Synthesis of poly[oxyethylidenecarbonylimino-(2-oxoethylene)] [poly(glycine-D,L-lactic acid)] by ring opening polymerization

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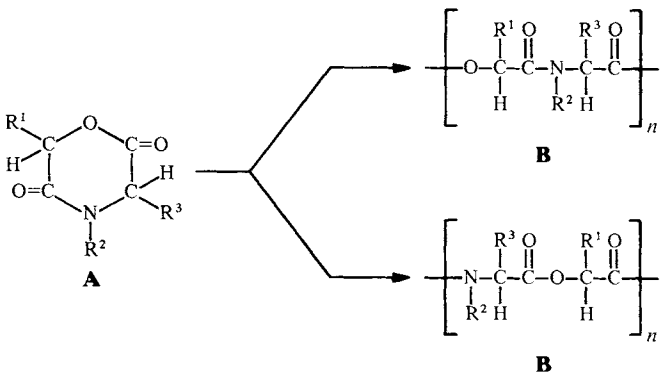
Introduction

Synthetic, absorbable, polymeric materials are frequently used in medicine and surgery[1,2], for example as sutures[3], carrier systems for the controlled release of drugs[4] or as resorbable prostheses in orthopedic surgery[5]. Two important classes of synthetic biodegradable polymers are poly(α-amino acid)s and poly(α-hydroxy acid)s. Copolymers of α-amino acids and α-hydroxy acids, which are called polydepsipeptides, may be a valuable addition to the existing series of synthetic biodegradable polymers.

Until now, linear, alternating polydepsipeptides were synthesized only at a very small scale, in order to study their conformational properties[6-11]. The starting materials for the polymerization were always preformed tetra- and sometimes didepsipeptide monomers, synthesized via a multi-step synthetic route.

High molecular weight polymers of lactic acid and glycolic acid can easily be obtained by melt polymerization of the cyclic monomers, dilactide and diglycolide, respectively[12]. Ring-opening polymerization of 2,5-morpholinedione derivatives (**A**) would be an attractive route to obtain various linear alternating polydepsipeptides **B** in a more facile way.



A **B** **B**

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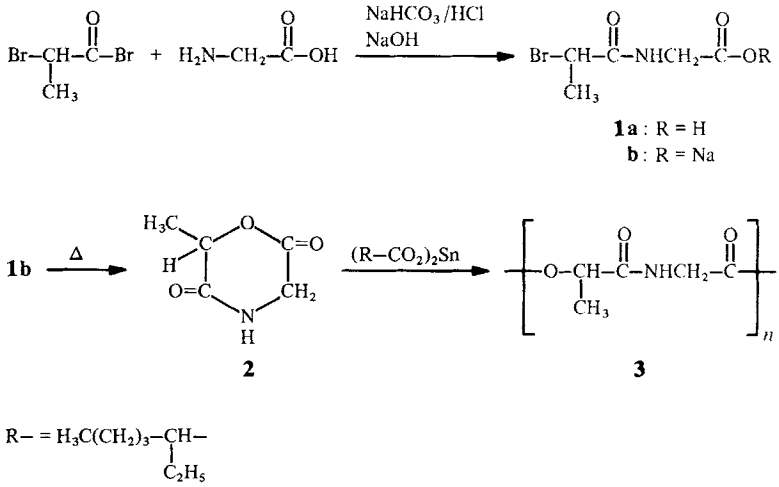
2,5-Morpholinedione can be considered both as a 6-membered lactone as well as a 6-membered lactam. Contrary to the polymerization of 6-membered lactones, the polymerization of 6-membered lactams is rather difficult[13,14].

2,5-Morpholinedione and its derivatives have only been mentioned occasionally in the literature. The synthesis of 6-methyl-2,5-morpholinedione (**2**) was reported by Chadwick and Pascu[15] and by Kazmierczak and Kupryszewski[16]. **2** was obtained by dry heating of respectively the brucine or sodium salt of D,L-*N*-(2-bromopropionyl)-glycine (**1b**) causing sublimation of **2**. Cook and Cox[17] studied the synthesis and stability of several, mainly *N*-substituted, 2,5-morpholinedione derivatives, which were prepared from 2-bromoacyl-α-amino acids by dry heating of the respective sodium salts or stirring in sodium carbonate solutions followed by the extraction with an organic solvent. Hwang et al.[18] and Kohn[19] described the synthesis and characterization of unsubstituted 2,5-morpholinedione. Attempted polymerization with this compound using tetraphenyltin as initiator, were not yet successful[19].

Recently, Shakaby and Koelmel[20] described the formation of polymers of *p*-dioxanone containing 1 to 15% of 2,5-morpholinedione or its 3-methyl and *N*-methyl derivatives. These copolymers were obtained by ring-opening copolymerization of the monomers using tin bis(2-ethyl hexanoate) as initiator.

Tin bis(2-ethylhexanoate) is extensively used as initiator for the ring-opening polymerization of lactones, including dilactide and diglycolide, although the actual initiation mechanism has not yet been elucidated[21,22].

This article reports on the synthesis and characterization of poly[oxyethylidene-carbonylimino(2-oxoethylene)] [poly(glycine-D,L-lactic acid)] (**3**), obtained by ring-opening polymerization of **2**.



Experimental part

Materials and methods: Glycine and D,L-2-bromopropionyl bromide were purchased from Merck (Darmstad, W-Germany) stannous octoate from Polysciences (Warrington, USA).

Melting points were obtained using capillary tubes and a Büchi melting point apparatus. IR spectra were recorded on a Beckman 33 IR spectrophotometer in the solid state as KBr pellets. ^{1}H NMR spectra were recorded on a Nicolet NMR-apparatus operating at 200 MHz using ($^2H_6$)-DMSO as solvent and TMS as internal reference. Viscosity measurements were carried out in DMF at 25 ± 0,002 °C with an Ubbelohde type viscometer. Limiting viscosity numbers [η] were determined by the usual extrapolation to zero concentration (conc. range 1,0 – 0,2 wt.-%)[23]. Static low angle laser light scattering (LALLS) measurements were performed in DMF at ambient temperature at 633 nm with the Chromatix KMX-6 photometer[23]. The necessary refractive increments (dn/dc) were determined with a Brice-Phoenix differential refractometer at 633 nm.

D,L-*N-(2-bromopropionyl)glycine* (**1a**): The synthesis of **1a** from D,L-2-bromopropionyl bromide and glycine under Schotten-Bauman conditions was based on the procedure described by Greenstein and Winitz[24] for the preparation of carbobenzoxy-L-threonine. **1a** was isolated by extraction of the aqueous solution with diethyl ether during 20 h in an apparatus for continuous extraction. Evaporation of the diethyl ether yielded an oil, which was crystallized by addition of hexane. The crude product was recrystallized from chloroform; m.p. 99 – 102 °C. Yield: 60%. (Lit.[15]: m.p. 100 – 103 °C).

6-Methyl-2,5-morpholinedione (**2**): 53,25 g of **1a** (0,254 mol) was dissolved in one equivalent of a 1 M aqueous sodium hydroxide solution (the solution may not become basic in order to prevent substitution of the bromine atom by a hydroxyl group). The solution was subsequently evaporated to dryness using ethanol to remove residual water. The dry sodium salt was heated at 125 °C i.vac. (0,7 – 1 mbar) for 1 h. Some product had sublimed onto the stillhead and was collected by dissolution in ethyl acetate. Most of the product, however, was obtained from the residue. The yellow residue was partly dissolved in ethanol and filtered. The filtrate was evaporated i.vac. to yield a yellow oil which was stirred about six times with 300 ml of ethyl acetate. Evaporation of the ethyl acetate yielded 12,74 g (39%) of crude product; m.p. 85 – 87 °C. For the polymerization the crude product was recrystallized five times from ethyl acetate. Melting points are given in Tab. 1. (Lit.[15]: m.p. 98 °C).

Poly[oxyethylidenecarbonylimino(2-oxoethylene)] poly(glycine-D,L-lactic acid) (**3**): Small polymerization tubes (10 ml) were silanized using dichlorodimethylsilane (23 wt.-% in toluene) and subsequently dried in an oven at 120 °C for at least 12 h. 1,98 g (0,015 mol) of **2** was placed in the polymerization tube and the corresponding amount of tin bis(2-ethylhexanoate), dissolved in a small quantity of dry toluene, was added (Tab. 1). After evaporation of the solvent i.vac., the tube was purged several times with dry nitrogen. The tube was sealed i.vac. (0,7 – 1 mbar) and placed in an oil bath at 130 °C. After the time indicated (Tab. 1) the tube was cooled and opened. The glassy-like, yellow-brown material was dissolved in 15 ml of DMF. The solution was added dropwise to 800 ml of diethyl ether with vigorous stirring yielding a yellow-white precipitate which was collected by filtration and dried i.vac.

Absorbed diethyl ether could be removed at a pressure of 0,7 – 1 mbar and a temperature of 80 °C. Yields ranged from 70 to 80%.

Results and discussion

Tab. 1 contains the first data on the ring-opening polymerization of 6-methyl-2,5-morpholinedione (**2**) with tin bis(2-ethylhexanoate), which results in the formation of poly[oxyethylidenecarbonylimino(2-oxoethylene)] [poly(glycine-D,L-lactic acid)] (**3**). These examples prove that ring-opening polymerization of 2,5-morpholinedione derivatives is a suitable route for the synthesis of polydepsipeptides. The molecular

Tab. 1. Melt polymerization of 6-methyl-2,5-morpholinedione (**2**) with tin bis(2-ethylhexanoate) at 130 °C

Sample No.	Mole ratio monomer/initiator	M. p. of monomer T/°C	Polym. time t/h	$[\eta]$ [a] / dl · g^{-1}	dn/dc / ml · g^{-1}	$10^{-4} \cdot \bar{M}_w$ [b]
1[c]	250	97 – 98	44	0,22	[d]	
2	1 000	99 – 100	28 – 40[e]	0,24	0,066	2,3
3	1 000	98 – 99	48	0,19	0,064	1,6
4	3 000	98 – 99	48	0,15	0,064	1,6
5[c]	3 100	96 – 97	90	No polymer		

[a] In DMF at 25 ± 0,002 °C.
[b] $\bar{M}_w$ was determined by static LALLS measurements in DMF.
[c] Tube was not silanized before polymerization.
[d] Refractive increment changed with time.
[e] Exact polymerization time not known.

weights of the polymers ranged from 1,6 to 2,1 · 10^4 as was determined by static low angle laser light scattering (LALLS) measurements.

After purification samples 3 and 4 of polymer **3** (Tab. 1) contained considerable amounts of diethyl ether as was indicated by NMR spectroscopy. The refractive increment of polymer sample 1 changed with time, which might be caused by the presence of diethyl ether. Diethyl ether could be effectively removed by drying several hours i. vac. at 80 °C.

Nissen et al.[6] prepared poly(L-valine-L-lactic acid) and poly(L-alanine-L-lactic acid) by thermal polymerization of the pentachlorophenyl esters of the appropriate tetradepsipeptide monomers. The yields ranged from 22 to 75% and from 4 to 50% for poly(L-valine-L-lactic acid) and poly(L-alanine-L-lactic acid), respectively. The intrinsic viscosity values (in dichloroacetic acid) ranged from 0,42 to 0,57 dl · g^{-1} and from 0,11 to 0,33 dl · g^{-1}. The weight-average molecular weights, $\bar{M}_w$, estimated by using the molecular weight — intrinsic viscosity relationships determined for poly(γ-benzyl-L-glutamate) and poly(L-lactic acid), ranged from 6,4 – 8 · 10^4 and from 1,3 – 5 · 10^4. It was reported[6] that the polymers are soluble in alcohols, chloroform, DMF, 1,4-dioxane, and slightly soluble in carbon tetrachloride. We found different solubility properties for polymer **3**, being soluble in DMF and DMSO but insoluble in a range of solvents like water, alcohols, chloroform, acetone, THF and 1,4-dioxane. The differences in solubility properties may be the result of the absence of substituents on the carbon atom next to the nitrogen atom in polymer **3**.

The data collected in Tab. 1 suggest that the purity of the monomer, as indicated by the melting point, is a critical factor for these polymerizations. Polymerization experiment 5 (Tab. 1) with the monomer having a melting point of 96 – 97 °C did not result in any polymer. To improve the molecular weight of polymer **3**, an investigation on the influence of different factors, like the monomer initiator ratio and polymerization time will be necessary.

Tab. 2. Chemical shifts in ^1H NMR spectra using ($^2H_6$)-DMSO as solvent

Compound	δ_{CH_2} [a]	δ_{CH} [a]	δ_{NH} [a]	δ_{CH_3} [a]
D,L-*N*-(2-Bromopropionyl)-glycine (**1a**)	4,65 (1H; q)	3,85 (2H; d)	8,60 (t)	1,70 (3H; d)
6-Methyl-2,5-morpholine-dione (**2**)	5,02 (1H; q)	4,18 and 3,99 (2H)[b]	8,4 (bs)	1,42 (3H; d)
Poly(glycine-D,L-lactic acid) (**3**)[c]	5,08 (1H; q)	4,0 (2H; bs)	8,5 (bs)	1,36 (3H; d)

[a] Chemical shifts in ppm vs. TMS.
[b] AB-part of ABX spin system (8 lines).
[c] Poly[oxyethylidenecarbonylimino(2-oxoethylene)]. It was difficult to remove residual amounts of DMF, which showed resonances at δ = 2,75, 2,90 and 7,97.

Tab. 3. Characteristic IR frequencies[a]

Compound	N—H Stretching vibration	Amide I	Amide II (*trans*)	C=O
D,L-*N*-(2-Bromopropionyl)glycine (**1a**)	3 320	1 625	1 550	1 755
6-Methyl-2,5-morpholinedione (**2**)	3 210	1 690	—	1 755
	3 270[b]	1 700[b]		1 760[b]
Poly(glycine-D,L-lactic acid) (**3**)	3 330	1 680	1 540	1 755

[a] In the solid state as KBr pellets; ν-values in cm^{-1}.
[b] Recorded by Kazmierczak and Kupryszewski[16] by the nujol suspension thin film technique.

Tab. 2 and 3 show the ^1H NMR and IR data, respectively. The chemical shifts of corresponding protons in **1a, 2** and **3** are very similar. The methylene protons of glycine in the cyclic compound **2** (at C^3) are clearly non-equivalent as indicated by the multiplet character of their proton resonance. In both acyclic derivatives, **1a** and **3**, these protons are apparently equivalent.

The IR-spectra of **1a** and **3** show *trans*-amide II absorptions. The *cis*-amide absorptions in the spectrum of **1a** are in part overlapped by other absorptions. The absence of the *trans*-amide II absorption in the spectrum of the cyclic compound **2** indicates that the amide bond in this compound is in the *cis*-conformation. This most probably implies that, in order to form the cyclic compound **2**, the acyclic precursor **1a** has to adopt a folded conformation in which the amide bond is in the *cis*-conformation rather than the more stable extended form, in which the amide bond is in the favoured *trans*-conformation. The *trans-cis* energy barrier for the non-alkylated amide bond is high[25]. This may explain the harsh conditions necessary for the formation of the cyclic compound **2** from its acyclic precursor **1a**. Probably the strong *trans*-preference of the amide group in 2-halogenacyl-α-amino acids disappears upon *N*-alkylation as in linear dipeptides derived from *N*-methylglycine[26]

which is consistent with the easy lactonization of several *N*-alkylated 2-bromoacyl-α-amino acids in aqueous sodium carbonate solution reported by Cook and Cox[17].

In conclusion, lactone ring-opening polymerization of **2** proved to be a suitable route for the synthesis of polymer **3**. Our current research includes the synthesis of other polydepsipeptides, the use of different initiators, improvement of monomer yield and of polymer molecular weight.

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