Storage Stability Studies of Anti-VEGF FpF Antibody Mimetics

H. Khalili[1], S. Brocchini[2], P. T. Khaw[2], S. Filippov[3]
[1]University of East London, [2]University College London, [3]Institute of Macromolecular Chemistry

Purpose

IgG antibodies and Fab fragments are used clinically, however Fabs suffer from monovalency and protein instability. IgGs also prone to aggregation and degradation especially within the hinge region. We have developed an antibody mimetic called Fab-PEG-Fab (FpF) (Fig 1) [1], which maintains the epitope bivalency of an IgG. The Fc region of an IgG is replaced in the FpF with a flexible, hydrophilic poly(ethylene glycol) (PEG) scaffold. The PEG scaffold is thought to enhance the stability and reduce the propensity for aggregation. The aim of this study was to evaluate FpF storage stability compare to both IgG and Fab.

Methods

An anti-VEGF FpF was prepared from the Fab obtained from the enzymatic digestion of bevacizumab and PEG-di(mono-sulfone) [1]. Storage stability studies were conducted in glass vials at 4oC with the anti-VEGF Fabbeva and FpFbeva for a period of 60 days. Thermal stability studies were also conducted in glass vials at 37oC with bevacizumab in its pharmaceutical formulation and the corresponding FpFbeva in PBS (pH 7.4) at double the bevacizumab concentration for a period of 30 days. Silicon oil (10 uL) was added on top of the solutions to avoid evaporation during incubation at 37oC. Aggregation and light/heavy chain dissociation were studied using a dynamic light scattering (DLS) DynoPro plate reader II and gel electrophoresis SDS-PAGE analysis.

Results

The Fabbeva that was freshly prepared from papain digestion of bevacizumab appeared as a band at 50 kDa (SDS-PAGE) with no trace of aggregation (Fig. 2A, lane 1). The freshly prepared Fabbeva also displayed a single DLS peak (DynoPro analysis) with an average diameter size of 9.1 ± 0.07 nm (Fig. 2B). Fabbeva, however, aggregated when it was stored in PBS buffer for 60 days at 4oC as shown by the higher molecular weight bands in Fig. 2A, lane 2. Two DLS peaks were observed for Fabbeva stored in PBS after 60 days, with hydrodynamic sizes of 15.9 ± 0.09 nm and 100 ± 0.10 nm (Fig. 2B), which suggested the formation of aggregates. The FpFbeva, however, did not aggregate or display any light/heavy chain dissociation when it was stored in similar buffer and storage conditions as Fabbeva (0.25 mg/mL; Fig. 2A, lanes 3-5). A single DLS peak was observed for the FpFbeva at day 0 and after 60 days at 4oC (diameter size of 11.4 ± 0.06 nm). In regard to bevacizumab (IgG1), chain dissociation (Fig. 2C, lane 7) and aggregation were observed when it was stored in its pharmaceutical formulation after 4 days at 37˚C at diluted concentration of 0.125 mg/mL. The FpFbeva was, however, remained stable after 30 days at 37˚C (0.250 mg/mL; Fig. 2C, lanes 1-6).

Conclusion

These results suggest that FpFs are more stable than Fabbeva and bevacizumab when stored in PBS without any excipients in their formulations. We will now determine the thermodynamic parameters of FpFs using isothermal titration calorimetry (ITC) as a mean to study the in-vivo KD [2] for a comparison with IgGs.

[1] H Khalili, et al. Bioconjugate Chem. 24(11), 2013, pp 1870-1882.
[2] L.A Hutton-Smith, et al. Molecular Pharm. 2016.

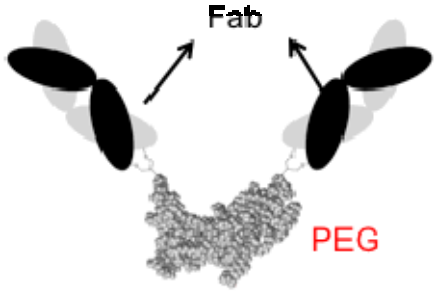

Figure 1. Structure of FpF_{beva}

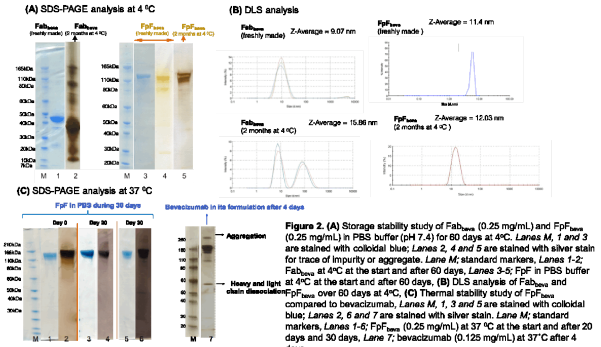

Figure 2. (A) Storage stability study of Fab_{beva} (0.25 mg/mL) and FpF_{beva} (0.25 mg/mL) in PBS buffer (pH 7.4) for 60 days at 4ºC. *Lanes M, 1 and 3* are stained with colloidal blue; *Lanes 2, 4 and 5* are stained with silver stain for trace of impurity or aggregate. *Lane M;* standard markers, *Lanes 1-2;* Fab_{beva} at 4ºC at the start and after 60 days, *Lanes* 3-5; FpF in PBS buffer at 4ºC at the start and after 60 days, **(B)** DLS analysis of Fab_{beva} and FpF_{beva} over 60 days at 4ºC, **(C)** Thermal stability study of FpF_{beva} compared to bevacizumab, *Lanes M, 1, 3 and 5* are stained with colloidal blue; *Lanes 2, 6 and 7* are stained with silver stain. *Lane M;* standard markers, *Lanes 1-6;* FpF_{beva} (0.25 mg/mL) at 37 ºC at the start and after 20 days and 30 days, *Lane 7;* bevacizumab (0.125 mg/mL) at 37˚C after 4 days.