid polymer: effects on biophysical properties and prolonged plasma half-life.

Abstract:
Chemical conjugation of small recombinant proteins with polyethylene glycol (PEG) is an established strategy to extend their typically short circulation times to a therapeutically useful range. We have investigated the production of a genetic fusion with a glycine-rich homo-amino-acid polymer (HAP) as an alternative way to attach a solvated random chain with large hydrodynamic volume. The anti-HER2 Fab fragment 4D5 was used as a model system and fused with either 100 or 200 residue polymers of the repetitive sequence (Gly(4)Ser)(n) to its light chain. Both fusion proteins were successfully produced in the periplasm of Escherichia coli and obtained as homogeneous preparations after two-step affinity chromatography via the His(6) tag fused to the heavy chain and the Strep-tag II fused to the extended light chain. Both modified Fab fragments showed binding activity towards the HER2 antigen indistinguishable from the conventional recombinant Fab fragment. When compared with the unfused Fab fragment, a significantly increased hydrodynamic volume, by ca. 120%, was observed during gel filtration for the 200 residue HAP fusion protein and, to a lesser extent, in the case of the 100 residue HAP. Difference CD measurements revealed a characteristic random coil spectrum for the 100 and 200 residue HAP fusion moieties. Finally,
pharmacokinetic experiments were carried out in mice after radioiodination of the recombinant Fab fragments. Although the 100 residue HAP fusion showed a behavior very similar to the unfused Fab fragment, with a terminal plasma half-life of ca. 2 h, the 200 residue HAPylated Fab fragment gave rise to a significantly prolonged half-life of ca. 6 h. While this moderate effect may so far be most beneficial for specialized medical applications, such as in vivo imaging, the genetic engineering of optimized HAP sequences should yield pharmacokinetic properties similar to PEGylation, yet without necessitating in vitro modification steps.

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