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Synthesis and preliminary characterization of polyesteramides containing enzymatically degradable amide bonds

L. Castaldo, P. Corbo, G. Maglio, and R. Palumbo*

Dipartimento di Chimica, Università "Federico II", Via Mezzocannone 4, I-80134 Napoli, Italy

Summary

Multiblock and regularly alternating biodegradable polyesteramides containing amide bonds susceptible of enzymatic cleavage were prepared and preliminarly characterized by viscosity, IR, ^{1}H-NMR and DSC techniques. L-phenylalanine was used to build up amide linkages containing a phenyl ring adjacent to the acyl group, enzymatically cleavable by chymotrypsin. Monomers having preformed ester groups or biodegradable amide bonds and/or telechelic oligomers of poly(L-lactide) were used as starting materials. Their chain length was regularly increased in order to improve the overall chain flexibility and, hence, to favour the enzymatic attack.

Introduction

The combination of good physical properties, biocompatibility and controlled degradability of polyesteramides makes this class of polymers worth of attention in view of biomedical applications as absorbable sutures or temporary implants, e.g., biodegradable devices for internal fixation of bone (1,2). We have previously prepared and characterized multiblock polyesteramides based on poly(L-lactide), (PLA), and polyamide 6,10 (3). Their degradability "in vitro" was also investigated in buffer solution at pH=8. A possible restriction of their use in the human body is the low degradability of the polyamide segments at physiological pH values. This drawback can be overcome introducing amide bonds susceptible to enzyme catalyzed hydrolysis into the polyamide segments. Here we report on the synthesis and preliminary characterization of both multiblock and regularly alternating polyesteramides containing enzymatically degradable amide bonds in the main chain.

Experimental

Materials

The solvents were purified according to standard procedures. The diols were predried over $CaCl_2$ overnight and distilled over metallic Na (1 g/100 mL) at reduced pressure. 1,6-Diaminohexane (HMD) was crystallized from anhydrous toluene. Sebacoyl chloride (Fluka Reagent) was distilled twice before use. Poly(L-lactide), $\bar{M}_n \approx 580$, was prepared according to the reported procedure (3). Other chemicals were used as received.

*Corresponding author

Techniques

The ^{1}H-NMR spectra were recorded using a Bruker AC-270 spectrometer operating at 270 MHz. $CDCl_3$ and $CDCl_3$/DCOOD were used as solvents for the monomers and the polyesteramides, respectively. TMS was the internal standard.
Thermal analysis was performed on 6-7 mg samples using a Mettler TA-300 differential scanning calorimeter with a heating rate of 10 °C/min.
Viscosity measurements were taken at 25.0 °C in m-cresol (c=0.5 g/dL) using a Cannon-Ubbhelohde viscometer.

Diester-diacid chlorides

Diester-diacids DE-DA262, DE-DA363 and DE-DA3TEG3 (see Table 1) were prepared according to ref. 4. DEDA3TEG3 is a colorless oil which was recovered from the reaction mixture by extraction with ethyl ether. The corresponding acyl chlorides were prepared by refluxing a benzene solution of the diester-diacid with oxalyl chloride (molar ratio 1:8) at 55°C for 2 h and for an additional 30 min at 70°C. Viscous liquids were recovered after evaporation of benzene and drying in vacuo. Attempts to purify the chlorides by high vacuum distillation were unsuccessful because of concurrent decomposition. Tetradecandioyl chloride was prepared following the above procedure and distilled under vacuum before use.

Diamide-diamines

Preparation of 1,2-di(phenylalaninamido)ethane (DD-DN2).

The bis(Z-diamine) was prepared by condensation of Z-L-phenylalanine with 1,2-diaminoethane (5). The corresponding hydrobromide salt, DD-DN2HBr, was obtained by reacting the bis(Z-1,2-diphenylalaninamido)ethane (12.72 g , 20.45 mmoles) with HBr/CH_3COOH 18% (63 mL) under stirring for 30 min. The precipitate, obtained by addition of anhydrous ethyl ether, was collected on a glass filter, repeatedly washed with ether and crystallized from methanol/ether (1:2) to give 7.87 g of the salt. 90 mL of a 0.80 M solution of Na_2CO_3 was added dropwise to DD-DN2HBr dissolved in 30 mL of water. The resulting suspension was extracted with $CHCl_3$ and the organic layer was dried overnight over anhydrous Na_2SO_4. The solvent was removed and the diamine was purified by column chromatography (Silica Gel 60, methanol/chloroform 1:3 as eluent) giving 3.80 g (57% yield) of a white product, m.p. 114 °C.
Preparation of 1,6-di(phenylalaninamido)hexane (DD-DN6).

A procedure as above was followed starting from Z-L-phenylalanine and 1,6-diaminohexane. The diamine, after purification by chromatography, melted at 127 °C.
All the intermediates were characterized by ^{1}H-NMR and FTIR spectroscopy and by elemental analysis.

Polymers

Preparation of DE-DA363/DD-DN6.

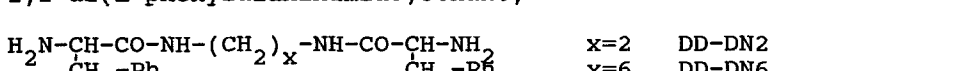
The following represents a typical procedure for the preparation of regularly alternating polyesteramides.
0.747g (1.95 mmol) of DE-DA363 dissolved in 10 mL of anhydrous $CHCl_3$ were quickly added to 70 mL of a water solution containing 0.807 g (1.95 mmol) of DD-DN6 and 0.163 g of NaOH in a blender under vigorous stirring. The stirring was continued

for 5 min. The precipitate was collected on a glass filter, repeatedly washed with water and dried in vacuo at 60°C giving 0.91 g of polymer (yield 65%), m.p. 167°C, η_{inh} 0.57 dL/g. Similar procedures were followed for all the polyesteramides. In the case of polymers derived from DE-DA3TEG3, addition of n-hexane was necessary in order to precipitate the polymer partially soluble in the $CHCl_3$ solution.
Random multiblock polyesteramides were prepared following the procedure reported in ref. 3.

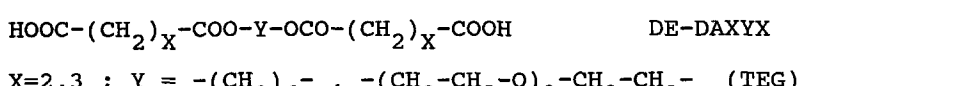
Results and Discussion

Monomers

As reported in the literature by several authors (6-11), the main factors affecting the enzymatic degradation of synthetic polymers are the steric configuration of the polymer itself and the nature of the substituents adjacent to the susceptible bond. The presence of a L-phenylalanine residue adjacent to the acyl groups of an ester or amide linkage has been reported as effective in enzymatic cleavage of such linkages by chymotrypsin (9). We have built up a synthetic procedure for biodegradable polyesteramides involving appropriate monomers containing preformed enzymatically cleavable amide linkages or ester groups. The two diamide-diamines, 1,6-di(L-phenylalaninamido)hexane and 1,2-di(L-phenylalaninamido)ethane,

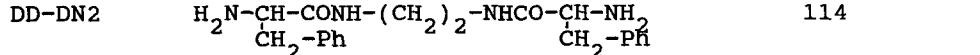
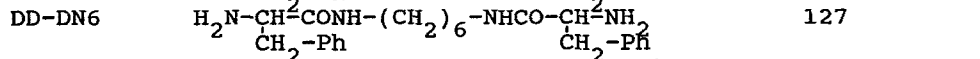
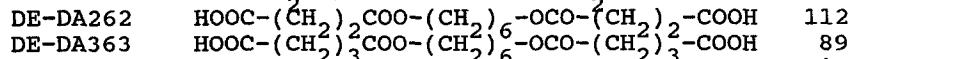
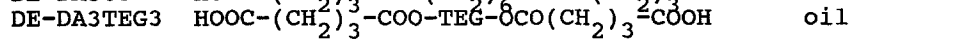


belong to the first class. They were prepared from L-phenylalanine and 1,6-diaminohexane or 1,2-diaminoethane, respectively, partially modifying a reported procedure (5). The following diester-diacids of different length and structure



belong to the second class and were prepared by reacting succinic or glutaric anhydride with a diol (4). The monomers of each series differ from one another in the length of the flexible spacers between the functional groups.

Table 1. Diester-diacids and Diamide-diamines Monomers

Code	Structure	T_m (°C)
DD-DN2		114
DD-DN6		127
DE-DA262		112
DE-DA363		89
DE-DA3TEG3		oil

Polyesteramides with different chain flexibility and interchain hydrogen bonding frequency may, therefore, be obtained. The

length and the flexibility of the main chain make the polymer able to fit into the enzyme active side, favouring the enzyme attack (9,10). In Table 1 are listed the prepared monomers together with their melting temperature, T_m.

Polymers

Two different classes of polyesteramides were prepared by using the diamide-diamines previously described: a) "random" multiblock ; b) regularly alternating.
The prepared polyesteramides are reported in Table 2.

Table 2. Properties of Polyesteramides

Code	η_{inh} b)	Yield	T_m	ΔH_m
	dl/g	%	°C	J/g
PLA/Se/DD-DN6 a)	0.51	63	212	15.0
PLA/Td/DD-DN6 a)	0.41	64	187	24.4
DE-DA363/HMD a)	1.24	82	138	35.0
DE-DA363/DD-DN6	0.57	67	167	40.0
DE-DA363/DD-DN2	0.33	65	199	23.1
DE-DA262/DD-DN2	0.34	55	231	19.0
DE-DA262/DD-DN6	0.26	56	184	34.7
DE-DA3TEG3/DD-DN2	0.32	64	200	23.7
DE-DA3TEG3/DD-DN6	0.18	60	120	15.9
Se/DD-DN6	0.53	70	214	58.3

a) PLA=Poly(L-lactide), Se=sebacic acid, Td=Tetradecandioic acid, HMD=1,6-diaminohexane. b) m-cresol, c= 0.5 g/dL, 25°C.

All the prepared polyesteramides are white powders, soluble in typical solvents of polyamides such as formic acid and m-cresol or in formic acid/chloroform mixtures. The inherent viscosity values seem to indicate moderate values of the polymerization degree. Attempts to obtain polymers of high molecular weight were in some case unsuccessful probably because of the difficulty of obtaining high purity diester-diacid chlorides.

a) Hydroxyl terminated poly(L-lactide), PLA, $\bar{M}_n$=535, sebacoyl or tetradecandioyl chloride and DD-DN6 were used in the synthesis of biodegradable multiblock polyesteramides following a two-step procedure reported in previous papers (3).

-CO-(PLA)-CO-NH-CO----NH-CO-(PLA)-CO-NH-CO------NH-
PA PA

PA=Polyamide block PLA= $-[O-\underset{CH_3}{\overset{H}{C}}-\overset{O}{C}-]_q-O-(CH_2)_4-O-[\overset{O}{C}-\underset{CH_3}{\overset{H}{C}}-O-]_p$

The presence of enzymatically degradable amide bonds, such as those preformed in the DD-DN6 diamine, should reasonably impart biodegradability to the polyamide segments of a block polyesteramide having degradable PLA oligomers as polyester segments. The copolymers were characterized by ^{1}H-NMR and IR spectroscopy and by DSC. The proton NMR spectrum of PLA/Se/DD-DN6 is shown in Figure 1 as an example. The composition, determined from the integral intensities of the lactide C<u>H</u> and of PA C<u>H</u>$_2$-NH resonanences, was 30% by wt of PLA,

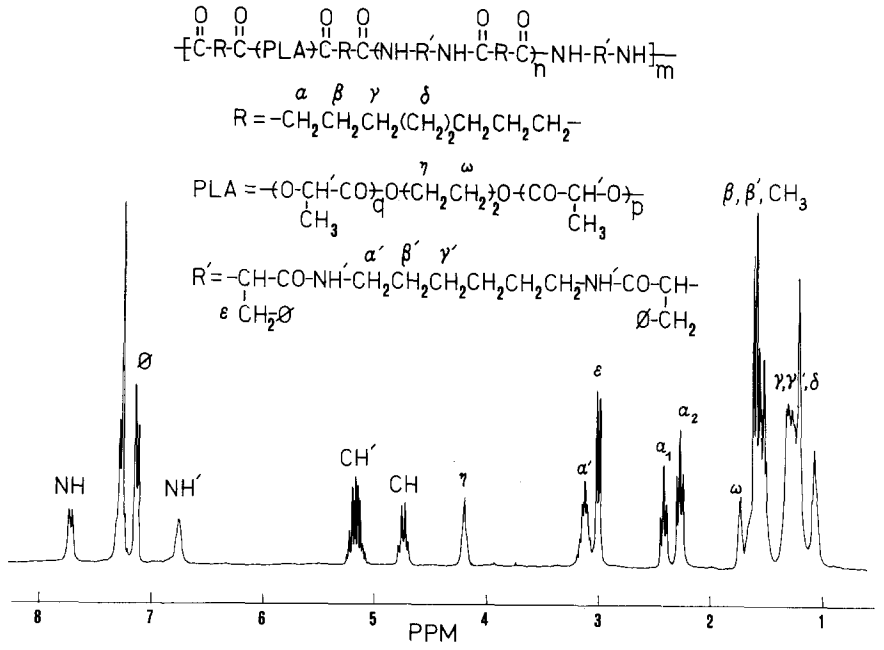
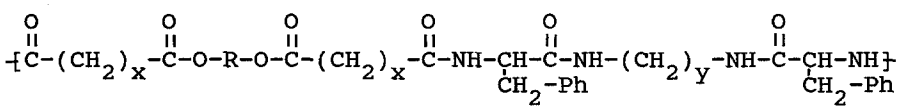


Figure 1. ^{1}H-NMR spectrum of the block polyesteramide PLA/Se/DD-DN6.

which is very close to the target value (33%). The block-like structure of the copolymers was confirmed by the presence of two distinct resonances, α_1 and α_2, for the CH_2-CO protons of the sebacic acid units. The higher field α_2 resonance was attributed to CH_2 groups adjacent to amide bonds and, therefore inserted in PA blocks, while the α_1 resonance was attributed to CH_2 groups adjacent to ester bonds and, therefore, belonging to acid units linking two heterotype blocks. The PA segments have an estimated average MW of about 1300 and are able to crystallize as shown by DSC. The melting temperature is related to the structure of the PA segments as shown comparing the T_m of PLA/Se/DD-DN6 (212 °C) with that of Se/DD-DN6 polyamide (214 °C). The lower value found for the polyesteramide containing the C14 acid can be attributed to the increased flexibility of the PA segments and to the decreased interchain hydrogen bond density.

b) Regularly alternating polyesteramides having the sequence EEAA'A'A (E=ester bond; A=amide bond; A'=enzimatically degradable amide bond) in the repeat unit



x=2,3 ; y=2,6 R = $-(CH_2)_6-$; $-(CH_2-CH_2-O)_3-CH_2-CH_2-$

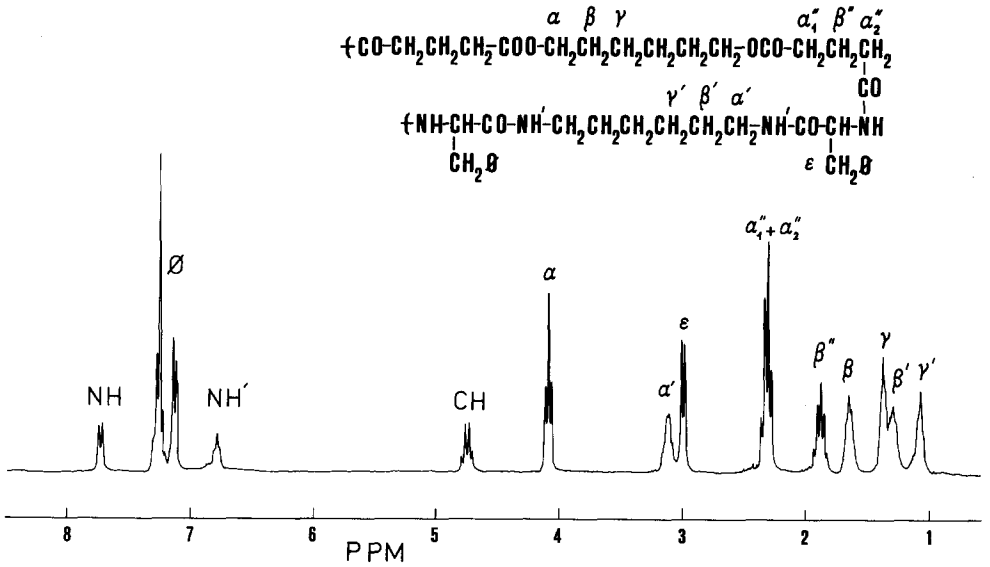


Figure 2. ^{1}H-NMR spectrum of the alternating polyesteramide DE-DA363/DD-DN6.

were prepared by interfacial polycondensation under stirring at room temperature using the acyl chlorides of the diester-diacids and diamide-diamines DD-DN2 and DD-DN6. The alternating structure of the copolymers was checked by ^{1}H-NMR spectroscopy: the spectrum of DE-DA363/DD-DN6, shown in Figure 2, is representative of this class of polyesteramides. All the resonances could be attributed on the basis of literature data and the spectra of the intermediates. All the copolymers are semicrystalline, as shown by the DSC results, (see Table 2). A great variability of T_m was found according to the structure of the repeat unit. The^{m} substitution of 1,6-hexanediamine in DE-DA363/HMD with DD-DN6 and DD-DN2 affords polyesteramides which melt 30° and 60°C higher, respectively. This effect is related to the increased amide bond density in the repeat unit and to increased intrachain interactions. On the basis of such considerations, it is not surprising that the polyesteramides containing DD-DN2 have the highest T_m. On the other hand, by using the same diamide-diamino, the melting temperature depends on the length of the diester-diacid according to the sequence: DE-DA262>DE-DA363>>DE-DA3TEG3.

Further work concerning the mechanical properties and the "in vitro" biodegradability of the prepared polyesteramides is currently in progress.

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