POLYPEPTOIDS FROM N-SUBSTITUTED GLYCINE N-CARBOXYANHYDRIDES: POTENTIAL AND LIMITATIONS

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Introduction

Since the first discovery of amino acid N-carboxyanhydrides (NCAs) by Leuchs more than a century ago[1], this class of heterocycles have been investigated intensively. While "regular" NCAs are a source for synthetic polypeptides, N-substituted NCAs (NNCAs) have primarily served as a tool to deepen the understanding of the reactivity of NCAs. A century of intensive investigation notwithstanding, every other year a novel, better, cheaper or more simply way how to polymerize NCAs to obtain well-defined polypeptides is discovered (recently summarized in Ref. 2).

Apart from the difficulties to synthesize well-defined polypeptides, some other limitations can be identified for polypeptides. To ensure protein-resistance and stealth properties in biomaterials, the polymer should be hydrophilic and have a zero net charge, feature hydrogen bond acceptors but lack donors.[3] Additionally flexible, amorphous chains may be beneficial. This combination of properties is, by and large, inaccessible for synthetic polypeptides. The amide proton plays an important role regarding both synthetic and physicochemical aspects of polypeptides. This can be exemplified particularly by comparison of polyglycine and its N-methylated analog poly(N-methylglycine), i.e. polysarcosine (PSar). While oligoglycines precipitates during polymerization due to formation of beta-sheets which are insoluble in all relevant solvents, PSar is not only excellently water soluble but also soluble in a range of organic solvents.[4]

Poly(N-substituted glycine)s are also known as polypeptoids (POI) and can be prepared by solid phase synthesis.[5] Such materials have been extensively investigated while polymerized POI were, until recently, limited to PSar. Within the group of pseudo-polypeptides, the polypeptoids play a particular role as they the are main-chain degradable by hydrolysis of the amide bond in the backbone.

Avantages and Potential Of Polypeptoids. Controlled or living polymerization is crucial for the preparation of advanced, defined and reproducible polymer materials, in particular for use as in polymer therapeutics or in nanomedicine. Also in this respect, POI are unique materials. The are accessible by solid phase synthesis, which allows the preparation of monodispers polymers and by living nucleophilic ring opening polymerization (NROP). In addition, the propagating species during NROP is a stable species, a secondary amine. Thus, in contrast to other controlled/living polymerization methods, permanent termination as well as chain coupling events are typically not detected. Thus, block copolypeptoids are available not only by sequential addition of new monomer but also through the use of purified and characterized polymer, even after prolonged storage under ambient conditions. To understand how far the limits of multi-block polymerization could be pushed, we studied the sequential polymerization of ten blocks of Sar-NCA. We deliberately chose to use ten consecutive blocks of the same monomer, as combination of different monomers would have made characterization of the product very difficult in general and virtually impossible by means of MALDI-ToF mass spectrometry. Again we were able to confirm that the propagating species is very stable of prolonged periods of time and we successfully prepared a decablock PSar. The dispersity decreased over the course of the polymerization, as is expected from theory. The dispersities for the decablock polysarcosine was determined to be Đ = 1.1 by gel permeation chromatography, however, preliminary MALDI-ToF experiments suggest that the disperstiy may be significantly smaller.

Moreover, the robustness of the NNCA polymerization was underlined by the introduction of the STOP-and-GO polymerization. Schlaad et al. used ammonium hydrochlorides and other acids to gain control over NCA polymerization.[6] We modified this concept and used the superacid trifluoromethanesulfonic acid to stop the NNCA polymerization transiently. After addition of various bases, the polymerization resumed to different extends. Surprisingly, non-nucleophilic bases of moderate strength, such as Hünig´s base were not able to quantitatively quench the protons from the polymer terminus. However, the strong non-nucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene was able to do so. The successful re-initiation of the polymerization was confirmed by MALDI-ToF MS. However, it should be noted that care must be taken not to initiate new polymer chains by the added base.

As mentioned before, POI are backbone degradable. The conditions to hydrolyse pseudo-polypeptides have been investigated to some extends using poly(2-oxazoline)s since an early report by Saegusa.[7] POI should be degraded similarly, but in contrast to POx, of which the product is the highly toxic poly(ethyleneimine) the degradation product of POI are the constituting N-substituted glycines. To which extend and in what time scale this would happen in a biological relevant setting remains unclear for both POx and POI but different reports suggest limited biodegradability for both.[8,9]

Limitations and Important Obstacles. Several issues can be identified that limit the applicability of POI as platform for advanced macromolecular engineering and as biomaterial. POI obtained from solid phase synthesis are difficult to produce in large scale and synthesis is cost- and time-intensive. In contrast, polypeptoids from NNCAs can be synthesized easily in multigram scale and further scale-up should not be a problem from an engineering point of view. However, monomer synthesis and stability are clearly the bottle-neck of NNCA polymerization. Although phosgene free synthesis is possible and the monomers can be obtained through straightforward three-step synthesis from readily available reagents, widespread application of NNCAs is likely to be limited by monomer availability**.**

Block copolymerization is an important aspect of advanced macromolecular engineering. Although block copolypeptoides are available through sequential monomer addition (*vide supra*), the steric influence of the substituent at the nitrogen must be expected to give rise to problems regarding the definition of block copolymers when a monomer with low steric influence (e.g. Sar-NCA) is to be polymerized after a block comprising a sterically demanding monomer (e.g. N-(n-propylglycine)-NCA). Here the conditions reflect a situation of slow initiation vs. fast propagation and polymers of low dispersities cannot be expected.

Whether POI are biodegradable remains to be elucidated. If they proof to be biodegradable within a reasonable time scale (several months to years), this platform may have a bright future. If not, it remains to be seen whether the materials properties can rival those of PEG or more prominent pseudo-polypeptides such as POx.

Conclusion

Polypeptoids from NNCAs other than polysarcosine have just emerged into polymer science. Their potential remains untapped and unclear, but a bright future may lay ahead.

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