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Histidylated poly(lysine) dendrimers for siRNA delivery

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SUMMARY

Since its discovery, small interfering RNA (siRNA) has revolutionised the field of gene silencing therapy because of its great potential in targeting diseases that were considered untreatable. Although several siRNA drugs are already in the market, many challenges remain to be addressed before siRNA therapies can realise their full potential including extra-hepatic delivery and improved endosomal escape. Dendritic polypeptides are attractive carriers for siRNA because of their wide range of defined structures that can be made. In this work, we report the on-going development of histidylated poly(lysine) dendrimers (G3KH) as carriers for siRNA. Histidine-capped lysine dendrimers were synthesised via HBTU/HOBt coupling reaction in solution. ^{1}H NMR data confirms the structure of G3KH dendrimers, although traces of impurities were also observed. Histidylated lysine dendrimers could efficiently condense short double strand DNA oligos (used as a model for siRNA) to form cationic nanoparticles of less than 100 nm in size.

INTRODUCTION

siRNA has gained an increasing interest because of its great promise in the field of gene silencing. siRNA is a short double strand RNA which mediates gene silencing by specifically targeting and degrading complementary mRNA via a mechanism called RNA interference (RNAi). Current siRNA medicines in the market are lipid- or bioconjugation-based and are mainly targeting liver cells. Extrahepatic delivery, toxicity and endosomal escape remain the greatest challenges for the wide clinical utility of siRNA. Highly branched lysine polymers have been widely investigated as biocompatible carriers for nucleic acid delivery, but they raise concerns of toxicity and low transfection efficiency[1]. The addition of histidine could reduce toxicity, enhance endosomal escape and improve transfection[2]. In this work, we investigate the synthesis of lysine and histidine-functionalised lysine dendrimers as potentially biocompatible and efficient carriers for siRNA delivery.

MATERIALS AND METHODS

Boc-lys(boc)-OH.DCHA, boc-his(trt)-OH, HBTU, HOBt, Hexamethylenediamine, anhydrous DMF, deuterium oxide were all purchased from Sigma-Aldrich.

Generation 3 lysine dendrimers were synthesised and capped with histidine (G3KH) (Fig. 1) following a divergent approach using HBTU/HOBt coupling reaction in DMF, using hexamethylenediamine as a core. Dendrimer structure was confirmed with 1H NMR (Bruker DRX-500). Short dsDNA oligos (21 bp) were used as a model for siRNA. Polyplexes were formed with G3KH at amine:phosphate (N:P) ratio 30:1. Complexation was confirmed with agarose gel electrophoresis and particle size and z-potential were measured by DLS (Zetasizer Ultra, Malvern).

RESULTS AND DISCUSSION

The structure of G3KH dendrimers was confirmed by ^{1}H NMR. The proton NMR spectrum of G3KH (Fig. 2)

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shows all the characteristic peaks corresponding to the protons of the internal lysine and external histidine residues. The ^{1}H NMR spectrum also suggests that there are still traces of DMF (δ = 2.8 (d) and 7.8 (s)) and HOBt (δ = 7.77 (d) 7.72 (d), 7.62 (t) and 7.51 (t).

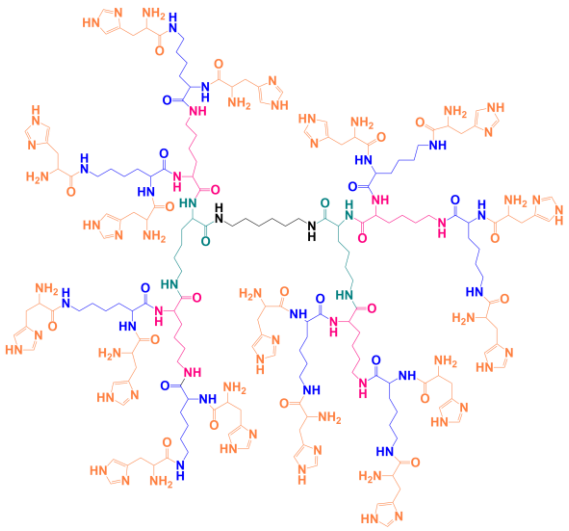

Fig. 1. *Structure of G3KH dendrimer. Generations 1, 2 and 3 of lysine dendrimers are in green, pink, blue respectively. External histidine residues are in orange.*

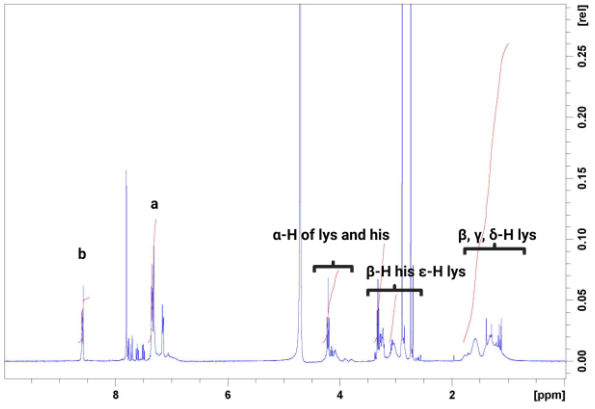

Fig. 2. *^{1}H NMR spectrum of G3KH dendrimers (500 MHz in D2O). δ 8.58 and 7.34 (CH in the imidazole ring of histidine b and a), 4.25 – 4.04 (α-protons of lysine and histidine) 3.5 – 2.88 (β-protons of histidine and ε-protons of lysine) 2 - 1 (β-, γ-, δ-protons of lysine residues).*

When complexed with dsDNA oligos, G3KH dendrimers could efficiently condense dsDNA oligos as shown by the absence of a fluorescent oligo band on the agarose gel electrophoresis (Fig. 3 – a). G3KH polyplexes have a positive zeta-potential of +24.5 ± 0.7 mV and a mean particle size of 77 ± 28 nm. The TEM image of G3KH polyplexes is shown in (Fig 3 - b) and it is consistent with the size data obtained by DLS.

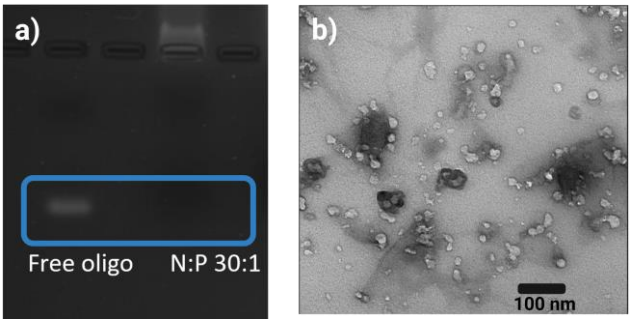

Fig. 3. *a) Gel electrophoresis of G3KH polyplexes. b) TEM image of G3KH polyplexes (N:P 30:1).*

CONCLUSIONS

This preliminary study reports the solution-phase synthesis of histidylated generation 3 lysine dendrimers. While further purification of the final dendrimers can still be achieved, G3KH dendrimers can efficiently condense dsDNA oligos into cationic nanoparticles <100 nm. Future work will investigate the impact of different histidine ratios on the complexation efficiency of these dendrimers with siRNA while assessing their cytotoxicity and transfection efficiency.

ACKNOWLEDGEMENTS

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