ADVANCES IN POLYMER SCIENCE 202

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Peptide Hybrid Polymers

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Preface

Nature is superior to mankind, including polymer scientists, in many respects. While nature produces perfectly monodisperse and sequence-specific polypeptide and nucleotide polymers, polymer chemists are still struggling to find better methods for controlled or "living" polymerization and have yet to find strategies to control themonomer sequence beyond that of simple random and block copolymers. Natural polymers, and in particular proteins, also beat synthetic polymers in terms of structure formation. Although much progress has been made with generating complex nanoscale structures using synthetic block copolymers, the hierarchically organized tertiary and quaternary structures of proteins are still unmatched in their complexity. Natural materials also often outperform their synthetic counterparts. The catalytic activity of many enzymes and the mechanical properties of spider silk, for example, are unparalleled. Interestingly, for proteins the macroscopic properties are intimately related to chain length and monomer sequence since these control protein folding and structure formation. For synthetic polymers such exquisite (molecular) structure–property relationships have yet to be developed.

Over the past decade or so, these remarkable achievements by nature have been recognized by the polymer science community. This has led to an increased interest in the use of biological concepts to synthesize polymers or to control the structure and properties of synthetic polymers. Of particular interest are peptide hybrid polymers. Combining peptide and synthetic polymer segments into a single macromolecule offers interesting possibilities to synergize the properties of the individual components and to compatibilize bio- and synthetic systems.

This volume of *Advances in Polymer Science* is an attempt to provide an overview of the state of the art in the area of peptide hybrid polymers. The five articles in this volume cover a broad range of topics, from chemical and biological synthesis, to solution and solid-state self-assembly, to medical applications.

We would like to express our sincere thanks to all authors and reviewers who contributed to this volume for their excellent work. We hope that the articles will inspire further development in this exciting field.

Lausanne and Golm, April 2006

Harm-Anton Klok
Helmut Schlaad

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Polypeptide and Polypeptide Hybrid Copolymer Synthesis via NCA Polymerization

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Abstract This article summarizes recent developments in the synthesis of polypeptides and hybrid peptide copolymers. Traditional methods used to polymerize α-amino acid-N-carboxyanhydrides (NCAs) are described, and limitations in the utility of these systems for the preparation of polypeptides are discussed. Recently developed initiators and methods are also discussed that allow polypeptide synthesis with good control over chain length, chain length distribution, and chain-end functionality. The latter feature is particularly useful for the preparation of polypeptide hybrid copolymers. The methods and strategies for the preparation of such hybrid copolymers are described, as well as analysis of the synthetic scope of the different methods. Finally, issues relating to obtaining these highly functional copolymers in pure form are detailed.

Keywords Polypeptide · Block copolymer · *N*-carboxyanhydride · Living polymerization · Hybrid copolymer

Abbreviations

NCA	α-amino acid N-carboxyanhydride
AM	activated monomer
GPC	gel permeation chromatography
NACE	non-aqueous capillary electrophoresis
PBLG	poly(γ-benzyl-L-glutamate)
PMLG	poly(γ-methyl-L-glutamate)

PMDG	poly(γ-methyl-D-glutamate)
PML/DG	poly(γ-methyl-*rac*-glutamate)
PZLL	poly(ε-carbobenzyloxy-L-lysine)
PBLA	poly(β-benzyl-L-aspartate)
PMA	polymethylacrylate
PEG	polyethylene glycol
depe	bis(diethylphosphino)ethane

1 Introduction

Biological systems produce proteins that possess the ability to self-assemble into complex, yet highly ordered structures [1]. These remarkable materials are polypeptide copolymers that derive their properties from precisely controlled sequences and compositions of their constituent amino acid monomers. There has been recent interest in developing synthetic routes for preparation of these natural polymers as well as de novo designed polypeptide sequences to make products for applications in biotechnology (artificial tissues, implants), biomineralization (resilient, lightweight, ordered inorganic composites), and analysis (biosensors, medical diagnostics) [2, 3].

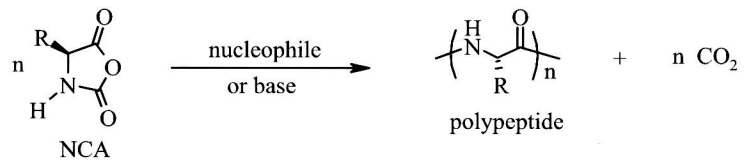
To be successful in these applications, it is important that materials can self-assemble into precisely defined structures. Peptide polymers have many advantages over conventional synthetic polymers since they are able to hierarchically assemble into stable ordered conformations [4]. Depending on the amino acid side chain substituents, polypeptides are able to adopt a multitude of conformationally stable regular secondary structures (helices, sheets, turns), tertiary structures (e.g. the β-strand-helix-β-strand unit found in β-barrels), and quaternary assemblies (e.g. collagen microfibrils) [4]. The synthesis of polypeptides that can assemble into non-natural structures is an attractive challenge for polymer chemists.

Synthetic peptide-based polymers are not new materials: homopolymers of polypeptides have been available for many decades and have only seen limited use as structural materials [5, 6]. However, new methods in chemical synthesis have made possible the preparation of increasingly complex polypeptide sequences of controlled molecular weight that display properties far superior to ill-defined homopolypeptides [7]. Furthermore, hybrid copolymers, that combine polypeptide and conventional synthetic polymers, have been prepared and combine the functionality and structure of peptides with the processability and economy of polymers [8, 9]. These polymers are well suited for applications where polymer assembly and functional domains need to be at length scales ranging from nanometers to microns. These block copolymers are homogeneous on a macroscopic scale, but dissimilarity between the block segments typically results in microphase heterogeneity yield-

ing materials useful as surfactants, micelles, membranes, and elastomers [10]. Synthesis of simple hydrophilic/hydrophobic hybrid diblock copolymers, when dispersed in water, allows formation of peptide-based micelles and vesicles potentially useful in drug and gene delivery applications [11]. The regular secondary structures obtainable with the polypeptide blocks provide opportunities for hierarchical self-assembly unobtainable with typical block copolymers or small-molecule surfactants.

Upon examining the different methods for polypeptide synthesis, the limitations of these techniques for preparation of hybrid copolymers readily become apparent. Conventional solid-phase peptide synthesis is neither useful nor practical for direct preparation of large polypeptides (> 100 residues) due to unavoidable deletions and truncations that result from incomplete deprotection and coupling steps. The most economical and expedient process for synthesis of long polypeptide chains is the polymerization of α-amino acid-N-carboxyanhydrides (NCAs) (Scheme 1) [12, 13]. This method involves the simplest reagents and high molecular weight polymers can be prepared in both good yield and large quantity with no detectable racemization at the chiral centers. The considerable variety of NCAs that have been synthesized (> 200) allows exceptional diversity in the types of polypeptides that can be prepared [12, 13].

Since the late 1940s, NCA polymerizations have been the most common technique used for large-scale preparation of high molecular weight polypeptides [14]. However, these materials have primarily been homopolymers, random copolymers, or graft copolymers that lack the sequence specificity and monodispersity of natural proteins. The level of control in NCA polymerizations has not been able to rival that attained in other synthetic polymerizations (e.g. vinyl addition polymerizations) where sophisticated polymer architectures have been prepared (e.g. stereospecific polymers and block copolymers) [15]. Attempts to prepare block copolypeptides and hybrid block copolymers using NCAs have always resulted in polymers whose compositions did not match monomer feed compositions and that contained significant homopolymer contaminants [16, 17]. Block copolymers could only be obtained in pure form by extensive fractionation steps, which significantly lowered the yield and efficiency of this method. The limitation of NCA polymerizations has been the presence of side reactions (chain termination and chain transfer) that restrict control over molecular weight, give broad mo-



Scheme 1

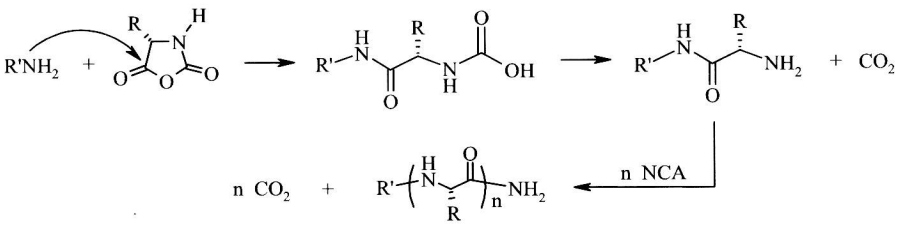
lecular weight distributions, and prohibit formation of well-defined block copolymers [19, 20]. Recent progress in elimination of these side reactions has been a major breakthrough for the polypeptide materials field.

2
Polypeptide Synthesis using NCAs

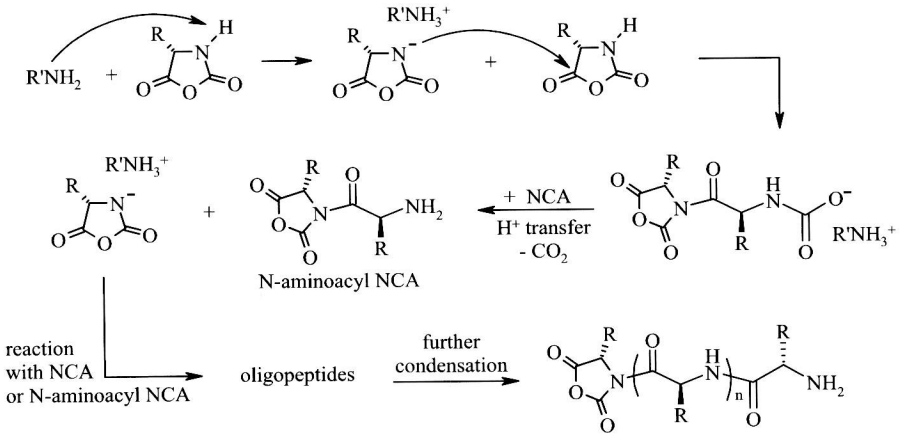
2.1
Conventional Methods

NCA polymerizations are traditionally initiated using many different nucleophiles and bases, the most common being primary amines and alkoxide anions [12, 13]. Primary amines, being more nucleophilic than basic, are good general initiators for polymerization of NCA monomers. Tertiary amines, alkoxides, and other initiators that are more basic than nucleophilic, have found use since they are in some cases able to prepare polymers of very high molecular weight where primary amine initiators cannot. Optimal polymerization conditions have often been determined empirically for each NCA and thus there have been no universal initiators or conditions by which to prepare high polymers from any monomer. This is in part due to the different properties of individual NCAs and their polymers (e.g. solubility) but is also strongly related to the side reactions that occur during polymerization.

The most likely pathways of NCA polymerization are the so-called "amine" and the "activated monomer" (AM) mechanisms [12, 13]. The amine mechanism is a nucleophilic ring opening chain growth process where the polymer could grow linearly with monomer conversion if side reactions were absent (Scheme 2). On the other hand, the AM mechanism is initiated by deprotonation of an NCA, which then becomes the nucleophile that initiates chain growth (Scheme 3). It is important to note that a given system can switch back and forth between the amine and AM mechanisms many times during a polymerization: a propagation step for one mechanism is a side reaction for the other, and vice versa. It is because of these side reactions that block copolypeptides and hybrid block copolymers prepared from NCAs using



Scheme 2



Scheme 3

amine initiators have structures different than predicted by monomer feed compositions and most likely have considerable homopolymer contamination. These side reactions also prevent control of chain-end functionality, which is crucial for preparation of hybrid copolymers.

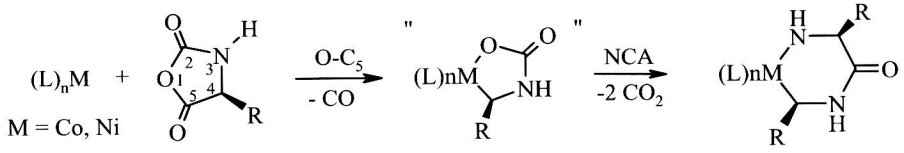
One inherent problem in conventional NCA polymerizations is that there is no control over the reactivity of the growing polymer chain-end during the course of the polymerization. Once an initiator reacts with a NCA monomer, it is no longer active in the polymerization and the resulting primary amine, carbamate, or NCA anion end-group is free to undergo a variety of undesired side reactions. Another problem is one of purity. Although most NCAs are crystalline compounds, they typically contain minute traces of acid, acid chlorides, or isocyanates that can quench propagating chains. The presence of other adventitious impurities, such as water, can cause problems by acting as chain-transfer agents or even as catalysts for side-reactions. Overall, the sheer abundance of potential reactions present in reaction media make it difficult to achieve a living polymerization system where only chain propagation occurs.

2.2 Transition Metal Initiators

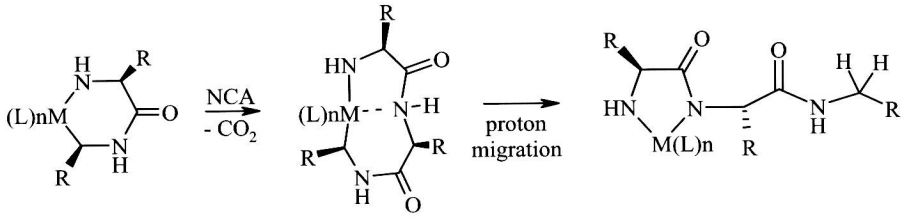
One strategy to eliminate side-reactions in NCA polymerizations is the use of transition metal complexes as active species to control addition of NCA monomers to polymer chain-ends. The use of transition metals to control reactivity has been proven in organic and polymer synthesis as a means to increase both reaction selectivity and efficiency [21]. Using this approach, substantial advances in controlled NCA polymerization have been realized in recent years. Highly effective zerovalent nickel and cobalt initiators (i.e. bpyNi(COD) [22, 23] and $(PMe_3)_4Co$ [24]) were developed by Deming that

allow the living polymerization of NCAs into high molecular weight polypeptides via an unprecedented activation of the NCAs into covalent propagating species. The metal ions can be conveniently removed from the polymers by simple precipitation or dialysis of the samples after polymerization.

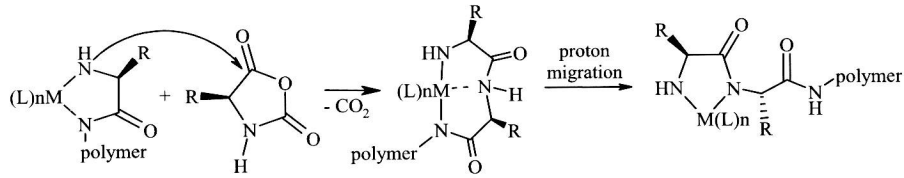
Mechanistic studies on the initiation process showed that both these metals react identically with NCA monomers to form metallacyclic complexes by oxidative addition across the anhydride bonds of NCAs [22–24]. These oxidative-addition reactions were followed by addition of a second NCA monomer to yield complexes identified as six-membered amido-alkyl metallacycles (Scheme 4). These intermediates were found to further contract to five-membered amido-amidate metallacycles upon reaction with additional NCA monomers. This ring contraction is thought to occur via migration of an amide proton to the metal-bound carbon, which liberates the chain-end from the metal (Scheme 5) [25]. The resulting amido-amidate complexes were thus proposed as the active polymerization intermediates. Propagation through the amido-amidate metallacycle was envisioned to occur by initial attack of the nucleophilic amido group on the electrophilic C_5 carbonyl of an NCA monomer (Scheme 6). This reaction would result in a large metallacycle that



Scheme 4



Scheme 5



Scheme 6

could contract by elimination of CO_2. Proton transfer from the free amide to the tethered amidate group would further contract the ring to give the amido-amidate propagating species, while in turn liberating the end of the polymer chain.

In this manner, the metal is able to migrate along the growing polymer chain, while being held by a robust chelate at the active end. The formation of these chelating metallacyclic intermediates appears to be a general requirement for obtaining living NCA polymerizations using transition metal initiators. These cobalt and nickel complexes are able to produce polypeptides with narrow chain length distributions ($M_w/M_n < 1.20$) and controlled molecular weights ($500 < M_n < 500\,000$) [26]. This polymerization system is very general, and gives controlled polymerization of a wide range of NCA monomers as pure enantiomers (D or L configuration) or as racemic mixtures. By addition of different NCA monomers, the preparation of block copolypeptides of defined sequence and composition is feasible [7, 27].

2.3 New Developments

In the past two years, a number of new approaches have been reported for obtaining controlled NCA polymerizations. These approaches share a common theme in that they are all improvements on the use of classical primary amine polymerization initiators. This approach is attractive since primary amines are readily available and since the initiator does not need to be removed from the reaction after polymerization. In fact, if the polymerization proceeds without any chain breaking reactions, the amine initiator becomes the C-terminal polypeptide end-group. In this manner, there is potential to form chain-end-functionalized polypeptides or even hybrid block copolymers if the amine is a macroinitiator. The challenge in this approach is to overcome the numerous side-reactions of these systems without the luxury of a large number of experimental parameters to adjust.

In 2004, the group of Hadjichristidis reported the primary amine-initiated polymerization of NCAs under high vacuum conditions [28]. The strategy here was to determine if a reduced level of impurities in the reaction mixture would lead to fewer polymerization side reactions. Unlike the vinyl monomers usually polymerized under high vacuum conditions, NCAs cannot be purified by distillation. Consequently, it is doubtful the NCAs themselves can be obtained in higher purity under high vacuum recrystallization than by recrystallization under a rigorous inert atmosphere. However, the high vacuum method does allow for better purification of polymerization solvents and the n-hexylamine initiator. It was found that polymerizations of γ-benzyl-L-glutamate NCA (Bn-Glu NCA) and ε-carbobenzyloxy-L-lysine NCA (Z-Lys NCA) under high vacuum in DMF solvent displayed all the characteristics of a living polymerization system [28]. Polypeptides could be prepared with