SYNTHETIC PATHWAYS TO POLYDEPSIPEPTIDES

Pieter J. Dijkstra and Jan Feijen*

Department of Chemical Technology and Institute for Biomedical Technology, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands

SUMMARY

The synthesis of polydepsipeptides through polycondensation of linear depsipeptides comprising an activated carboxylic endgroup and an amine end group or through ring-opening polymerization of morpholine-2,5-diones has been reviewed. Polydepsipeptide synthesis allows the preparation of a large variety of biodegradable polymers which are either unsubstituted, alkyl substituted or contain functional groups in the side chains of the polymers.

INTRODUCTION

During the last three decades the research on synthetic materials for medical and pharmaceutical applications has been rapidly growing. These materials are called 'biomaterials' and have been defined as ‘nonviable’ materials, used in a medical device application, that is intended to interact with a biological system (Ref. 1). A wide variety of metals, ceramics, polymers and composites are currently applied as biomaterials. Each biomaterial has to fulfill a set of requirements with respect to its specific application. Biomaterials can be divided in two main classes, i.e. nondegradable and degradable materials. Nondegradable biomaterials are relatively inert in a biological environment. Typical examples of nondegradable biomaterials are metals such as titanium alloys, ceramics such as aluminium oxides and synthetic polymers such as poly(ethylene), poly(tetrafluoroethylene) and silicone elastomers. Nondegradable biomaterials are mainly used for permanent prostheses such as heart valves, arterial and vascular prostheses and artificial joints. However nondegradable biomaterials are also used for temporary therapeutic aids such as sutures, bone plates and screws, which have to be surgically removed after healing.

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Degradable biomaterials are designed to degrade in vivo in a controlled manner over a predetermined implantation period. The application of degradable biomaterials for temporary artificial implants can have important advantages. Firstly, degradable biomaterials do not have to be removed after use by a second operation, because the degradation products formed can be excreted from the human body via natural pathways. Secondly, the use of biodegradable materials may lead to a better recovery of the biological system, because progressive loss of mechanical strength of the implant will lead to a continuous stimulation of the healing tissue (Ref. 2-4).

Biodegradable polymers are the most important representatives of degradable biomaterials, especially because polymers can be properly adjusted by various chemical and physical means such as copolymerization, chemical functionalization and blending. Important classes of biodegradable polymers are the polyanhydrides, polyorthoesters, polyamino acids and aliphatic polyesters.

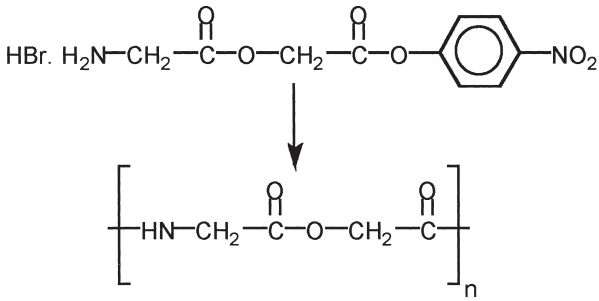
Degradation of most of these polymers takes place by hydrolysis of (enzymatically) hydrolytically unstable bonds (e.g. ester, amide and anhydride bonds) present in these materials. Biodegradable polymers have already found widespread applications and are increasingly investigated for possible use in a wide variety of temporary medical aids such as sutures, bone plates and screws, tissue engineered products like artificial skin substitutes, vascular grafts and especially in carrier systems for the controlled release of drugs (Ref. 5-8).

It is essential that the degradation products of biodegradable polymers are non-toxic. A useful approach to obtain degradation products with low toxicity is to design polymers which are derived from metabolites found in the human body. Poly(α–hydroxy acid)s and poly(α–amino acid)s are examples of biodegradable polymers developed on the basis of this approach and during the past decades the emphasis of studies has been on poly(α–hydroxy acid)s. Several years ago the synthesis of novel polyesteramides based on α–hydroxy acids and α–amino acids was initiated. Up to now several papers have been published on the synthesis and properties of these polymers. Here we summarize the approaches which have been used in the synthesis of these polymers, also called polydepsipeptides.

SYNTHESIS OF POLYDEPSIPEPTIDES

Polydepsipeptides, copolymers of α–hydroxy acids and α–amino acids, are the most important representatives of biocompatible biodegradable polyesteramides. The synthesis of sequential polydepsipeptides was first reported by Stewart (Ref. 9). This investigator synthesized poly(Gly-Glc), poly(L-Pro-L-Ala-Glc) and poly(L-Ala-L-Ala-Glc) (Glc=

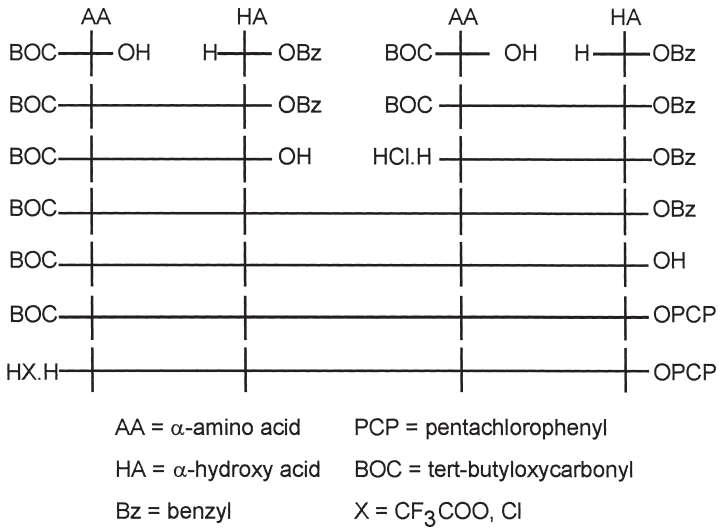
glycolic acid) by solution polymerization in dimethylformamide of preformed di- or tridepsipeptides of which the carboxyl group was activated with p-nitrophenol.



Scheme 1. Synthesis of Poly(Gly-alt-Glc) from a linear depsipeptide containing an activated ester group. (Gly = glycine, Glc = glycolic acid).

The yields varied from 68 to 90%, and the molecular weights of the polymers were probably quite low. The synthesis of polydepsipeptides was more extensively studied by Goodman et al. and Katakai et al. (Ref. 10,11).

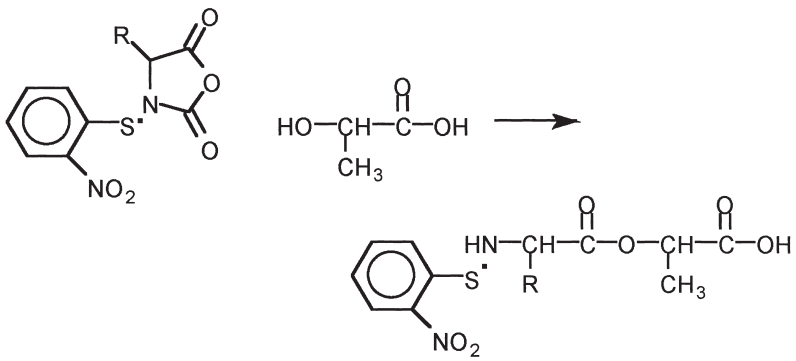
The necessary tri-, tetra- and pentadepsipeptide monomeric units were synthesized via multi-step synthetic routes, using synthetic procedures commonly used in the peptide chemistry.



Scheme 2. Synthesis of tetradepsipeptide monomeric units

To illustrate these synthetic routes the synthesis of tetradepsipeptide activated esters is outlined in Scheme 2. First the α–amino acid was N-protected by the tert-butyloxycarbonyl (BOC) group, while the carboxylic acid group of the α–hydroxy acid was protected by the benzyl group. The BOC α–amino acid and the α–hydroxy acid benzyl ester were coupled by formation of an ester bond. Subsequently either the BOC protective group or the benzyl protective group was selectively removed with hydrochloric acid or catalytic hydrogenation, respectively. The differentially deprotected didepsipeptide moieties were coupled by formation of an amide bond to give the protected tetradepsipeptide units. The benzyl group was removed by catalytic hydrogenation and the resulting carboxylic acid group was transferred into a pentachlorophenyl ester. Finally the BOC group was removed by reaction with hydrochloric acid or trifluoroacetic acid to yield the tetradepsipeptide pentachlorophenyl ester hydrochlorides or trifluoro acetate salts. Tri- and pentadepsipeptide monomeric units were prepared by modification and coupling reactions of the intermediate differentially deprotected depsipeptide moieties.

The synthetic procedure outlined in Scheme 2 could not be used in the synthesis of depsipeptide moieties containing glutamic acid and lysine residues. During the synthetic procedure the pendant groups of these residues (carboxylic acid and amine groups, respectively) had to be blocked by protecting groups, which were simultaneously removed in the deprotection reaction of the benzyl group of the α–hydroxy acid residue using catalytic hydrogenation. To avoid this problem an alternative synthetic route was developed involving the coupling of an α–amino acid and an α–hydroxy acid without protection of the carboxylic acid group of the α–hydroxy acid (Scheme 3) (Ref. 12).



Scheme 3. Coupling of an α–amino acid and an α–hydroxy acid based on an (o-nitrophenylsulphenyl)-N-carboxy-α–amino acid anhydride (NPS-NCA). R = pendant functional group.

This route was based on the use of (o-nitrophenylsulphenyl)-N-carboxy-α–amino acid anhydrides (NPS-NCA). The NPS-NCA derivatives were reacted with unprotected lactic acid, whereafter the carboxylic acid group of the resulting didepsipeptides was esterified with pentachlorophenol to give the NPS protected didepsipeptide pentachlorophenyl esters. The NPS group of the active esters was removed by treatment with hydrochloric acid. The resulting didepsipeptide active ester hydrochlorides were coupled with NPS protected α–amino acids to give tridepsipeptides. The NPS group was removed by reaction with hydrochloric acid to yield the tridepsipeptide activated ester hydrochlorides. These compounds were either polymerized or coupled with another NPS protected α–amino acid, followed by deprotection, to provide tetradepsipeptide monomeric units. The synthetic procedure outlined in Scheme 3 has also been used for the synthesis of depsipeptide monomeric units with alkyl side chains (Ref 13).

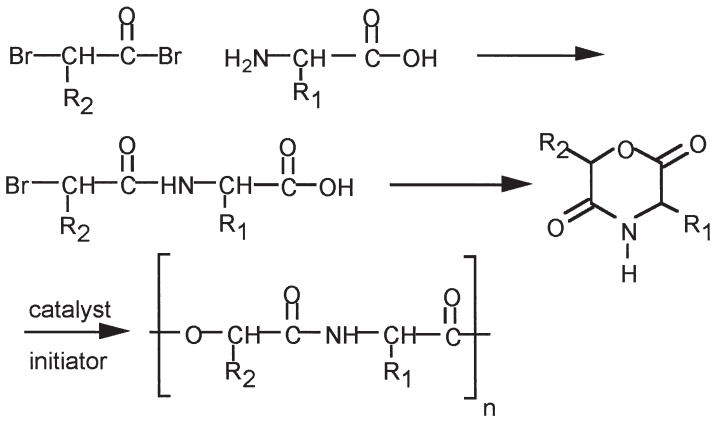
For the synthesis of polydepsipeptides without protected pendant functional groups, such as poly(L-Val-alt-L-Lac) and poly((L-Ala)$_n$-co-L-Lac) with n = 1, 2 or 3, best results were obtained by thermal polymerization of trifluoro acetate salts of tri- or tetradepsipeptide pentachlorophenyl esters which had been precipitated on an inert matrix of Celite. Intrinsic viscosities up to a value of [η] = 0.57 dl/g (dichloroacetic acid, 25 ^{o}C) were obtained. Solution polymerization of trifluoro acetate salts or hydrochlorides of tri- or tetradepsipeptide pentachlorophenyl esters in dimethylsulfoxide (DMSO) in the presence of equimolar amounts of triethylamine gave polymers with low molecular weights ([η] , 0.14 dl/g (dichloroacetic acid, 25 ^{o}C)). When salts of didepsipeptide activated pentachlorophenyl esters were used as monomers in the polymerization reaction, the main products were cyclic didepsipeptides.

Polydepsipeptides having protected pendant functional groups, such as poly(L-Ala-co-γ-benzyl L-Glu-co-L-Lac), poly((L-Ala)$_n$-co-γ-ethyl L-Glu-co-L-Lac) with n = 1, 2 or 3 and poly(L-Ala-co-N$^{\varepsilon}$-(benzyloxycarbonyl)-L-Lys-co-L-Lac), were prepared by solution polymerization of the depsipeptide pentachlorophenyl esters in DMSO or N,N-dimethylacetamide in the presence of triethylamine. The intrinsic viscosities of the polydepsipeptides ranged from [η] = 0.17 - 0.84 dl/g (dichloroacetic acid, 25 ^{o}C).

Ring-opening Polymerization

The multi-step synthetic routes discussed above are inadequate for a large scale preparation of polydepsipeptides. Moreover, depsipeptides composed of a single amino acid and a single hydroxy acid residue do easily cyclize to 6-membered rings, the morpholine-2,5-diones. In order to prepare alternating copolymers linear depsipeptides

comprising two hydroxy and two amino acid residues in an alternating sequence must be prepared as monomers. Based on these disadvantages Feijen et al. (Ref. 14,15) suggested that ring-opening polymerization of cyclic didepsipeptides (morpholine-2,5-dione derivatives) could be an attractive alternative to obtain (alternating) polydepsipeptides in a more facile way (Scheme 4). The underlying rationale for this idea came from the facile preparation of poly(glycolic acid) and poly(lactic acid) by ring-opening polymerization of the six-membered cyclic dimers glycolide and lactide, respectively. Morpholine-2,5-dione derivatives were prepared by reaction of α–amino acids with α–halo acid halides, followed by cyclization of the intermediate N-(2-halogenacyl)-amino acids (Scheme 4). Because a variety of α–amino acid residues can be used to synthesize morpholine-2,5-dione derivatives, ring-opening polymerization of these monomers provides a method to prepare a wide variation of alternating polydepsipeptides.



Scheme 4. Synthetic route to an alternating polydepsipeptides through ring-opening polymerization of a 3-methyl-morpholino-2,5-dione

Helder et al. (Ref.14) investigated the ring-opening polymerization of (6RS)-methylmorpholine-2,5-dione (Scheme 4, R_1 = H, R_2 = Me). Poly(Gly-alt-DL-Lac) with the highest molecular weight was obtained when the polymerization was carried out in the bulk using stannous octoate as an initiator, with molar monomer to initiator ratios (M/I) of 500 - 1750 and at relatively low reaction temperatures (110 ^{o}C). The molecular weights of the glycine-alt-DL-lactic acid polymers (Mw ~ 2 x 10^4) were considerably lower than

the molecular weights of P(L)LA (Mw~10^5) obtained in the stannous octoate initiated bulk polymerization of L-lactide using similar reaction conditions. Poly(Gly-alt-DL-Lac) is an amorphous polymer with a glass transition temperature of 109 ^oC. Yonozawa et al. (Ref. 16) reported the ring-opening polymerization of (6RS)-isopropylmorpholine2,5-dione. Ring-opening polymerization in the bulk at 185 ^oC with ZnO as an initiator gave the corresponding polydepsipeptide with low molecular weights ($\eta inh \leq 0.12$ dl/g (N,N-dimethylacetamide, 30 ^oC)). The polymerization of (3S,6RS)-3,6-dimethyl morpholine-2,5-dione (Scheme 4, R_1 = Me, R_2 = Me) in the bulk at 140 - 145 ^oC using stannous octoate as an initiator (M/I = 500 - 2500) yielded L-Ala-alt-DL-Lac polymers with molecular weights upto a value of Mw = 2.3 x 10^4. The polymers are amorphous with a glass transition temperature of 94 ^oC (Ref. 17).

Fung and Glowaky (Ref. 18) investigated the ring-opening polymerization of several 3-alkyl substituted morpholine-2,5-diones (Scheme 4, R_1 = alkyl group, R_2 = H). The polymerizations were carried out in the bulk with stannous octoate or zirconium acetoacetonate as initiators (M/I = 3000). Polydepsipeptides with inherent viscosities ranging from η_{inh} = 0.61 - 0.77 dl/g (dichloracetic acid, 25 ^oC) were obtained. The polymerization of (3S)-methylmorpholine-2,5-dione (Scheme 4, R_1 = Me, R_2 = H) yielded semi-crystalline poly(L-Ala-alt-Glc) with a melting temperature of 232 ^oC, whereas the polymerization of (3RS)-methylmorpholine-2,5-dione gave a completely amorphous polymer. Polydepsipeptides with N-alkyl substituted α–amino acid residues, such as poly(N-methylglycine-alt-DL-lactic acid) and poly(N-isopropylglycine-alt-DL-lactic acid) could not be obtained by ring-opening polymerization of the corresponding N-alkyl substituted morpholine-2,5-diones.

The reported synthesis of alternating polydepsipeptides by ring-opening polymerization of morpholine-2,5-dione derivatives having different alkyl substituents offers a variety of polymeric materials. Recently Jörres et al. did synthesize several alkyl substituted morpholine-2,5-diones and studied the ring-opening polymerization using stannous octoate as a catalyst or stannous acetylacetonate as an initiator. It has been shown that racemization takes place in the hydroxy acid residue and not in the amino acid residue (Ref. 19,20). The ring-opening (co)polymerization of morpholine-2,5-dione derivatives with pendant functional groups has been investigated by several researchers (Ref. 21). The use of trifunctional α-amino acids like e.g. L-aspartic acid, L-glutamic acid, L-lysine, L-serine and L-cysteine in the synthesis of morpholine-2,5-dione derivatives offers a synthetic route to biodegradable polymers with pendant carboxylic acid, amine, hydroxyl and thiol groups, respectively. Therefore, α-amino acid residues with protected side chain functional groups should be incorporated into morpholine-2,5-dione derivatives, which

can be ring-opening (co)polymerized, followed by deprotection of the pendant functional groups.

The synthesis of morpholine-2,5-dione derivatives, in which R_1 is a protected side chain carboxylic acid, amine or thiol group, has been reported by different groups. The ring-opening homopolymerization, as well as the ring-opening copolymerization of these morpholine-2,5-dione derivatives with either DL-lactide, L-lactide, glycolide or ε-caprolactone and the deprotection of the pendant protected functional groups of the intermediate copolymers has been described. The synthesis of these polymers is analogous to that given in Scheme 4.

Morpholine-2,5-dione derivatives having substituents with benzyl protected carboxylic acid, benzyloxycarbonyl protected amine and p-methoxybenzyl protected thiol groups, respectively, were prepared by reaction of N-[(2RS)-bromopropionyl]amino acids with TEA in DMF. The ring-opening homopolymerization of morpholine-2,5-dione derivatives with protected functional substituents failed, due to the low reactivity of the monomers. However, these derivatives could be copolymerized with ε-caprolactone and DL-lactide, to give polyesteramides with pendant protected functional groups. The selective removal of the benzyl and benzyloxycarbonyl protective groups by catalytic hydrogenation yielded copolymers with pendant carboxylic acid and amine groups, respectively.

Successful homopolymerization of a morpholine-2,5-dione constructed from a glycolic acid and a protected aspartic acid residue was firstly reported by Ouchi et al. (Ref. 21). The poly(Glc-alt-Asp) was synthesized using stannous octoate as a catalyst and was obtained as a polymer with molecular weight of ~ 5000. Deprotection of the benzyl protecting group was accomplished with trifluoromethanesulfonic acid-thioanisole in trifluoroacetic acid. Similarly the poly(Glc-alt-Glu) and poly(Glc-alt-Lys) were prepared. The homopolymerization of the poly(Glc-alt-Asp) was recently again described by Wang and Feng (Ref. 21). Morita described the ring-opening polymerization of a protected serine glycolic acid morpholine dion and its deprotection (Ref. 21).

Copolymerization of morpholine-2,5-dione derivatives with other lactones provides a possibility to prepare various biodegradable polydepsipeptides with a wide range of properties which depend on the composition of the copolymers. Comonomers used comprise p-dioxanone, lactide, caprolactone and glycolide (Ref. 17,18,21,22).

Copolymerization of morpholine-2,5-dione with caprolactone or lactides generally affords random copolymers. Molecular weights are higher than found in the homopolymerization of the cyclic depsipeptides but do decrease with increasing morpholine-2,5-dione in the feed. In many cases the polymers will be amorphous or in case of copolymerization with

L-Lactide the melting temperature and crystallinity rapidly decreases with increasing morpholine-2,5-diones ratios.

CONCLUSIONS

Polydepsipeptides can be synthesized from linear depsipeptides through polycondensation of activated depsipeptide esters constructed from at least three amino/hydroxy acid units. Cyclic depsipeptides (morpholine-2,5-diones) are formed as a main product when dimers composed of an amino and hydroxy acid unit are polymerized. Morpholine-2,5-diones can be conveniently ring-opening polymerized to alternating polydepsipeptides. Polymers with molecular weights around 10.000 are generally obtained. Ring-opening proceeds through cleavage of the ester group of the morpholine-2,5-diones. Racemization of the hydroxy acid residue has always been observed in these polymerization and most likely results from the high polymerization temperatures needed. Morpholine-2,5-diones comprising e.g. protected Aspartic acid or Lysine residues do polymerize to give functionalized polymers with pendant carboxylic acid or amine groups after deprotection. This shows the versatility of polymers that can be prepared from these morpholine-2,5-diones.

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