Bioconjugation of biotinylated PAMAM dendrons to avidin†

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The biotin-terminated PAMAM dendron has been synthesized and the asymmetric dendron used to modify the protein avidin *via* non-covalent bioconjugation.

Application of proteins and peptides as human therapeutics is developing rapidly with the discovery of novel peptides and proteins.[1,2] Many peptide derived molecules can be destroyed in the digestive system and in the circulatory system, removed by proteolytic digestion and rapid renal excretion.[3] Covalent attachment of synthetic macromolecules is an effective way to improve protein stability with reduced immunogenicity and extended plasma half-lives.[4–11] Secondary properties can be introduced, for example in temperature-sensitive,[9–15] photosensitive[16–21] and pH-sensitive[22] conjugates.

Poly(amidoamine) PAMAM dendrimers have attracted interest in various fields since their discovery by Tomalia *et al.* in 1985.[23,24] Their globular and well-defined structure has led PAMAM dendrimers and dendrons to be used in a range of constructs, including protein/enzyme mimics, and as gene delivery reagents.[25–32] Herein we report the work to modify a protein with PAMAM dendrimer. Previously Davis *et al.* have attached tetra terminal dendrons to the enzyme SBL[33] and Smith *et al.* have conjugated polyamine dendrons with spermine surface groups to Class II hydrophobin.[34]

Avidin was chosen as the model protein for two reasons: (1) avidin is formed from four subunits, each subunit is able to bind one biotin molecule with an extraordinarily high affinity (affinity constant $\sim 10^{-15}$ M). (2). The avidin–biotin system has been studied as a model of protein–ligand interaction[34,35] and has led to a large number of applications such as tumor pre-targeting,[36] improved clinical diagnostics[37] or protein labelling and proteomics and is also used as a research tool in surface engineering, self assembly studies, and drug delivery[38] and in a similar approach by Maynard *et al.* to attach polymers to avidin[10,11]

Michael addition of the non-symmetrical initiator, biotin (2-aminoethyl)amine (**2**) to methyl acrylate (MA) followed by amidation with a large excess of ethylenediamine doubled the number of surface amino groups. Dendron growth continued by alternate Michael addition and amidation to form the final target dendron **G#3.5**, Scheme 1.

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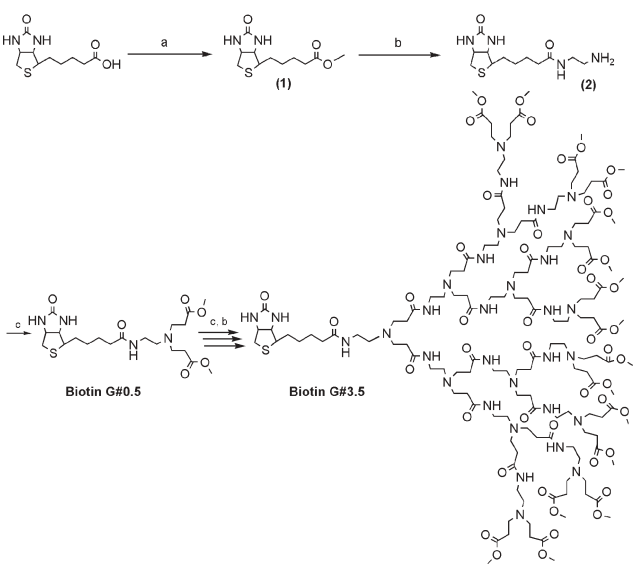
The ^{1}H NMR spectrum of the biotin terminated PAMAM dendron **G#3.5** shows peaks at 4.46 and 4.31 ppm corresponding to the protons on the biotin ring, whilst the peak at 3.65 ppm is from the peripheral methyl groups, Fig. 1. The integration ratio of the three peaks = 1 : 1 : 48, agrees with the theoretical values. The dendron has narrow polydispersity (1.04 by SEC), and the molecular weight by MALDI-TOF = 3260 amu, again the same as predicted, indicating successful synthesis of the biotinylated dendron.

In order to test the bioavailability of the biotinylated dendron to the protein receptor, a competitive binding evaluation was undertaken. 2-(4-Hydroxyphenylazo)benzoic acid (HABA) binds to avidin in the same position as biotin. Compared with the native HABA (maximum UV absorbance = 350 nm), the maximum UV absorbance of the avidin–HABA complex shifts to λ = 500 nm. However, avidin's affinity to HABA is much weaker ($K_d = 10^{-6}$ M) than biotin ($K_d = 10^{-15}$ M), thus HABA should be replaced by a biotinyl reagent when it is added to a solution of avidin–HABA complex, Fig. 2.

When the biotinylated dendron **G#3.5** was added into the avidin–HABA complex solution, the pale red color (avidin–HABA complex) immediately disappeared, with the emergence of a new peak and the disappearance of the peak at λ = 500 nm and



Scheme 1 Synthesis of biotinylated PAMAM dendron **G#3.5**. (a) H+ resin/methanol, 48 h at ambient temperature; (b) ethylene diamine–methanol, 48 h at 60 °C; (c) methyl acrylate–methanol, 48 h at ambient temperature.

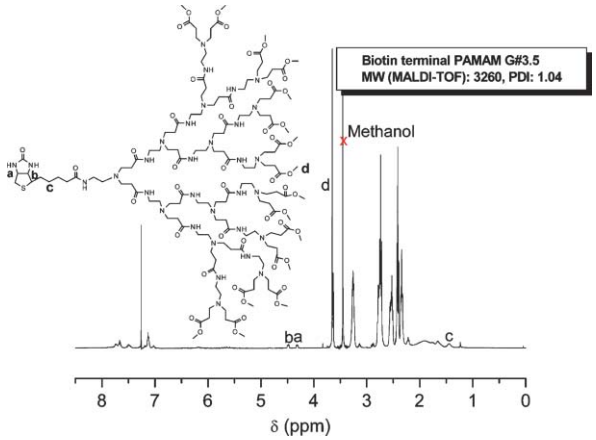


Fig. 1 ^{1}H NMR spectrum of biotin PAMAM dendron **G#3.5**.

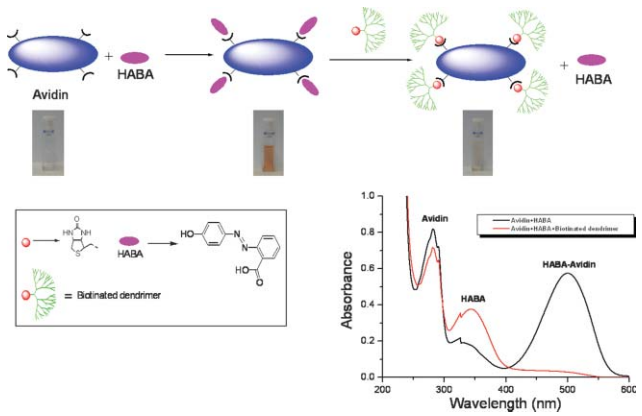


Fig. 2 Schematic of HABA binding test, UV/Vis spectra of the HABA–avidin complex before and after the addition of the biotinylated dendron **G#3.5**.

reappearance of the peak at 350 nm from free HABA, showing the interaction of the avidin–biotin dendron.

The reverse phase HPLC data, Fig. 3, shows the peak corresponding to the avidin–dendrimer conjugate shifted when compared with the peak of native avidin. Also demonstrating that the avidin–biotin dendrimer conjugate has been formed, this shift is due to the conjugate becoming less polar than the free protein. This proved more successful than SEC HPLC as, even with the addition of four dendrons, the change in molecular size was not sufficient for satisfactory resolution.

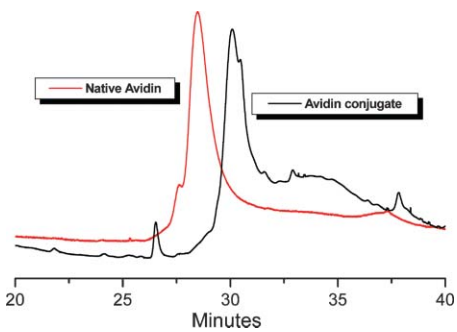


Fig. 3 Reverse phase HPLC of native avidin and avidin–**G#3.5** dendron conjugate.

In summary, a biotinylated PAMAM dendron has been successfully prepared. The biotin end group was employed to link onto the avidin surface, and the UV results of the HABA test indicate the occurrence of an avidin–biotin interaction. Reverse phase HPLC curves give a further indication of the conjugation between avidin and terminally biotinylated PAMAM.

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