Active Polycondensation: From Peptide Chemistry to Amino Acid Based Biodegradable Polymers

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Summary: New polycondensation (PC) methods of polymer synthesis using non-traditional active derivative of dicarboxylic acids are reviewed. The new PC methods are named by general name "Active Polycondensation" (APC) to tell them from traditional low-temperature PC. The most of these methods are based on well known in peptide chemistry approaches to the activation of carboxylic groups. In the present paper the syntheses of heterochain polymers of basic classes – polyamides, polyesters, polyurethanes, polyureas, and polybenzazoles by interaction of various active diesters with di- and polyfunctional nucleophiles are discussed in brief. Special attention is given to the synthesis of non-conventional heterochain macromolecular systems, in particular poly(ester amide)s (PEAs), composed of naturally occurring α–amino acids and other non-toxic building blocks like fatty diacids and diols - synthetic analogues of naturally occurring amino acid based polymers – peptides and proteins. The synthesis and properties, biodegradation, and some practical applications of PEAs are discussed in brief.

Keywords: α–amino acids, active polycondensation, biodegradable polymers, biomedical applications, heterochain polymers, poly(ester amide)s

Introduction

Polycondensation (PC) is traditionally subdivided into two types: high-temperature PC (HTPC) and low-temperature PC (LTPC).[1-3] In this paper the overview of the PC methods is chiefly confined by the PC reactions based on aminolysis of derivatives of dicarboxylic acids (polyamidations).

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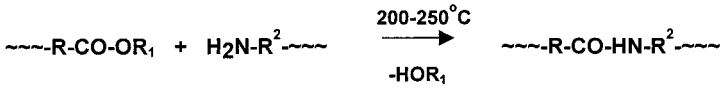
The HTPC was developed mostly in 20-30s of the last century. Under the conditions of HTPC (commonly bring about at 200-250°C in a melt or in a high-boiling solvent) monomers with low reactivity (e.g. dicarboxylic acids or their alkyl esters) are used:

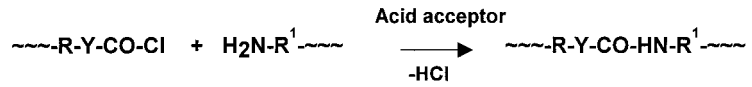


where: $R_1 = H, CH_3$, etc.

Scheme 1.

These reactions, however, in most cases proceed with low rates and are accompanied by numerous side reactions which lead to decomposition of the functional groups (and hence lead to chain-termination) as well as to the formation of anomalous units in the polymeric backbones ("unit heterogeneity").[4] In case of low-activity aromatic diamines high-molecular-weight polyamides under the conditions of HTPC are not formed at all.[5]

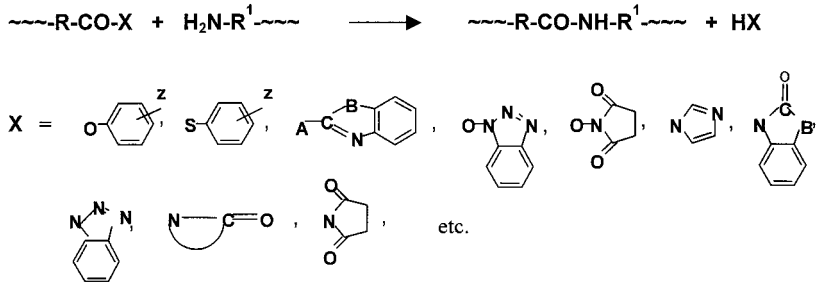
In such cases LTPC, developed mainly in 50-60s of the last century and commonly brought about in temperature range *c.a.* from –30 to 50°C in organic solvents or interfacially, is effective by far. This method is based on the use of chemically activated monomers like diacid chlorides (**Y=none**) and *bis*-chloroformates (**Y=O**):[2,3]



Scheme 2.

The low-temperature PC promoted further advancement of macromolecular chemistry and helped to solve many practical problems, however it is characterized by a number of drawbacks, among which numerous side reactions ("multi-channeling" of the process) leading to chain-termination and unit-heterogeneity,[2-4] as well as to synthetic limitations, should be noted. Among these side reactions undesirable interactions of aliphatic diacid chlorides with tertiary amines should be specially emphasized. These parasitic reactions prevent the formation of high-molecular-weight polyamides by solution PC.[3]

All the drawbacks of traditional PC methods mentioned above, determined in a significant extent the development in 70-80s of the last century of new PC methods based on non-traditional ways of activating monomers.[6] Among the five approaches developed the method called as "Leaving groups" method looks the most universal and promising. Came from peptide chemistry this method is based on the activation of that part of derivatives of carboxylic acids – esters or amides – which are considered as leaving groups (**X**), i.e. are liberated as low-molecular-weight by-products **HX** after PC, as is shown in Scheme 3.



A = **O, S;** **B** = **O, S, NH;** **Z** = electron-withdrawing substituents: **F, Cl, Br, NO_2, CN,** etc.;

Scheme 3.

In details active diesters (O- and rarely S-containing leaving groups) and active diamides (N-containing leaving groups) as new polycondensation type monomers, their synthesis and structure, the nature of activating effects, reactivity, etc. are analyzed in ref.[6]

Heterochain Polymers Synthesized *via* Active Diesters Aminolysis Reactions

Polyamides and Polybenzazoles

Active polyamidation proceeds according to Scheme 3. In this scheme R and R^1 can be Alkylene or Arylene. This means four different pairs of interacting monomers followed by the synthesis of all four possible classes of high molecular weight polyamides by APC.

Active polyamidation is especially promising for the synthesis of high-molecular-weight aliphatic polyamides (R and R^1 = Alkylene) by *solution* PC in contrast to traditional LTPC based on the interaction of alkylenediamines with aliphatic diacid chlorides that leads to the synthesis of low-molecular-weight or branched/cured polyamides under the same conditions.[2-4] A wide range of organic solvents both with or without functional groups – from alcohols (like ethanol, 2-propanol, etc.) to common organic (chloroform, methylene chloride, dioxane, THF, etc) and amide type solvents (like DMF, DMA, NMP, etc.) were successfully used as a medium of APC that is allowed by high inertness of active diesters towards these solvents.[7] The *p*-nitrophenoxide leaving group was found to be the best one for synthesizing polyamides from fatty diamines taking into account both availability of *p*-nirtophenol and high-molecular-weights of PEAs obtained. In case of aromatic diamines diesters of higher activity - derivatives of dinitrophenols or pentafluorophenol had to be used.

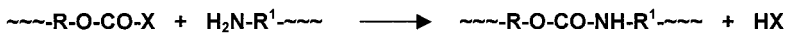
A high stability of aliphatic active diesters towards tertiary amines[7] allowed to use the salts of diamines instead of free diamines in APC (Tertiary amines in this case play a role of acid acceptor transforming *in situ* salts of diamines into free bases). This approach is promising for synthesizing polymers on the basis of diamines unstable as free bases, e.g. diamines containing ester linkages in the molecules, etc. We successfully used this new schemes of polyamidation for the synthesis of biodegradable poly(ester amide)s, discussed below.

A high selectivity of active diesters towards nucleophilic functional groups of close reactivity like *ortho*-amino groups in aromatic *tetra*-amines, turned out suitable for synthesizing high-molecular-weight (η_{red} up to 2.4 dL/g) soluble polybenzimidazoles.[6] The first stage of the synthesis of polybenzimidazoles represents active polyamidation reaction with participating

one, the most active *ortho*-amino group with subsequent involvement of the next *ortho*-amino group in benzimidazole ring formation.

Polyurethanes and Polyureas

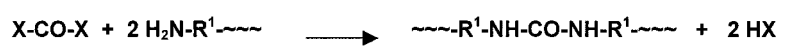
The synthesis of polyurethanes by APC is based on the aminolysis of active *bis*-carbonates of diols, as is shown in Scheme 4.



Scheme 4.

This reaction was found to be the most suitable for synthesizing linear aliphatic polyurethanes (i.e. when R and R^1 = Alkylene) revealing excellent film and fiber-forming properties.[8,9] Like the synthesis of aliphatic polyamides above the *p*-nitrophenoxide leaving group is found to be the best one for synthesizing polyurethanes by APC.

The synthesis of polyureas by APC *via* active carbonates follows Scheme 5.



Scheme 5.

This scheme is suitable for synthesizing unbranched and soluble high-molecular-weight polyureas from both aliphatic (η_{red} up to 0.75 dL/g) and aromatic diamines (η_{red} up to 1.34 dL/g) .[10]

Amino Acid Derived Polymers

Synthesis

It is not surprising that the methods of APC, based on the achievements of peptide chemistry, turned out especially suitable for constructing non-conventional hetero-chain macromolecular systems composed of α–amino acids. These polymers can be considered as synthetic analogues of naturally occurring amino acid derived polymers – proteins, and are promising for numerous biomedical applications. Using the methods of APC developed, we

have synthesized a large variety of these polymers which we called as Amino Acid Based Bioanalogous Polymers (AABBPs) (See ref.[11] and refs. cited therein).

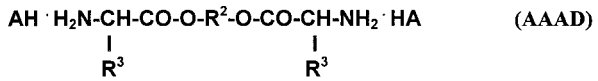
Four types of polycondensation monomers based on polyfunctional α–amino acids and dimeric forms of hydrophobic α–amino acids were used for constructing AABBPs:

Polyfunctional α–Diaminocarboxylic acids (mostly lysine and cystine) with protected C-terminus are used as diamines (in N,N'-*bis*-trimethylsililated or salt forms), and α–Amino dicarboxylic acids (L-aspartic and L-glutamic acids) with protected N-terminus are used as dicarboxylic acids (in active esters forms). These compounds are useful for incorporating lateral functionalal groups into AABBPs[10,12-15], and synthesizing non-canonical poly-(dipeptides).[13]

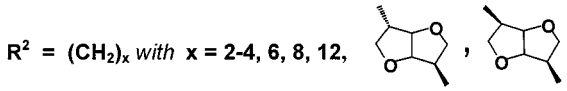
Dimeric forms of hydrophobic α–amino acids - N,N'-diacyl-*bis*-α-amino acids and *bis*-α-(L-amino acid) α,ω-alkylene diesters were also used as monomers for constructing AABBPs. N,N'-diacyl-*bis*-α-amino acids – both diamide diacids[16-19] and diurethane diacids[20] (in forms of active diesters[18,20] or *bis*-azlactones[16,17,19]) are suitable monomers for incorporating various hetero-linkages (amide, urethane) into polymeric backbones as well as for synthesizing AABBPs with di-[18] and tripeptide fragments.[17]

For constructing biodegradable polymeric backbones, however, the most promising as building blocks are *bis*-α-(L-amino acid) α,ω-alkylene diesters (AAADs) containing two ester linkages per molecule which can undergo either nonspecific (chemical) or specific (enzymatic) hydrolysis. As alkyl esters of α-amino acid,[21] AAADs are unstable as free bases and enter into undesirable side reactions. Therefore, they are prepared as stable salts of general formula (Scheme 6).

Di-*p*-toluenesulfonic acid salts were found as the most suitable monomeric forms of AAADs. They were synthesized in a nearly quantitative yields according to very simple procedure – by direct condensation of hydrophobic α-amino acids (2 moles) with fatty diols (1 mole) in refluxed benzene (or toluene)[11,22-25] in the presence of *p*-toluenesulfonic acid monohydrate. Various diols – widely available polymethylene diols[11,22] along with diols from renewable resources – dianhydrohexitols[23,25] were used for preparing AAADs.



(AAAD)



R^3 = CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, CH_2Ph, $(CH_2)_2$-S-CH_3.
HA = HCl, HBr, *p*-Toluenesulfonic acid.

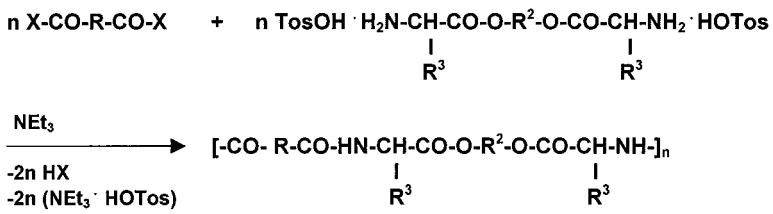
Scheme 6.

Neither traditional PC methods could be applied to AAADs due to instability of these compounds (exactly free bases) at elevated temperatures (HTPC) and necessity to use tertiary amines as *p*-toluenesulfonic acid acceptor (LTPC) which, as noted above, cause parasitic reactions with aliphatic diacid chlorides. And only APC proved to be a suitable method for preparing AABBPs from AAADs: poly(ester amide)s (PEAs) by PC with active diesters of dicarboxylic acids,[11,22-25] poly(ester urethane)s (PEURs) and poly(ester urea)s (PEUs) by PC with active *bis*-carbonates of diols and active diphenyl carbonates, accordingly.[26] Due to page limitations this paper deals with the synthesis, properties and some practical applications of PEAs only.

Bis-electrophilic partners of AAADs we used for preparing PEAs – di-*p*-nitrophenyl esters of dicarboxylic acids were synthesized by interaction either a) traditional diacid chlorides with *p*-nitrophenol in the presence of tertiary amines or b) free diacids with *p*-nitrophenol in the presence of condensing agent.[6] The latter allows to realize one of the substantial advantages of APC – wider synthetic possibilities - and to synthesize PEAs based on higher homologues of fatty diacids, dichlorides of which are problematic to be purified up to polycondensation grade. At the same time polymers composed of higher homologues of diacids could be of interest for various biomedical applications due to their anticipated mechanical, physico-chemical and biochemical properties.

The synthesis of AAAD based PEAs was carried out according to Scheme 7.

The APC proceeded smoothly under mild conditions in common organic solvents ($CHCl_3$, DMF, DMA) resulting in high molecular weight (M_w ranged from 24,000 to 167,000 depending on the AAAD and active diester used) PEAs with narrow polydispersity (Mw/Mn ranged from 1.20 to 1,81). The obtained PEAs, having regular but adirectional structure, in most cases were amorphous materials with T_g ranged from 11 to 102°C,[11,23] and only several samples (mostly composed of L-phenylalanine) showed semicrystallinity with T_m in the range 104-124°C.[11]



Scheme 7.

The PEAs obtained had a wide range of mechanical, physico-chemical and biodegradation properties, however, they did not contain any functional groups (except for terminal ones) which could be used for the attachment of active molecules (drug, bioactive compounds, recognition agents, adhesion promoter, probe, etc.). At the same time, one of the important criteria for the use of synthetic polymers in biomedical applications seems to be that polymers should be not only biodegradable but also have functional groups to which active molecules can be attached covalently or non-covalently. Therefore, very recently[27,28] we obtained functional co-poly(ester amide)s (F-co-PEAs) with lateral COOH groups, using di-*p*-toluenesulfonic acid salt of L-lysine benzyl ester (i.e. α-diaminocarboxylic acid derivative) as a co-monomer with AAAD in APC, followed by debenzylation of the lateral benzyl ester groups of PEAs by catalytic (Pd black) hydrogenolysis.

Biodegradation

To examine the biodegradation property of these regular PEAs a systematic *in vitro* biodegradation study in the presence of hydrolases like trypsin, α-chymotrypsin, lipase and a complex of proteases of Papaya (the last enzyme was used for modeling the catalytic action of nonspecific proteases) were carried out using both automatic potentiometric titration[29] and gravimetric (weight loss) methods.[30] It was found that, in the most cases studied, the PEAs were biodegraded by erosive mechanism, according to the first order kinetics. Spontaneous immobilization (absorption) of the enzymes onto the PEAs surfaces was observed. The surface immobilized enzyme not only accelerated the erosion of the PEAs but also was able to catalyze the hydrolysis of both low-molecular-weight (ATEE) and high-molecular-weight (protein) external substrates. The enzymes could also be impregnated into the PEAs to make them «self-destructive» at a target rate. A comparison of the PEAs' *in vitro* biodegradation data with polylactide (PDLLA) showed that PEAs exhibited a far more tendency towards enzyme catalyzed biodegradation than PDLLA, and PEAs' erosion rates (10^{-1}-10^{-3} mg/cm^2*h) were comparable with erosion rates of polyanhydrides[31] – the fastest biodegradable polymers to date.

A preliminary *in vivo* biodegradation study of the selected PEA sample (implanted as films subcutaneously to rats)[30] with and without lipase-impregnation showed that that PEA was completely absorbed within 1-2 months postimplantation (for the lipase-impregnated ones), and 3-6 months (for the lipase free ones) without any trace of tissue reaction. These findings prompt us to suggest that these new PEAs may have a great potential for designing drug sustained/controlled release devices as well as implantable surgical devices.

Applications

An artificial skin "PhagoBioDerm": this preparation represents a novel wound-healing device consisting of biodegradable PEA impregnated with an antibiotic and lytic bacteriophages. Licensed recently for sale in Georgia, PhagoBioDerm showed an excellent therapeutic effect in the management of infected wounds and ulcers (of both trophic and diabetic origin).[32]

Selected representative of the F-co-PEAs, revealing high-elastic properties along with

enzyme catalyzed biodegradation, was used for stent coating.[33] The *in vivo* biocompatibility was tested in porcine coronary arteries, comparing the polymer-coated stents with bare metal stents in 10 pigs. All animals survived till sacrifice 28 days later and follow-up angiography prior to sacrifice revealed identical diameter stenosis in both groups. Histology confirmed similar injury scores, inflammatory reaction, and area stenosis. The results of this study suggest that the polymer is biocompatible and should not elicit an inflammatory reaction, an important pre-requisite for a drug-carrying polymer.

Conclusion

It has been shown that new Active Polycondensation method could be applied for synthesizing hetero-chain polymers of various classes. This method proved to be especially useful for preparing biodegradable poly(ester amide)s composed of nontoxic building blocks like naturally occurring α-amino acids, fatty dicarboxylic acids and diols. Some representatives of the PEAs showed high biodegradation rates along with good biocompatibility and are of interest for various biomedical applications, e.g. as medicated wound dressing/healing materials, stent coatings, drug carriers, etc.

Acknowledgements

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