Polymer Chemistry

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Chemical Composition and Topology of Poly(lactide-*co*-glycolide) Revealed by Pushing MALDI-TOF MS to Its Limit**

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Poly(lactide-*co*-glycolide) (PLGA) is a copolymer used extensively in the medical sector as a material for drug-delivery implants, bone screws, and absorbable sutures. Especially in the field of drug delivery, the use of PLGA has grown rapidly over the last few decades owing to its good biocompatibility and biodegradability. As the degradation products of the polymer can be metabolized in the human body to carbon dioxide and water, surgical removal of the implants is generally not required. An understanding of the chemical structure and topology of the polymer and their correlation with its physical properties and morphology is of great importance in the endeavor to control the degradation characteristics of the polymer matrix. Although various studies on structure–property relationships have been performed, little is known about the exact topology of PLGA, which can form as a random, block, alternating, or gradient structure.

PLGA can be synthesized by direct melt polycondensation of the hydroxyacids lactic and glycolic acid, as well as by ring opening of lactide and glycolide (Scheme 1). Ring-opening polymerization can take place cationically,[1] anionically,[2] and by a metal-catalyzed coordination–insertion mechanism.[3] The latter method has drawn the most attention and is widely employed, as stereospecific polymers with high molecular weights can be obtained.[4] These different pathways each leave their fingerprint behind in the topology of the polymer. Generally, microstructures of a particular copolymer are characterized by high-resolution ^{13}C NMR spectroscopy, in which mainly the carbonyl signals are used because of their sensitivity to sequence effects. However, to obtain a highly resolved spectrum, long measuring times are required as well as powerful NMR instruments. Moreover, the spectra are complex, and the assignment of the peaks is not always straightforward.

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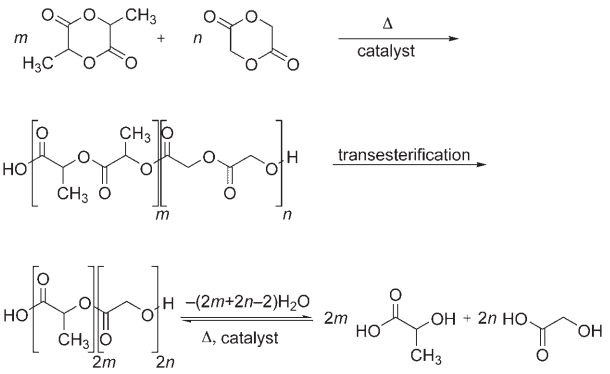

Scheme 1. Ring-opening polymerization of lactide and glycolide, and polycondensation of lactic and glycolic acid.

Albeit challenging, matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) might be a suitable tool to disclose the fine structure even of complex copolymers, such as poly(lactide-*co*-glycolide). Initially, MALDI-TOF MS was developed for protein research, but because of the highly accurate information it provides on chemical structures, this characterization technique has gained increasing attention in polymer chemistry. Herein, we report a study on the composition and topology of PLGA which makes use of a recently developed method based on MALDI-TOF MS. Software developed in house enables not only the elucidation of individual chain structures, but a full characterization of the copolymer, including even its chemical composition and topology (random, gradient, block, or alternating).

Poly(lactide-*co*-glycolide) used in this study was synthesized by ring-opening polymerization of L- (LL) or D,L-lactide (DLL) and glycolide in the presence of tin(II) 2-ethylhexanoate ($Sn(Oct)_2$). The copolymer with a molar ratio of 80 L/20 G was characterized by DSC, SEC, and NMR spectroscopy. PLLGA (80 L/20 G) synthesized from the monomers L-lactide and glycolide had a number-average molecular weight of 9.9 $kg\,mol^{-1}$ with a polydispersity of 1.5, and the synthesis of PDLLGA (80 L/20 G) resulted in a polymer with a number-average molecular weight of 32.6 $kg\,mol^{-1}$ with a polydispersity of 1.6. Both copolymers were amorphous, with a T_g of 50.9 °C for PLLGA and 43.8 °C for PDLLGA. The ratio of lactide to glycolide in the polymer represented the feed ratio as determined by ^1H NMR spectroscopy.

MALDI-TOF MS spectra were generally recorded with potassium trifluoroacetate as the cationization agent, in the reflectron mode for good mass resolution. Software developed in house was used to simulate the spectrum and required the molar mass of 1) the repeating units, 2) possible end groups, and 3) the cation of the cationization agent as input data. The program makes use of Equation (1) to assign a

$$m_{th} = n_G M_G + m_L M_L + E_I + E_{II} + M^+ \quad (1)$$

certain combination of glycolyl and lactyl units with a given end group to an experimental value of m/z.[5]

E_I and E_{II} represent the masses of the end groups at either end of the chain, M_G represents the mass of the repeating glycolyl unit, M_L the mass of the repeating lactyl unit, and M^+ the mass of the cation. Multiple peak assignment, that is, more combinations of $n_G M_G$ and $m_L M_L$ found for a particular experimental value of m/z, is common, and an independent technique is required, such as ^1H NMR spectroscopy, to determine the correct chemical composition. Moreover, the presence of chains with different end groups and the small difference in mass between lactyl and glycolyl units resulted in overlapping isotope patterns.

A pattern of alternating high- and low-intensity isotope distributions was detected for both PLLGA and PDLLGA. The difference in m/z between adjacent isotope distributions is 14, which corresponds to the exchange of one glycolyl unit (58.01 $g\,mol^{-1}$) for one lactyl unit (72.02 $g\,mol^{-1}$) within a single chain (see enlargement of spectrum in Figure 1). The difference in m/z of 28 between the most abundant isotope distributions corresponds to the exchange of one glycolydyl unit (116.01 $g\,mol^{-1}$) for a lactydyl unit (144.04 $g\,mol^{-1}$).

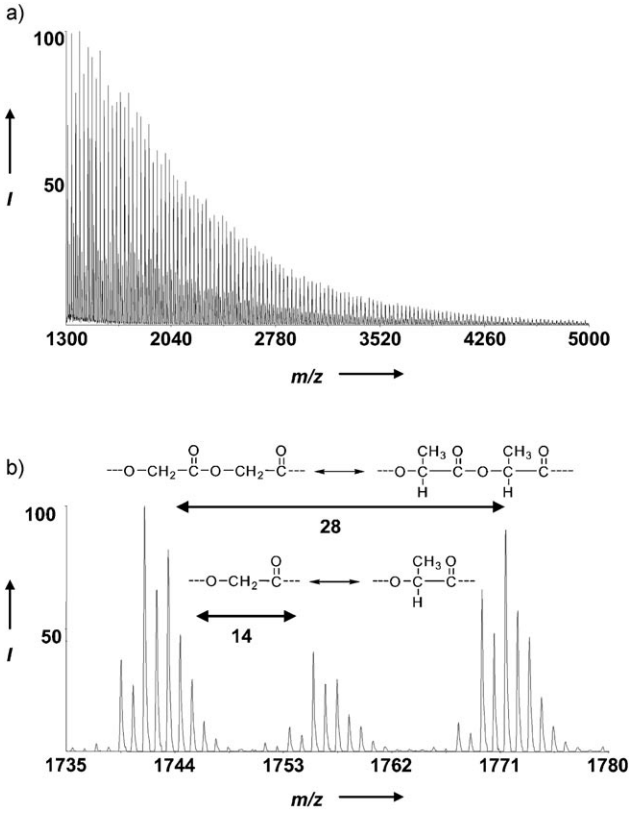

Figure 1. a) MALDI-TOF MS spectrum of PDLLGA and b) enlargement of part of the spectrum.

The ring opening of lactides generally results in linear chains end capped with hydroxy and carboxylic acid groups (referred to as an H–OH end group) and cyclic polymer structures (Figure 2 a,c). Occasionally, catalyst residues give rise to additional end groups, for example, an octanoyl end group as reported by Kowalski et al.[6] (Figure 2 d). Simulation of the experimental MALDI-TOF MS spectrum did not afford a unique solution, as cyclic structures, chains with an octanoyl end group, and chains with an H–OK end group

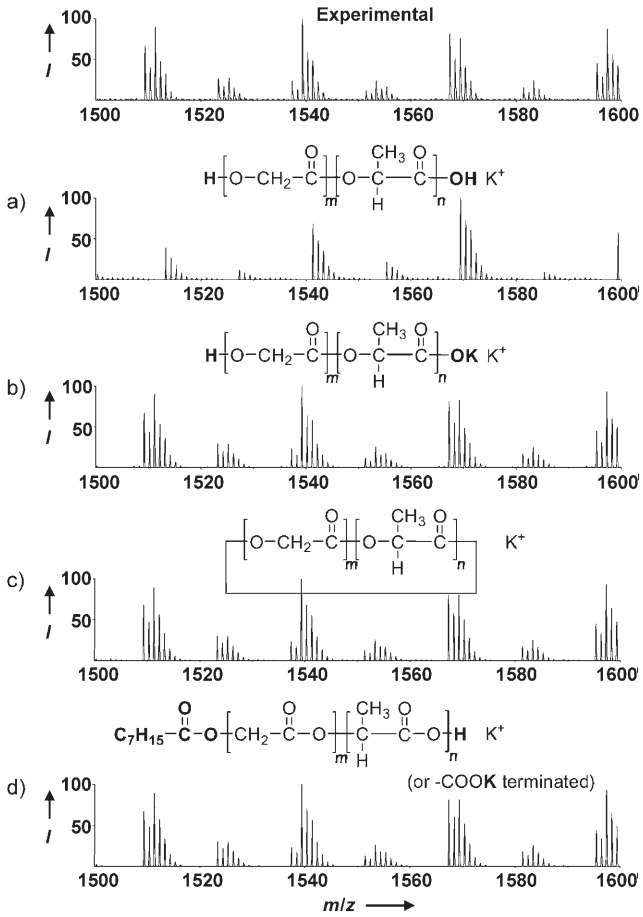

Figure 2. Experimental and a)–d) simulated isotope patterns for different end groups. (A complete spectrum was simulated, but for clarity only a selected range is shown.)

yielded virtually identical spectra (Figure 2). The occurrence of an H–OK end group is an artifact of MALDI-TOF MS, as the hydrogen atom of the carboxylic acid end group of an H–OH end-capped polymer is in this case exchanged by a cation of the cationization agent (Figure 2b). Comparison of the MALDI-TOF MS spectra recorded with potassium and sodium trifluoroacetate helped to determine the true end group. The spectrum measured with the sodium cationization agent was shifted −16 in m/z with respect to the spectrum measured with the potassium agent (Figure 3). This result automatically excludes the H–OK end group as a possible solution. The difference between an H–OK-terminated chain with a K^+ adduct and an H–ONa-terminated chain with a Na^+ adduct would be shown by a shift in the spectrum measured with sodium of −32 in m/z. The occurrence of H–OH-terminated chains can be ruled out as well for three different reasons: The first is that statistically, with the large excess of salt used, the exchange of a certain percentage of acidic protons for K^+/Na^+ would take place and result in the occasional difference of 32. Secondly, the presence of H–OH-terminated chains alone can not explain all the isotope distributions present. Finally, the calculated composition for H–OH-terminated chains differs too much from the composition of the copolymer as determined by ^{1}H NMR spectroscopy.

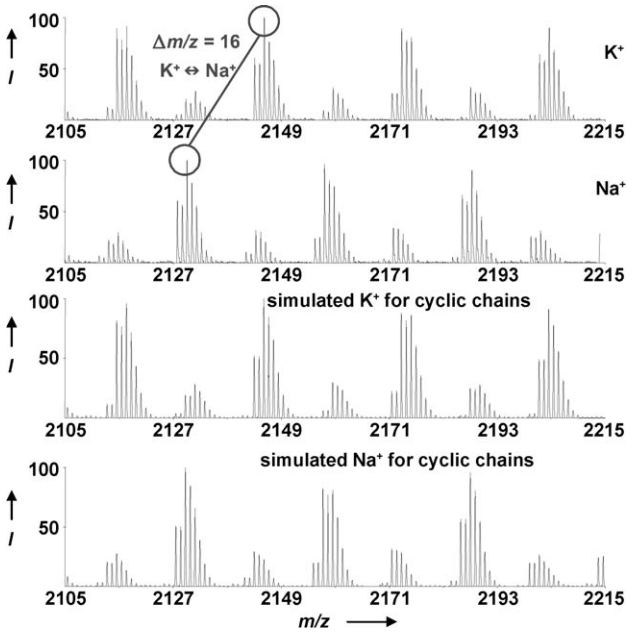

Figure 3. Enlargements of experimental and simulated spectra of PDLLGA recorded with K^+ and Na^+.

Although the presence of chains containing octanoyl end groups that originate from catalyst residues can not be excluded, their contribution is expected to be marginal because of the low catalyst concentration. Furthermore, these polymers should also undergo occasional deprotonation of the carboxylic acid end group; the expected shift of −32 m/z which would result was not detected. Therefore, the sequential difference of 16 m/z observed strongly suggests the presence of mainly cyclic structures. Both experimental and simulated isotope patterns of cyclic species ionized with potassium and sodium are shown in Figure 3. For sufficient mass resolution, MALDI-TOF MS is restricted to lower-molecular-weight fractions. The fact that the MALDI-TOF MS spectrum only shows cyclic structures does not exclude the possibility that linear chains are present in the higher-molecular-weight fraction of the material.

The software developed in house was also used to compute a contour plot, which represents the fingerprint of the polymer. Such contour plots were constructed to make the complex MALDI-TOF MS spectra of copolymers more accessible in general and are based on the work of Wilczek-Vera et al.[7,8] The contour plot is a 3D representation of a normalized matrix of the mass spectrum with lactyl units, glycolyl units, and the corresponding peak intensity on the axes. The shape and position of the plot reveals information on the topology of the polymer.[5,9,10] The contour plots appear to have been cut off, but this effect is simply a result of the fact that these MALDI-TOF MS spectra were not recorded at m/z values lower than 1250.

Interestingly, the computed contour plots show a peculiar pattern, which is characteristic for the presence mainly of chains both even- and odd-numbered in glycolyl units but only even-numbered in lactyl repeating units (Figure 4). The horizontal lines in the plot represent the polymer chains containing complete lactydyl units. This striped pattern would

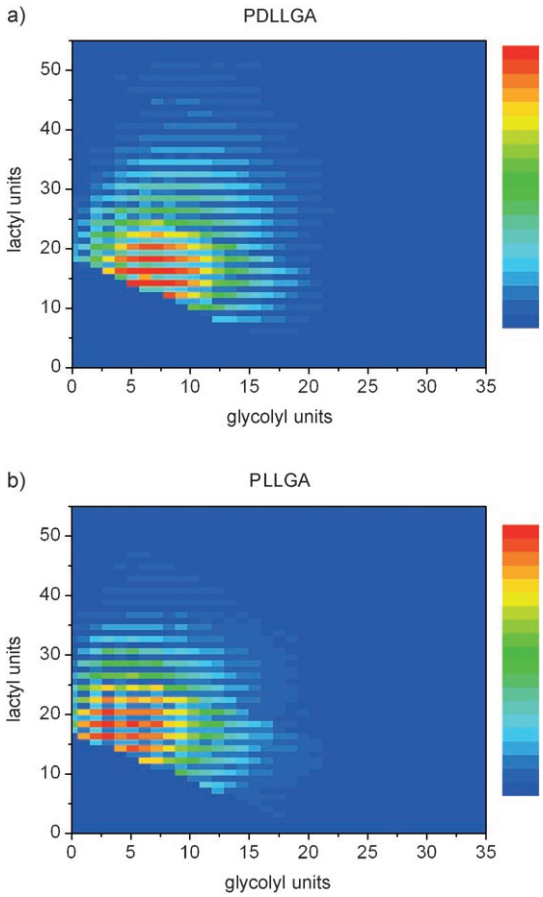

Figure 4. Contour plots of a) PDLLGA and b) PLLGA for cyclic structures. The colors indicate intensity; red represents the most abundant chains and corresponds to the isotope distributions of highest intensity in the spectrum shown in Figure 1.

be absent if chains with half-lactydyl (lactyl) units were present in an appreciable amount, which suggests that the $Sn(Oct)_2$ readily transesterifies glycolydyl units, but is not capable of transesterifying the lactydyl ester bond within the given reaction time. Similar contour plots are obtained for copolymer synthesized with D,L-lactide (racemic mixture of D-lactide and L-lactide) to those for copolymer synthesized with L-lactide, which indicates that $Sn(Oct)_2$ does not discriminate between the enantiomers in the transesterification. As expected, contour plots of PLGA synthesized by polycondensation of the hydroxyacids showed a homogeneous area, which is indicative of an equal distribution of even-numbered and odd-numbered chains.

As mentioned earlier, the contour plot reveals information on the topology of the polymeric material. A line drawn through the center of the contour plot is a measure of the average chemical composition. If this line crosses the origin and the slope remains constant the copolymer can be classified as random. If the line is curved but still crosses the origin, the plot represents a gradient copolymer. Finally, in the case of a block copolymer, the line does not cross the origin.[9,10]

Because of the discontinuity in the area, the shape and position of the contour plot in Figure 4 do not show unambiguously if we are dealing with a block or a random copolymer. Better insight into the topology is obtained when the plot is simulated with complete lactydyl units instead of lactyl units (Figure 5). Surprisingly, the contour plot of

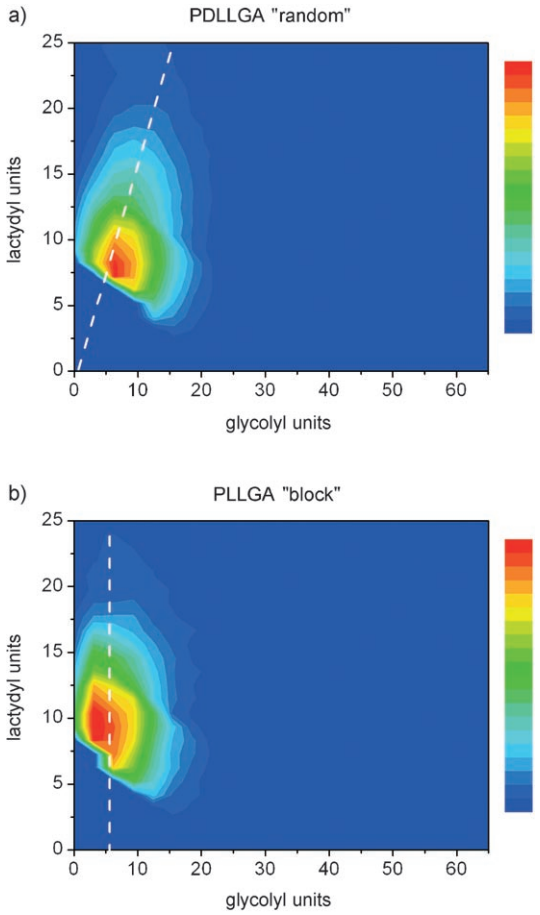

Figure 5. Contour plots of a) PDLLGA and b) PLLGA for cyclic structures plotted with lactydyl units.

PLLGA suggests a block copolymer, whereas that of PDLLGA points to a more random copolymer. Apparently, the configuration of lactide has an influence on the randomness of the copolymer. The more random character of PDLLGA relative to that of PLLGA suggests either that the rate of incorporation is higher for D-lactide than for L-lactide or that the transesterification of the D-LA–D-LA ester bond is more facile. As similar striped patterns are observed in the contour plots of PDLLGA and PLLGA, transesterification is unlikely to be the origin of this difference (Figure 4). Therefore, the topologies of both polymers are probably determined by a higher rate of incorporation of the racemic mixture of D-lactide and L-lactide relative to that of enantiomerically pure L-lactide.

In summary, MALDI-TOF MS has been used successfully to determine the composition, end groups, and topology of poly(lactide-*co*-glycolide). It was shown that the molecular characterization of copolymers by MALDI-TOF MS is a challenging task, but that intelligent software can be used to derive more information than would otherwise seem possible. Overlap of isotope distributions and multiple peak assign-

ment are common and make the spectra more complicated, especially when comonomers only differ by a methyl group. Evidence was found for selective transesterification by Sn(Oct)$_2$ in the form of the presence of complete lactydyl units, as shown by a fingerprint of the MALDI-TOF MS spectrum. When these contour plots are used, MALDI-TOF MS can be employed as a powerful tool for the elucidation of even the topology of the polymer. Used together with NMR spectroscopy, the MALDI-TOF MS method presented can give a much better understanding of mechanistic processes, such as transesterification. Further studies on the MALDI-TOF MS analysis of PLGA synthesized enzymatically and by polycondensation have been carried out, the results of which will be published in the near future.

Experimental Section

Synthesis: A three-necked flask equipped with a mechanical stirrer was charged with a mixture of lactide (5.0 g, 34.7 mmol) and glycolide (1.0 g, 8.7 mmol). Tin(II) 2-ethylhexanoate (0.1 mol‰) was added after the monomers had melted completely. The mixture was stirred for 6 h under an argon atmosphere at 160 °C. The polymer was then dissolved in chloroform and precipitated with diethyl ether. The purified product was dried in vacuo at 40 °C for 48 h.

Analysis: MALDI-TOF MS analysis was performed with a Voyager DE-STR instrument (Applied Biosystems) equipped with a 337-nm nitrogen laser. An accelerating voltage of 25 kV was applied. Mass spectra were recorded in the reflectron mode (1000 shots). The polymer samples were dissolved in THF at a concentration of 1 mg mL^{-1}. The cationization agents used were potassium trifluoroacetate (Fluka, > 99 %) or sodium trifluoroacetate (Fluka, > 99 %) dissolved in THF at a concentration of 1 mg mL^{-1}. The matrix *trans*-2-(3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene)malononitrile (DCTB; Fluka) was dissolved in THF at a concentration of 40 mg mL^{-1}. Solutions of matrix (10 μL), salt (1 μL), and polymer (5 μL) were mixed, and the mixture was spotted by hand onto a stainless-steel MALDI target and left to dry. Baseline corrections and data analysis were performed by using Data Explorer version 4.0 from Applied Biosystems.

DSC Analysis: The glass transition temperatures of the purified material were measured using a TA Instruments Q100 DSC equipped with a refrigerated cooling system (RCS) and autosampler. The DSC cell was purged with nitrogen gas at a flow rate of 50 mL min^{-1}. Experiments were performed in aluminum hermetic pans using heating and cooling rate of 10 °C min^{-1}. The T_g was determined from the second heating curve by applying the half extrapolated tangent method.

SEC Analysis: SEC analysis was carried out using a Waters model 510 pump, a model 410 refractive index detector (at 40 °C), and a model 486 UV detector (at 254 nm) in series. Injections were done by a Waters model WISP 712 autoinjector using an injection volume of 50 μL. The columns used were a PLgel guard (5-μm particles) 50 × 7.5-mm^2 column, followed by two PLgel mixed-C (5-μm particles) 300 × 7.5-mm^2 columns at 40 °C in series. THF was used as eluent at a flow rate of 1.0 mL min^{-1}. For calibration polystyrene standards were used (Polymer Laboratories, $M_n = 580$ to 7.1×10^6 g mol^{-1}). Data acquisition and processing were performed using Waters Millennium 32 (v4.0) software.

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