Molecular dynamics study of 20kDa PEG reagents with different architectures

S. Carmali, T. S. Barata, A. Godwin, S. Brocchini, M. Zloh

1UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK; 2PolyTherics Ltd., The London BioScience Innovation Centre, 2 Royal College Street, London NW1 0NH, UK; 3University of Hertfordshire, College Lane, Hatfield AL10 9AB, UK

ucnvsca@ucl.ac.uk

ABSTRACT SUMMARY

Two different architectures of bis-alkylating PEGylation reagents were synthesised and their reactivity determined. Molecular dynamics simulations (MD) in a fully solvated system were used to understand the different reactivity shown by the synthesised PEG reagents. The linear reagent was shown to be more reactive, which is consistent with the distance measured between the chain ends. In contrast, the end of one of the PEG on of the chains from the branched reagent was in close proximity to the linker possibly contributing for a steric hindrance effect.

INTRODUCTION

Protein and peptide therapeutics are associated with several limitations, including short circulating half-life, immunogenicity, proteolytic degradation and low solubility. One of the strategies that emerged to improve the efficacy of protein and peptide biopharmaceuticals is PEGylation.

We have developed bis-alkylating PEGylation reagents, such as 1, to conjugate PEG site-specifically to proteins, using two different architectures, linear and branched. These reagents undergo conjugation by an addition-elimination mechanism that allows the formation of a three-carbon bridge between the two cysteine sulphurs of a reduced native bond. These reagents also undergo site-selective conjugation with protein His-tags.

In this study we assessed their reactivity and used molecular modelling techniques to gain insight into their dynamic properties and relative reactivity.

Figure 1 - Chemical structure of bis-alkylating PEG reagent 1.

EXPERIMENTAL METHODS

Conjugation study: DTT was added to a Fab solution (Ranibizumab, 0.1 mg/mL) in 50 mM sodium phosphate buffer, pH 7.4 containing 20 mM EDTA. After 30 minutes at room temperature, DTT was removed using a desalting column. The reduced Fab was then incubated with linear (MW 20kDa) and branched (MW 2x20kDa) bis-alkylating PEG reagents (1.5 equiv. 1 mg/mL) for 5 hours at 25°C. Crude reactions were analyzed by SDS-PAGE with the gel stained using Coomassie blue (Figure 1).

Molecular Dynamic Study: The initial structures were built with Maestro toolkit (Schrodinger). The structures were minimised (OPLS2005, 2500 iterations with extended cut off, water) and a short implicit solvation simulation ran with Macromodel (v9.9, Schrodinger) (1 ns, 300 K and the shake algorithm selected). To remove the bias of the starting point the reagents were subjected to a Simulated Annealing (SA) protocol (1 ns) using Desmond (Table 1).

Table 1. Three stage protocol used for the SA simulation of the PEG reagents.

<table>
<thead>
<tr>
<th>Stages</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (ps)</td>
<td>100</td>
<td>300</td>
<td>600</td>
</tr>
<tr>
<td>Temperature (K)</td>
<td>300-400</td>
<td>450-300</td>
<td>300</td>
</tr>
</tbody>
</table>

Finally, a 10 ns MD simulation with Desmond was performed using water as solvent; no ions were added to the solution. For the trajectory structures were recorded every 4.8 ps. The trajectory analysis was also performed with Desmond and analysis tools from Maestro.

RESULTS AND DISCUSSION

To evaluate conjugation reactivity, linear and branched reagents were each incubated with Fab, ranibizumab at pH 7.4 for 5 hours and the crude reactions were analyzed by SDS-PAGE (Figure 2). Both reagents afforded the mono-PEGylated compound (band visible at 80kDa), however the linear reagent gave a higher yield, which suggested a better reactivity.

Figure 2. SDS PAGE gel stained with coomassie blue showing: MW – Protein standards, Lane 2 – native Fab, Lane 3 – reduced Fab, Lane 4 and 5 – Reactions with 1.5 equiv. of linear and branched reagent, respectively.
MD simulations were performed to gain insight about the molecular properties of each reagent that could contribute to their reactivity. Preliminary trajectory analysis has revealed some interesting features of these two molecules.

The distance between the end of the PEG chains and the linker was monitored for the duration of the simulation (Figure 3). The linear PEG reagent shows some variation along the trajectory, expected due to the flexibility of the polymer. However, the smaller distance values are around 12 Å, which is still reasonably far and occur for short periods only, resulting in minimal effect on reactivity.

Considering the branched reagent. One of its PEGs (branch 1, Fig.3) follows the same trend as the linear reagent. However the second PEG (branch 2, Fig.3) revealed a completely different behaviour. The distance of the PEG terminus to the linker decreased throughout most of the trajectory. A slight plateau at 10 Å was observed in the middle of the trajectory and at the end of the simulation the distance values were around 4 Å. This indicates the possible interaction of the linker with the rest of the chain, resulting in steric hinderance of the linker and preventing its interaction with the target, consequently reducing reactivity. This situation occurred towards the end of the trajectory. PEG molecules are flexible. Therefore, the distance from the PEG terminus to the linker can rapidly increase, allowing the linker to be available only part of the time. This can result in reduced reactivity for the branched reagent. Solvent accessible surface area values will further elucidate the availability of the linker in both reagents.

Gyration radius gives a measure of how compact the structure is. The results for the analysed reagents (Figure 4) show no significant difference between the linear and branched molecules. There were also no significant changes for each molecule along the trajectory. Thus no dramatic changes in the structure occurred during the simulation. Despite the presence of the two PEG chains, the branch reagent’s overall structure still shows a high level of folding.

Further experiments and analysis of the trajectory are underway to better characterise these reagents.

CONCLUSION
The linear reagent was shown to be relatively more reactive. This is consistent with the distance measurements where one of the chains from the branched reagent was much closer to the linker. PEG steric shielding effects on the linker may interfere with interaction with the protein. Studies are ongoing with a family related reagents.

REFERENCES

ACKNOWLEDGMENTS
SC is grateful to UCL, School of Pharmacy and to PolyTherics Ltd. for funding her PhD project. Funding (TB and SB) from the UK Engineering & Physical Sciences Research Council (EPSRC) for the EPSRC Centre for Innovative Manufacturing in Emergent Macromolecular Therapies is gratefully acknowledged. Financial support from the consortium of industrial and governmental users is also acknowledged. MZ is grateful for support from the University of Hertfordshire.