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Controlled branching of polyglycidol and formation of protein–glycidol bioconjugates via a graft-from approach with “PEG-like” arms†

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The control of the branching in polyglycidol as semibranched alternatives to traditional polyglycidols is presented. The relative abundance of dendritic carbons is lowered by five-fold compared to hyperbranched polyglycidols. It is the first example of tailoring the branching in polyglycidol and creating protein–glycidol bioconjugates as alternatives to pegylated biostructures.

Branched and hyperbranched polyglycidols have been widely investigated as soluble catalyst supports in organic synthesis, controlling agents for biomineralization, and as carriers of potential vaccine models. Typical polymerization methods involve a cationic or anionic polymerization to form extensively hyperbranched systems in a ring-opening multibranching polymerization (ROMBP). Linear polyglycidols have been prepared via the polymerization of protected glycidol units mostly from anionic polymerization procedures. Highly hydrophilic polymers are of great interest as they can act as protective solubilizing scaffolds to improve the biological half-life and increase the circulation of biological structures. One of the most well studied hydrophilic polymers is polyethylene glycol (PEG), which has been the material of choice to enhance the duration of pharmacological activity in protein drugs. However, recent reports have linked the linear structure of PEG to increased toxicity and immunogenicity when administered for longer periods of time. Shorter PEG species, below 20 k, with branched structures are preferred. An example of such a system is branched poly(ethylene glycol) methyl ether methacrylate (PEGMA) which is currently being developed. Controlled radical polymerization (CRP) techniques such as atom transfer radical polymerization (ATRP) and reversible addition–fragmentation chain transfer (RAFT) polymerization have been the methods of choice in these efforts. In other approaches, linear polyglycidols and hyperbranched systems have been conjugated to proteins in graft-to methods. However, polyglycidols grown from proteins in a graft-from approach with tunable branching have not been accomplished yet. A practical graft-from approach in buffer or also organics would give the possibility to study the unknown advantages of semibranched polyglycidols. During our recent work investigating Sn(OTf)2 as the catalyst for the polymerization of polyester and polycarbonates, we found that residual amounts of the catalyst react readily with epoxide groups. The Sn(OTf)2 catalyst has not been investigated in polymerization reactions of glycidols and a thorough investigation of this monomer family to form controlled branched polymers seemed to be attractive. Polyglycidols with tunable degrees of branching are envisioned as ideal materials to overcome the limitations of traditional polyethylene glycol in protein therapeutics.

The ring-opening polymerization (ROP) reactions were performed at a range of temperatures to kinetically control the ring-opening of the oxirane group in glycidol monomers that would favor the formation of a more linear polymer species. In general, linear units can be differentiated through the presence of secondary hydroxyl groups (L1,4) or primary hydroxyl groups (L1,3). Typically, in hyperbranched polyglycidols, the ratio of L1,4 : L1,3 linear units is increased by 3-fold, favored by a lower steric hindrance in combination with higher stability of primary active sites. In most cases, it is sought to maximize the degree of branching to form dendritic type architectures which are mostly derived from the propagation of the primary units of L1,3. Conversely, we seek to control and increase the amount of L1,4 units and thereby decrease the degree of branching to promote more linear structures. Therefore, a kinetically controlled polymerization, and catalysis with Sn(OTf)2 might be favorable to understand and tailor the degree of branching in polyglycidols. Reaction temperatures of −20 °C, 0 °C, 20 °C and 40 °C were selected for the homopolymerization of distilled glycidol, employing isooamy alcohol as the ROP initiator and Sn(OTf)2 as the catalyst. To ensure constant temperature control, a polycrystalline refrigerator circulator was implemented with a specially formed jacketed flask. The polymerizations were run to completion and the viscous polymers were precipitated in hexanes.
The analysis of the $^{13}$C NMR gives relative integrals and the absolute abundance of the four structural units: linear $L_{2,1}$, linear $L_{1,4}$, terminal (T), and dendritic (D, branched). The degree of branching (DB) gives one possible measure of the dendritic characteristics of the polymer and was established to compare a hyperbranched structure to the respective perfect dendrimer. It is expressed in an entity between 0–1 and is calculated through a previously introduced equation by Frey (ESI†), with 0 representing linear and 1 being a perfect dendritic structure. Additionally, for all the temperatures studied, we quantified the relative abundance of the dendritic structural unit to have a better understanding of the polymeric architectures produced. In Fig. 1 we summarize the findings of the kinetic study and the detailed $^{13}$C NMR analysis. It was observed that the reaction temperature influences the degree of branching and the abundance of the dendritic unit. A DB of 0.24 was determined at 40 °C, which was decreased to 0.2 at 0 °C and then to 0.15 at the lowest temperature of −20 °C. Furthermore, the relative abundance of the dendritic species declined from 10.5% to 5.2%. Comparing these results to traditional cationic and anionic strategies in which a DB of 0.6 to 0.7 is common for AB₄ polycondensation reactions, the developed metal catalyzed polymerization with Sn(OTf)₂ afforded polglycidol with DB values that are drastically reduced. The reduction in the relative abundance of dendritic units is even more prominent with reductions of up to one fifth of previously reported values. We can conclude that, by implementing this newly formulated polymerization method we have developed a valuable tool to alter the degree of branching in polglycidol to achieve semi-branched structures that are not available with previously developed techniques.

To further characterize the prepared homopolymers, MALDI-TOF analysis was performed. An average polymer mass of 2–3 K was determined. Frey and Mühlaupt reported that it was not possible to obtain reliable data for polglycidol polymers with masses exceeding 4 K. An evaluation via gel permeation chromatography (GPC) was performed to obtain the PDI values. We observed PDI values ranging from 1.25 to 1.36, with lower PDI's at lower temperatures (ESI†) which is considered narrow for a semi-branched system.

The ease of the conducted polymerizations motivated us to conjugate a maleimide alcohol to a model protein (BSA) using the thiol group available on BSA to create a macroinitiator for a graft-from approach (Fig. 2). The subsequent polglycidol polymerizations using the BSA-OH initiator were investigated at RT with three different solvents such as DMSO, DMF and phosphate buffer at pH 6.0 (PB). In these trials we chose two different concentrations of glycidol monomer and allowed the reactions to run for 24 h. The resulting materials were dialyzed against deionized water and lyophilized. MALDI-TOF characterization was found to be an ideal tool to determine the success of the graft-from approach and to quantify the molecular weight increase. In the first set of experiments with a concentration of 0.33 M glycidol we increased the molecular weight of BSA by 1 K (ESI†) in all samples. To demonstrate a more apparent change in molecular weight, the concentration of glycidol was increased four-fold from 0.33 M to 1.33 M and an increase in the molecular weight of the BSA of up to 10 K (Fig. 2) was observed. Most surprisingly, the reactions conducted in buffer yielded species with the highest molecular weight increase. This finding may indicate a catalytic promotion of the slightly acidic buffer (pH 6.0) accelerating the polymerization of the glycidol and is the subject of further studies. With this, we found a practical method to grow polglycidol from proteins in physiological environments and organic solvents.

Polyacrylamide electrophoresis (PAGE) was employed to further support the evidence of a successful polymer-protein conjugation. Fig. 2 shows lanes with BSA (lane 2), BSA-OH macroinitiator (lane 3) and conjugated protein-polymer products (lanes 4–6) in the three selected solvents and the highest concentration of the glycidol monomer (see also ESI†). The PAGE confirmed the molecular weight increases of the samples via the graft-from approach and the products appear as high molecular weight bands slightly above the BSA standard. The polymer-protein conjugate reaction conducted in buffer showed a substantial molecular weight increase in comparison to other samples. To determine the bioactivity of the polymer-protein conjugates, the species were allowed to react with 4-nitrophenyl acetate and the absorbance of the resulting hydrolysis product was measured using UV/Vis spectroscopy (ESI†). The results indicated only a slight decrease in activity in comparison with the unmodified BSA protein. In future studies we will attempt to achieve sufficient solubilization and stability with the shortest polglycidol chains possible to limit adverse effects on bioactivity. Additionally, a circular dichroism experiment was conducted to
confirm the retention of the protein structure. All produced species, the one that was modified in buffer without the addition Sn(O\textsubscript{2})\textsubscript{2} and the two species modified in organic solvents (DMF and DMSO) and metal catalyst present showed a preserved protein structure.

We have developed a polymerization method to kinetically control the degree of branching in polyglycids. These low branching polymers define a new class of polyglycids that are further developed to nanomaterials or as PEG alternatives in protein therapeutics. The use of the Sn(O\textsubscript{2})\textsubscript{2} catalyst and depressed reaction temperatures led to an average of 8% relative abundance of dendritic units, a fivefold decrease from that observed in hyperbranched polymers. Furthermore, semi-branched structures can be grown in a grafting-from fashion onto a protein in biologically compatible conditions at room temperature. This work describes a novel polymerization methodology and is applied to grow branched, PEG like, hydrophilic polymers in situ, forming protein–polymer conjugates.

Notes and references