

Conjugation of PolyPEG[®], Linear PEG and Branched PEG to a Thiol-Modified Aptamer

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Introduction

Aptamers are well known for their short in vivo half-life and rapid renal clearance. They are frequently modified by conjugation to linear or branched polyethylene glycol (PEG) in order to overcome these limitations. PolyPEG[®] comprises a 'comb-like' arrangement of short PEG chains attached to a polymethacrylate backbone by ester bonds¹⁻² (Fig 1).

In the present study, the conjugation to a thiol modified MUC1 aptamer of maleimide terminated linear PEG (20kDa), branched PEG (20 and 40kDa) and PolyPEG (17-60kDa), and the binding activity of the conjugates to the target peptide, have been studied.

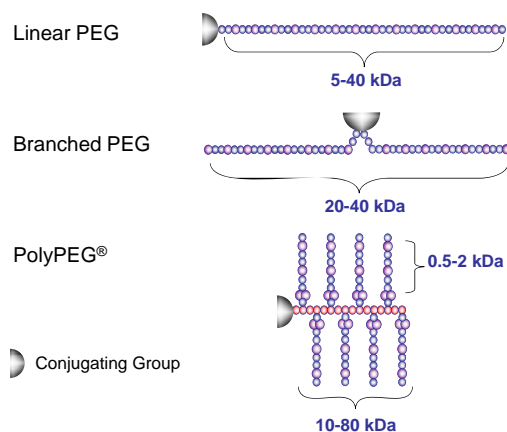


Fig 1. Schematic representation of the structure of linear, branched PEG and PolyPEG[®]

Methods

Thiol-modified aptamer was reduced with TCEP and conjugated to PEGs under various conditions at 37°C. Conjugation was monitored by anion exchange HPLC, and gel electrophoresis.

Results

Conjugation of PEGs to aptamer was monitored by HPLC (Fig 2) and gel electrophoresis (Fig 3.) Aptamer conjugated rapidly and in high yields to all PEGs at ratios between 1:1 and 5 (reduced-Apt:PEG). All PEGs conjugated to similar extents under appropriate conditions (Fig 4).

Determination of aptamer-target binding activity indicated that linear PEG reduces the affinity of binding slightly, whereas binding was not affected by branched PEGs or PolyPEGs (Fig 5).

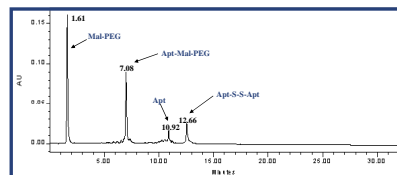


Fig 2. Ion-exchange HPLC chromatogram of the aptamer's PEGylation reaction mixture

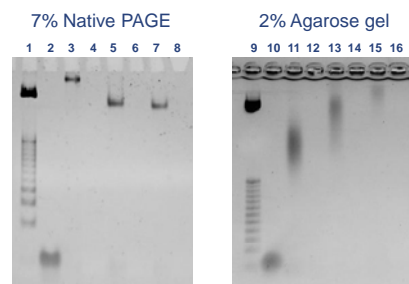


Fig 3. Native PAGE (left) and agarose gel (right) of aptamer-PEG conjugates

Lanes: 1: 25bp ladder; 2: apt; 3: BrPEG40-Apt; 4: free BrPEG40; 5: LinPEG20-Apt; 6: free LinPEG20; 7: BrPEG20-Apt; 8: free BrPEG20; 9: 25bp ladder; 10: apt; 11: PolyPEG17-apt; 12: free PolyPEG17; 13: PolyPEG40-apt; 14: free PolyPEG40; 15: PolyPEG60-apt; 16: free PolyPEG60.

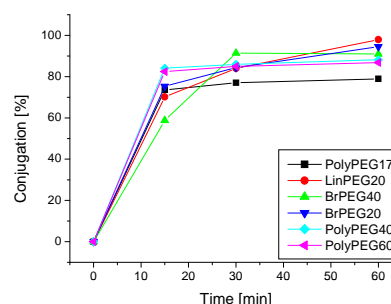


Fig 4. Kinetic study of the conjugation of the aptamer to the different PEGs at 37°C, ratio 1:5 (reduced aptamer: PEG)

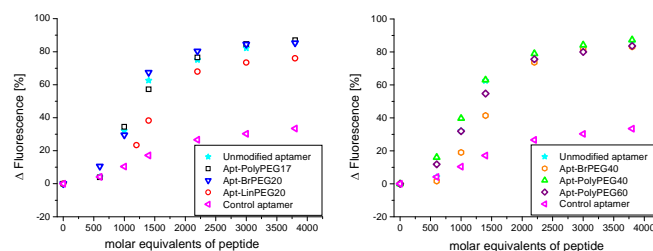


Fig 5. FID studies of aptamer-target binding affinity

Conclusions

Conjugation of thiol-modified aptamers can be accomplished similarly by PolyPEG and conventional PEGs, without compromising binding activity. The novel conjugates with PolyPEG allow further study of the in vivo properties of these products, and the determination of the impact of higher molecular weight PEGylated biologicals.

References

- Veronese, F.N. *Biomaterials* 2001, 22, (5), 405-417
- Mantovani, G.; Lecolley, F.; Tao, L.; Haddleton, D.M.; Clerx, J.; Cornelissen, J.J.L.M.; Velonia, K. *J. Amer. Chem. Soc.* 2005, 127, (9), 2966-2973.