Synthesis of Polypeptides with Activated Amino Acid Monomers

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Commonly, three different synthetic pathways are used to prepare peptides in the laboratory: via the polymerization of amino acid N-carboxyanhydrides (NCAs), amino acid N-thiocarboxyanhydrides (NTAs), and N-phenoxycarbonyl amino acids (NPCs); via various stepwise coupling reactions of α-amino acids, such as during solid phase peptide synthesis (SPPS); or via recombinant DNA techniques for expressing peptides in microorganisms. Polypeptides that are synthesized through SPPS have controlled primary sequences and can fulfill certain functionalities, but it is difficult to create high molecular weight polypeptides above 100 residues, due to the inevitable side reactions. Recombinant DNA techniques can create polypeptides with specific sequences and high molecular weights. Moreover, they allow for peptide production on a very large scale. However, specialized equipment, which is not readily available in most synthetic laboratories, is needed for this method. The polymerization of activated amino acid monomers enables the formation of bioactive and high molecular weight polypeptides in a facile and expedient manner. The process begins with the conversion of amino acids into the corresponding activated monomers; afterwards, polymerization is initiated in the presence of certain initiators. Although polypeptides that are synthesized in this way lack precise sequence control, their synthetic advantages make this method attractive and economical for synthesizing polypeptides in large quantities.

Keywords: α-Amino Acid N-Carboxy Anhydrides ; polypeptide synthesis ; polypeptide

1. Introduction

Cancer is the second leading cause of death worldwide. The efficacy of many anti-tumor drugs is often reduced via rapid blood clearance, non-specific biodistribution, or poor accumulation and retention in tumor sites [1]. In addition, many anti-tumor drugs have inherent limitations, such as poor water solubility and low cellular uptake [2]. Therefore, many kinds of drug delivery systems (DDS), based on polypeptides, polyesters, mesoporous silica, gold nanoparticles, etc., have been developed to address these deficiencies [3].

Among them, polypeptides have received extensive attention due to their innate biocompatibility and degradability [2]. When dispersed in water, polypeptides with hydrophilic/hydrophobic segments can form micelles, vesicles, hydrogels, and capsules [4][5]. Due to the above-mentioned versatile structures, and their biocompatibility and biodegradability, polypeptides are extensively studied as carriers for drug delivery [6]. With the development of a variety of well-controlled polymerization chemistries, polypeptides can be easily integrated into other materials to synthesize hybrid materials with even more versatile features for self-assembly and controlled release [2][4][7]. These polypeptides and polypeptide-based hybrid materials exhibit controlled drug-release properties because of their natural amino acid residues with innate stimuli-responsive characteristics or other responsive moieties from their hybrid materials [8].

2. Ring-Opening Polymerization (ROP) of α-Amino Acid N-Carboxy Anhydrides (NCAs)

During 1906 and 1908, Hermann Leuchs published three papers that described the synthesis and properties of α-amino acid N-carboxyanhydrides (NCAs) [9]. NCAs were discovered by coincidence when Leuchs attempted the purification of N-ethoxycarbonyl α-amino acid chlorides. However, Leuchs changed his area of research completely from NCAs to the chemistry of strychnine alkaloids after 1907, due to the lack of proper analytical methods for NCA-polymerized products and the wrong estimation of their structure. As a result of his pioneering work, NCAs are commonly referred to as Leuchs' anhydrides [9].

Presently, the most important and economical method for synthesizing NCAs is called the Fuchs–Farthing method, in which phosgene or its derivatives are used as a cyclizing agent. In 1922, Friedrich Fuchs described the preparation of NCA of N-phenylglycine via the phosgenation of N-phenylglycine in an aqueous solution. Based on Fuchs' reaction, A. C.

Farthing made some modifications and prepared some other NCAs such as NCAs of glycine, DL-β-phenylalanine, L-leucine, etc., in 1950 [10]. Using this system, it is typical to prepare an NCA via the reaction of an α-amino acid with phosgene in ethyl acetate (also dichloromethane and dioxane) at elevated temperatures (~60 °C) under an inert atmosphere [11][12][13].

The ring-opening polymerization (ROP) of NCAs can produce 'living' polypeptides. The expression "living polymers" was first described by M. Szwarc in 1956, when he tried to synthesize polystyrene via the polymerization of styrene [14]. The term "living" mainly means that when the polymerization is terminated either by 100% conversion of the monomers, by cooling, or by precipitation, the reactive end group that is responsible for chain growth remains unchanged (alive). In this way, polymers with controlled lengths and low polydispersity can be prepared. Moreover, it is also possible to form block copolymers via the sequential addition of different monomers [15].

A suitable initiator is essential for the polymerization of NCAs. Because of the numerous reactive sites on the five-membered NCA ring, i.e., the 2- and 5-carbonyl groups, 3-NH and 4-CH, there is a wide range of initiators available for the initiation of NCA polymerizations, e.g., protonic nucleophiles, nonprotonic bases, metal salts, organometallics, transition metals, and their analogues of strong bases [15][16]. All of the initiators and reaction mechanisms are explained in detail in the reference [6]. Primary amines are presently one of the most common initiators for the ROP of NCAs, because of the following two reasons. Firstly, using primary amines as initiators could prepare polypeptides with a living end group (amino group). Secondly, the highly nucleophilic and sterically unhindered nature of primary amines allows them to initiate polymerization rapidly, resulting in smaller polydispersity values [9]. The polymerization involves three steps: carbonyl addition, ring opening, and decarboxylation. In a first step, a nucleophilic primary amine bearing a lone pair of electrons attacks the C_5 carbon of the NCA to initiate ring opening. This is followed by a decarboxylation step and regeneration of the amine. Finally, the regenerated amine attacks the molecule of another NCA to increase the length of the polypeptide chain. This pathway allows for the synthesis of co-polypeptides with several different blocks, with defined terminal structures and with narrow polydispersities [9].

However, there are two main drawbacks that limit the use of NCA on an industrial scale. Firstly, with phosgene or its derivatives, very poisonous educts are needed for the synthesis of NCA. Secondly, the storage of NCA is very difficult, due to its sensitivity to moisture and heat. Thus, alternative monomers for the large-scale synthesis of polypeptides have been extensively studied over the years [16][17].

3. Schemes of Ring-Opening Polymerization (ROP) of α-Amino Acid N-Thiocarboxyanhydrides (NTAs)

α-Amino acid N-thiocarboxy anhydrides (NTAs), as thio-analogues of NCAs, are promising alternative monomers, due to the fact that they are tolerant to moisture and heat, and because their synthesis does not require the use of phosgene derivatives. In 1950, Aubert et al. first reported the synthesis of the NTA of glycine. This reaction involves two steps [18]. In the first step, the potassium salt of glycine is reacted with ethyl alkoxydithioformate to give N-alkoxythiocarbonyl glycine. Then, the NTA of glycine is obtained via the cyclization in presence of PBr_3 or PCl_3. This method is most widely used to synthesize NTA monomers, and many kinds of amino acid NTA were prepared over the next few decades using this method [19].

NTAs were usually used for the stepwise syntheses of polypeptides, but rarely for polymerizations before 2000. The ROP of NTAs was first investigated by Kricheldorf et al. in the 1970s. They initiated the polymerizations of several amino acid NTAs with a primary amine, and demonstrated that the polymerization underwent the normal amine mechanism (NAM) as in primary amines-initiated NCA polymerization[19]. However, they thought that NTAs were not promising candidates for the preparation of high molar mass polypeptides, because of the lower yields and lower DPs [20]. Jun Ling's groups believe that a low reaction temperature and inappropriate solvents are also responsible for the uncontrollable polymerization. Therefore, they developed NTA polymerization in polar solvents in a controlled manner, using Tyr-NTA and DOPA-NTA as examples. This polymerization produced polypeptides at a high yield (over 80%) and with predictable molar masses. However, the DPs of the products were below 50 [21]. There is still a great challenge to improve the controlled NTA polymerization in polar solvents. Carbonyl sulfide, a toxic gaseous compound, is released during the ROP of NTAs, which also limits the utilization of NTAs at a larger scale.

The ROP of N-substituted NTA (NNTA), which produces polypeptoids, was quite well-controlled, and many kinds of polypeptoids were synthesized with high yields and predictable MW because of the absence of N-H and the better solubility of the polypeptoids [19].

4. Polymerization of N-Phenoxycarbonyl Amino Acids (NPCs)

NPCs are another class of monomers that can be used for polypeptide synthesis [17]. Inspired by Kricheldorf's synthesis of polyamides using the monomer R-(N-aryloxycarbonyl)amino-ω-carboxylalkane, in 2008, Endo et al. first synthesized several amino acid NPCs and studied their polymerization [22][23]. NPCs are also called amino acid urethane derivatives (UDs) if hydrogens at the phenoxy group of the NPC are substituted. Two routes are mainly used to prepare NPCs. One is via a reaction between amino acids and phenyl chloroformate. The other one is via a reaction between amino acids and diphenyl carbonate (DPC) in the presence of an excess base (Et_3N or tetrabutylammonium hydroxide). Using these two methods, Endo's group prepared a large variety of NPCs over the course of 10 years, and proved that NPCs are promising alternatives for polypeptide synthesis [17].

The controlled polymerization of these NPCs can be achieved via a reaction in DMAc solution at 60 °C with a primary amine as an initiator, which can form polypeptides with predictable MW and narrow MW distribution (less than 1.2). The described polymerization may follow the same mechanism as the polymerization of NCAs, because they observe the *in situ* formation of NCAs using ^1H NMR before and during the polymerizations [17].

In the following, the history of Endo's research on the polymerization of NPCs will be briefly introduced, because the synthesis allows for the production of polypeptides efficiently without using hazardous chemicals such as phosgene. In 2008, Endo et al. investigated the potential of three activated urethane-type derivatives of λ-benzyl-L-glutamate as monomers for polypeptide synthesis [22]. They studied the influence of different factors on the polymerization reaction, including solvents, temperature, monomer concentration, and monomer types. They concluded that polar solvents such as Dimethylacetamide (DMAc) and elevated temperatures (60 °C) were essential for polymerization. Among the three urethanes, (N-phenyloxycarbonyl-λ-benzyl-L-glutamate, 4-chlorophenyloxycarbonyl-λ-benzyl-L-glutamate, and 4-nitrophenyloxycarbonyl-λ-benzyl-L-glutamate), 4-nitrophenoxycarbonyl-λ-benzyl-L-glutamate was the most reactive urethane for producing poly(BLG) efficiently, as was expected due to the high electron deficiency of the nitrophenoxycarbonyl group. At that time, they did not add any initiators, and terminal structures were also not defined. Later they used N-(4-nitrophenoxycarbonyl)-γ-benzyl-L-glutamate as a monomer, and butylamine as an initiator for the polymerizations, and they revealed that butylamine was incorporated into the terminal end of poly(BLG), while the other terminal end was endowed with an amino group [23]. Later, in 2013 and 2014, they reported the synthesis of a series of hydrophilic polypeptides, including poly-L-leucine, poly-L-phenylalanine, poly-L-valine, etc., as well as a series of hydrophobic polypeptides, including poly-L-serine, poly-L-cysteine, poly-L-asparagine, etc., with predictable molecular weights, narrow molecular weight distributions, and well-defined terminal structures [24][25]. Based on these years of research, Endo and Sudo have recently written a very good review, in which more knowledge about the history and development of NPCs can be found [17].

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